



Universitat Autònoma de Barcelona

Programa de doctorado en Medicina

Departament de Medicina

Facultat de Medicina

Universitat Autònoma de Barcelona

## TESIS

# Biomarcadores farmacodinámicos y predictivos de respuesta en la toma de decisiones en ensayos clínicos Fase I de terapias dirigidas contra el cáncer.

Jordi Rodón Ahnert

Director de tesis

Dr. Josep Tabernero Cartula

Barcelona, Junio de 2015

Programa de doctorado en Medicina

Departament de Medicina

Facultat de Medicina

Universitat Autònoma de Barcelona

**Biomarcadores farmacodinámicos y predictivos de respuesta en la toma de decisiones en ensayos clínicos Fase I de terapias dirigidas contra el cáncer.**

TESIS

Jordi Rodón Ahnert

Tutor de tesis

Dr. Josep Angel Bosch Gil

Departamento de Medicina

Facultad de Medicina

Universitat Autònoma de Barcelona

Director de tesis

Dr. Josep Tabernero Cartula

Departamento de Medicina

Facultad de Medicina

Universitat Autònoma de Barcelona

Barcelona, Junio de 2015

A mis padres Jordi y Rocio y a mi hermana Laura

## Agradecimientos

A los diferentes mentores que durante las diferentes etapas de mi vida me han ido guiando desinteresadamente y me han ayudado a crecer como persona. A Pepe Rodriguez, por haberme levantado el listón y haber exigido a ese adolescente soñador. Al Dr. Pepe Alegre, por haberme inculcado una admiración a la Medicina con mayúsculas. Al Dr. Josep Ramon Germà y el Dr. Ricard Mesia así como otros compañeros del Institut Català d'Oncologia, por haberme inculcado una formación integral del paciente con cáncer. A la Dra. Ana Montes y al Dr. Ramon Salazar, por haberme abierto horizontes profesionales que no me había imaginado antes. Al Dr. Anthony Tolcher, Dr. Chris Takimoto, Dra. Amita Patnik, Dr. Kyriakos Papadopoulos y Dra. Kurzrock, por haber confiado en un medicucho español recién especializado y haberlo transformado en un investigador clínico. Al Dr. Jose Baselga y el Dr. Josep Tabernero, por las oportunidades ofrecidas, proyectos compartidos y confianza depositada. A todos vosotros os debo el haberme formado en el médico que presenta esta tesis.

A mis padres, a quienes les debo además del cariño, ejemplo y educación en la libertad y responsabilidad, el haberme dado todas las herramientas necesarias para que me formara como persona y profesional. A mi padre, Jordi, del que aprendí a trabajar con "seny", sin orgullo sino por hacer bien las cosas. A mi madre, de la que aprendí a ver las cosas diferentes, a ser creativo, inconformista. A mi hermana Laura, cómplice y amiga desde la infancia.

A todos los que me han ayudado con sus consejos, los que me han escuchado pacientemente y animado en el camino. A Josemaría, quien con su ejemplo y autenticidad me ha servido como guía de comportamiento en circunstancias tan diversas.

A Ben, Irene, Rodrigo, Analía, Cinta, María, Guillem, Cristina... porque sin vuestro gran trabajo diario y la confianza que me habéis depositado, la UITM no sería posible. A Gemma Sala, con quien, gracias a su trabajo, confianza y amistad, hemos puesto las bases de lo que es hoy la UITM. A Ana Vivancos y Paolo Nuciforo, con los que es una suerte poder contar. Ellos son el alma del Programa de Prescreening Molecular. A todos los compañeros de trabajo, en especial aquellos que vertebran la UITM.

Esta tesis tampoco hubiera sido posible sin tantas otras personas, que es difícil no olvidarse a alguien. A Beatriz García, que con su perseverancia ha conseguido que me organize y la presente satisfactoriamente. A mis colegas investigadores, en Vall d'Hebron y en otras instituciones: la investigación hoy en día es multidisciplinar y multiinstitucional. No entiendo otra forma de avanzar que mediante la colaboración. A todos vosotros, gracias.

Especiales agradecimientos a todos los pacientes y familias que depositan en nosotros su confianza. Sin su esfuerzo y valentía, su generosidad y altruismo, la investigación clínica no sería posible. Vosotros sois los auténticos protagonistas de los descubrimientos y avances.

## INDICE

Agradecimientos	3
1. Introducción	6
1.1 Apertura de una nueva era en la biología molecular del cáncer y reclasificación de la enfermedad	6
1.2 Un cambio de paradigma en el desarrollo clínico precoz de fármacos oncológicos causado por la irrupción de las terapias dirigidas	11
1.3 Desarrollo simultáneo de biomarcadores y fármacos en las terapias dirigidas y en el desarrollo clínico precoz de fármacos	13
1.4 Biología molecular de Fosfoinositol 3 quinasa en el cáncer	16
1.5 Biología molecular de la vía Sonic Hedgehog en el cáncer	21
1.6 Establecimiento de un programa de preselección en el contexto de un programa de desarrollo clínico precoz de fármacos	23
2. Hipótesis y objetivos	29
3. Primera parte: Inhibidores de PI3K y mutaciones en PIK3CA	31
3.1 Implementación de un programa de preselección para identificar alteraciones de PI3K que permitan reclutar pacientes para ensayos clínicos de fase I con tumores con alteraciones de PI3K	32
3.2 Evaluación clínica de la seguridad, farmacología (farmacocinética y farmacodinamia) y biomarcadores predictivos de dos inhibidores de PI3K: Pilaralisib ó XL147 y Buparlisib ó BKM120.	34
3.2.1 Primer manuscrito: Estudio de fase I sobre la farmacocinética y farmacodinamia de SAR245408 (XL147), un pan-inhibidor de PI3K de Clase I, en pacientes con tumores sólidos avanzados.	35
3.2.2 Segundo manuscrito: Estudio de fase I de aumento de dosis y expansión de Buparlisib (BKM120), un pan-inhibidor oral de PI3K de Clase I, en pacientes con tumores sólidos avanzados.	50
3.2.3 Implementación de un programa de preselección para identificar alteraciones de PI3K que permitan reclutar pacientes para ensayos clínicos de fase I con tumores con alteraciones de PI3K.	64
3.4 Resultados y discusión	82
3.5 Conclusiones y seguimiento de líneas de investigación	88

4. Segunda parte: El inhibidor de SHH Sonidegib, activación de la vía y desarrollo de una firma de activación de SHH	94
4.1 Evaluación clínica de la seguridad, farmacología (farmacocinética y farmacodinamia) y marcadores predictivos de un inhibidor de SMO: Sonidegib o LDE225.	96
4.1.1 Tercer artículo: Este es el primer estudio multicéntrico, abierto, de fase 1 con aumento progresivo de la dosis realizado en humanos del inhibidor oral de Hedgehog Sonidegib (LDE225) en pacientes dultos con tumores sólidos avanzados.	97
4.2 Resultados y discusión	110
4.3 Conclusiones y líneas de investigación de seguimiento	114
5. Conclusiones	118
5.1 Los estudios de fase I como entorno para probar hipótesis y realizar ensayos en poblaciones de pacientes seleccionados molecularmente	118
5.2 Evolución del programa de prescreening molecular fundamentada en las experiencias aquí descritas	121
5.3 Estudios clínicos en el área de la Medicina Genómica	123
5.4 Controversias y dificultades del desarrollo clínico precoz de fármacos basado en el empleo de biomarcadores	125
5.5 Observaciones finales	128
6. Anexos	130
Anexo 1 Estudios y publicaciones del grupo en el área de trabajo descrita	130
Anexo 2 Articulo “Desarrollo de una firma de cinco genes de Hedgehog como herramienta para la preselección de pacientes para terapia con inhibidores de Hedgehog en el meduloblastoma”	139
Anexo 3. Resumen de los datos del ensayo clínico “A Study of BYL719 in Adult Patients With Advanced Solid Malignancies, Whose Tumors Have an Alteration of the PIK3CA Gene”	150
Anexo 4 Estudios con Pilaralisib, Buparlisib y Sonidegib	170
Anexo 5. Estudios en Medicina Genómica	186
7. Bibliografia	191

# 1.Introducción

## 1.1 Apertura de una nueva era en la biología molecular del cáncer y reclasificación de la enfermedad

Uno de los principales objetivos de la biología moderna es conocer el comportamiento de las células tumorales y normales. En las últimas dos décadas se han producido enormes avances en el conocimiento de la patogénesis del cáncer. Está actualmente aceptado que el cáncer se genera mediante un proceso mutagénico que consta de diferentes fases a través del cual las células adquieren una serie de características comunes entre las que se encuentran un potencial de proliferación ilimitado, autosuficiencia en las señales de crecimiento y resistencia a las señales apoptóticas y antiproliferativas.

El comportamiento así como el control del crecimiento celular tanto en células normales como tumorales son extremadamente complejos. Para desenmascarar estos procesos, se ha optado por analizar una serie de vías de señalización concretas que representan una compleja red molecular a través de la cual se controla el comportamiento celular. Hanahan y Weinberg(1) conceptualizaron bastante trabajo previo al enumerar los sellos distintivos de las células cancerígenas y los tumores, esto es, un potencial de replicación ilimitado, resistencia a la apoptosis, inactivación de las señales supresoras del crecimiento, autosuficiencia en las señales de crecimiento, capacidad para invadir tejidos adyacentes o migrar a órganos distantes (metástasis) y angiogénesis continua. Además de estas, poseen otras características que recientemente han sido identificadas como cruciales en el desarrollo tumoral(2), tales como la capacidad para evitar la vigilancia inmunológica(3), daños significativos en el ADN, una elevada replicación del ADN causada por las altas tasas de replicación, y defectos en la reparación del ADN en las células tumorales(4), estrés mitótico, inestabilidad cromosómica(5), estrés

metabólico, mayor uso de la glicólisis, estrés oxidativo y un mayor nivel de especies reactivas del oxígeno en las células tumorales(6).



**Figura 1.** Los sellos distintivos del cáncer. Al desentrañar la biología del cáncer se han podido

identificar con mayor detalle las características de las células tumorales.

Los últimos avances en la investigación del cáncer están cambiando nuestra manera de entender la biología del cáncer y su tratamiento. Entre dichos avances se encuentran las tecnologías de secuenciación masiva de próxima generación, así como iniciativas mundiales de investigación como el *1000 Genomes Project*(7), el *Atlas del Genoma del Cáncer* (TCGA)(8), el Consorcio Internacional del Genoma del Cáncer (ICGC)(9), y catálogos de libre acceso como el *Catálogo de mutaciones del cáncer* (COSMIC)(10) y *Genomics of Drug Sensitivity in Cancer*(11).

Muchos de los rasgos fenotípicos anteriormente descritos son debidos a alteraciones genéticas como las mutaciones de ganancia de función, amplificación y/o la sobreexpresión de

oncogenes clave, junto con mutaciones de pérdida de función y la supresión y/o silenciamiento epigenético de genes supresores de tumor clave(12). A pesar de la multitud de alteraciones genéticas y epigenéticas que se encuentran en los distintos tipos de cáncer, puede que simplemente unos pocos cambios sean los relevantes para la malignización tumoral(13), aquellos resultantes de la ganancia de un oncogén o de la pérdida de un gen supresor tumoral o de la activación de algunas vías de señalización. En el análisis Pan-Cancer del TCGA, por ejemplo, se caracterizaron diversos patrones repetitivos de eventos funcionales comunes a múltiples tumores con origen en distintos tejidos, patrones que codifican componentes de las cuatro principales principales vías oncogénicas (RTK-RAS-RAF, PI3K-AKT-mTOR, ciclo celular y reparación del ADN mediante la vía p53)(14). En este estudio, en la mayoría de las muestras de diferentes tumores se observaron alteraciones en al menos una de estas vías. En ese mismo proyecto, también se observó que una parte significativa de las alteraciones descritas eran susceptibles de tratamiento directo o indirecto con fármacos anticáncer aprobados o experimentales. La expresión "dependencia oncogénica"(15) fue acuñada para describir el fenómeno mediante el cual el mantenimiento del tumor suele depender de la actividad continuada de algunos oncogenes o vías de señalización, lo cual ha sido demostrado *in vivo* en varios oncogenes como *MYC*(16), *HRAS*(17), *BCR-ABL*(18) y *KRAS*(19).

Actualmente se están reclasificando los tumores según criterios genéticos. Las patologías más comunes se están dividiendo según su análisis genómico, como es el caso del cáncer colorrectal (tumores con *KRAS/BRAF/NRAS/PIK3CA* salvaje o nativo, tumores con mutaciones o combinaciones de mutaciones en *KRAS*, *NRAS*, *BRAF* y *PIK3CA*); el melanoma (tumores con mutaciones en *BRAF*, *NRAS* ó *KIT*), y el cáncer de pulmón no microcítico (carcinomas con mutaciones en *EGFR*, *KRAS* y *BRAF*, y carcinomas con traslocación de *ALK* o *ROS1*). Las actuales tecnologías de secuenciación también han permitido recientemente descubrir nuevas mutaciones oncogénicas, distintas de las aberraciones más frecuentes ya descritas en muchos tipos de tumores, algunas de los cuales tienen una clara relevancia terapéutica. Por ejemplo,

tras la secuenciación completa del transcriptoma se detectó la fusión de transcritos de *KIF5B* (el gen 5B de la familia de quinesinas) y el oncogén *RET* en el 1-2% de los adenocarcinomas de pulmón(20). Otros ejemplos son la fusión *MAGI3-AKT3*, que desencadena la activación constitutiva de la quinasa AKT hallada en el cáncer de mama triple negativo(21), que se inhibe mediante inhibidores de moléculas pequeñas, así como translocaciones genéticas que desembocan en la desactivación del PTEN en algunos subtipos de cáncer de próstata(22). Todo esto justifica la realización de ensayos clínicos en los que se investigue la actividad de algunos inhibidores de vías concretos en subgrupos de pacientes(23).

Además, la generación de predicciones genéticas de respuesta farmacológica en contextos preclínicos como la *Drug Sensitivity in Cancer*(24) y la *Cancer Cell Line Encyclopedia*(25) están siendo de gran ayuda en la traslación de datos genómicos a regímenes terapéuticos, tal como evidencia la alta sensibilidad que muestran a los inhibidores PARP las células del sarcoma de Ewing, que albergan una translocación del gen EWS-FLI1(23). Una consecuencia directa de estos avances es que cada vez se obtienen mayores tasas de respuesta en los ensayos clínicos donde se selecciona a los pacientes según las características moleculares de sus tumores. Entre estos avances se encuentra vemurafenib, con un 48% de índice de respuesta en los pacientes con melanoma que albergan la mutación V600E del gen BRAF(26), así como olaparib, con un 41% de índice de respuesta en el cáncer de ovario recurrente con mutación del gen *BRCA1/2*(27), y crizotinib, con un 57% de índice de respuesta en los pacientes con cáncer de pulmón con la translocación *EML4-ALK*(28). Estas impresionantes tasas de respuesta obtenidas en poblaciones seleccionadas de pacientes contrastan significativamente con el índice aproximado de respuesta del 5% obtenido en los ensayos fase I llevados a cabo en pacientes no seleccionados(29).

Una estrategia tal como seleccionar una cohorte de pacientes en base a biomarcadores moleculares está cambiando el paradigma del tratamiento oncológico hacia uno que permita

la práctica de la medicina personalizada. Esto abre una nueva era llamada "la era del teragnóstico" en la que la combinación de terapias y tests diagnósticos moleculares permitirá asociar a una terapia dirigida los resultados de una prueba diagnóstica mediante la cual se identifique a los pacientes con mayor probabilidad de responder o no a un régimen de tratamiento. Los prometedores resultados obtenidos con los abanderados de esta estrategia en tumores sólidos, esto es, imatinib en GIST con mutaciones en c-KIT(30) y trastuzumab en el cáncer de mama HER2 positivo(31), avalan la continuación de este tipo de investigaciones en esa dirección. Actualmente existe una pléthora de ejemplos ilustrativos, tal como se muestra en la Tabla 1.

Marcador/Población	Fármaco	Mecanismo de acción	Respuestas*
Cáncer de mama HER2 sobreexpresado/amplificado	Trastuzumab	Anticuerpo anti-HER2	12%
	Trastuzumab-DM1	Anticuerpo inmunoconjuguado anti-HER2	44%
GIST con CD117 sobreexpresado	Imatinib	Inhibidor de c-KIT, PDGFR	54%
Ca mama, ovario y próstata BRCA1/2 mutado.	Olaparib	Inhibidor de PARP	47%
Melanoma BRAF V600E mutado CPNM con ALK traslocado	Vemurafenib Dabrafenib Crizotinib	Inhibidor de BRAF Inhibidor de BRAF Inhibidor de ALK, MET	75% 60% 57%
Ca medular de tiroides (con mutación de RET, expression de MET y VEGF)	Cabozantinib	Inhibidor de MET, VEGFR2, RET	29%
Ca Mama con amplificación de FGFR1	E-3810	Inhibidor de FGFR, VEGFR	70%

ALK: anaplastic lymphoma kinase; GIST: gastrointestinal stromal tumor; MET: mesenchymal epithelial transition; CPNM: cáncer de pulmón no Microcítico; PARP: poly(ADP-ribose) polymerase; PDGFR: platelet-derived growth factor receptor; VEGF: vascular endothelial growth factor receptor.  
 \* disminución de tamaño > 20%

**Tabla 1.** Índice de respuesta a terapias dirigidas efectivas en poblaciones seleccionadas

A medida que el análisis completo de múltiples estudios de secuenciación van arrojando luz sobre los mecanismos subyacentes en los complejos procesos de invasión del cáncer y metástasis, todo indica que tan solo un limitado número de vías de señalización desempeñan un papel importante en la génesis y progresión del cáncer en humanos.

En base a esto, es aparente que los avances en las terapias moleculares dirigidas producirán cambios revolucionarios en el tratamiento de muchos tipos de tumores. A la vez que se exploran multitud de nuevas vías y estrategias de tratamiento, los avances realizados en el descubrimiento y síntesis de fármacos han permitido modular terapéuticamente vías difíciles como la vía de fosfoinositol 3 quinasa (PI3K) y la vía de señalización de Sonic Hedgehog (SHH). Una vez se disponga de fármacos selectivos para inhibir dichas vías, será crucial investigar cómo administrar de forma segura dichos inhibidores, el uso de marcadores de la inhibición de la vía en el contexto clínico y el desarrollo de marcadores predictivos que indiquen aquellos tumores con mayor probabilidad de tener adicciones génicas.

## **1.2 Un cambio de paradigma en el desarrollo clínico precoz de fármacos oncológicos causado por la irrupción de las terapias dirigidas**

A principios de los años 90, una nueva generación de oncólogos afrontó el desafío de desarrollar una nueva clase de nuevos fármacos, los llamados “terapias dirigidas”. Estas nuevas investigaciones se realizaron en un contexto dominado en gran medida por experiencias anteriores en las que se trataba a los pacientes únicamente con agentes citotóxicos. Anteriormente, el desarrollo de agentes citotóxicos se basó en una relación directa de dosis-respuesta, esto es, cuanto más alta la dosis, mayor el efecto. Sin embargo, a altas dosis, la mayor reducción de la carga tumoral iba acompañada de un mayor grado de toxicidad. Por el contrario, en nuestros días el desarrollo clínico de nuevos fármacos se ha alejado de los citotóxicos, pasando a centrarse en los agentes dirigidos molecularmente(32). No obstante, sigue sin existir una definición clara de lo que son las terapias dirigidas. Por concretar, los agentes dirigidos son terapias que actúan modulando una serie de procesos biológicos como los llamados "sellos distintivos del cáncer" (*hallmarks of cancer*). El desarrollo de estas terapias dirigidas debe ser diferente del de los agentes citotóxicos. Puede que, a grandes dosis, se pierda la especificidad de un efecto dado y que aumentando una dosis concreta más allá de la biológicamente efectiva no se obtenga mayor beneficio. En consecuencia, este paradigma

requiere la integración de mediciones de efectos biológicos con los llamados “marcadores farmacodinámicos”(33).

Se han revisado una gran cantidad de recomendaciones para actualizar las normas de desarrollo de fármacos y adaptarlas para las terapias dirigidas. Para evaluar la eficacia de los fármacos, se han revisado los criterios RECIST(34) y se han propuesto una serie de modificaciones para adaptarlos a unos mecanismos de acción concretos. Así mismo, se desarrollaron los criterios de Choi(35) para representar los cambios metabólicos que en ocasiones se observan con estos fármacos. Así mismo, se han desarrollado los criterios RECIST relacionados con el sistema inmunológico (IR RECIST)(36) con el fin de evaluar los efectos de la inmunoterapia en los tumores sólidos. Del mismo modo, se han desarrollado algunos principios orientativos para evaluar si la enfermedad estable es en sí misma un criterio evaluable de eficacia, reflejando el retraso de la progresión de la enfermedad(37). También se han modificado las directrices para evaluar los efectos secundarios, en un intento por representar las nuevas toxicidades que se producen con el uso de las terapias dirigidas. Entre ellas se encuentran la hipertensión, las erupciones acneiformes, la hiperglucemia y la hiperfosfatemia, entre otras(38). Otro aspecto a considerar es si las toxicidades específicas (*on-target toxicities*) deben ser tratadas de forma diferente a las toxicidades inespecíficas e inesperadas (*off-target toxicities*). El sentido común indica que las primeras, a menos que resulten intolerables, no deberían llevar a una reducción de la dosis, mientras que las segundas deben evitarse(39).

La capacidad de obtener el perfil de aberraciones moleculares susceptibles de tratamiento de cada paciente oncológico de forma rentable y precisa mediante el empleo de tecnologías de alto rendimiento ha abierto un mundo de posibilidades a la hora de administrar terapias dirigidas a una población de pacientes seleccionados(40). En la última década, hemos sido testigos de cómo la investigación clínica y traslacional en el ámbito de la oncología tienden a

converger en un proceso a través del cual se pretende desarrollar los mejores agentes dirigidos alineándolos simultáneamente con los mejores biomarcadores predictivos, con el fin de orientar su uso ya en las primeras fases del proceso de desarrollo de fármacos.

De este modo, la identificación de biomarcadores de efectos farmacológicos (marcadores farmacodinámicos), así como de posibles subpoblaciones de pacientes diana ha pasado de producirse en las últimas fases de desarrollo de fármacos a ocurrir en los ensayos clínicos de fase I. De este modo, los ensayos de fase I están adquiriendo un papel cada vez más importante en el plan de desarrollo de nuevos agentes. Algunos ejemplos recientes de dicha evolución son los inhibidores ALK en el cáncer de pulmón no microcítico. En el desarrollo de crizotinib, la primera generación de inhibidores de ALK, los pacientes fueron seleccionados basándose en una alteración molecular (empleando test que se desarrolló paralelamente), obteniendo respuestas espectaculares(28). Una vez bien definida la población de pacientes e identificado un biomarcador de selección, bastaron los datos obtenidos en el ensayo de fase I para aprobar ceritinib, el inhibidor de segunda generación de ALK (41, 42).

Tal como hemos indicado, hemos pasado de una estrategia de desarrollo de fármacos de orientación empírica, en el que la respuesta a los fármacos observada en algunos tipos de tumor concretos orientaban los siguientes pasos de la experimentación, a la investigación translacional mediante la cual los biomarcadores predictivos y los fármacos se desarrollan simultáneamente desde las primeras fases de desarrollo hasta la aprobación del fármaco(43).

### **1.3 Desarrollo simultáneo de biomarcadores y fármacos en las terapias dirigidas y en el desarrollo clínico precoz de fármacos.**

El diseño de los ensayos clínicos en la era de los agentes dirigidos dependerá del tipo de biomarcador a probar o desarrollar(44, 45). Los biomarcadores predictivos proporcionan información al investigador sobre la actividad antitumoral de una terapia concreta. Los biomarcadores pronósticos aportan datos sobre el riesgo de recurrencia, progresión de la

enfermedad o muerte. Los biomarcadores farmacogenómicos indican la respuesta de los pacientes a un fármaco con respecto a su toxicidad o eficacia. Durante el proceso de desarrollo hay que determinar la validez analítica y clínica, así como la utilidad clínica de los biomarcadores. La validación analítica es la confirmación de que la prueba mide lo que se pretende medir, con la sensibilidad y especificidad necesarias. La validez clínica de un biomarcador hace referencia a la eficacia de la prueba a la hora de identificar a aquellos pacientes que mejor o peor van a responder a una terapia concreta. Finalmente, entendemos por utilidad clínica que la medición y uso del biomarcador para la toma de decisiones terapéuticas es más beneficioso para los pacientes que el manejo convencional(46).

El desarrollo simultáneo de biomarcadores y fármacos es esencial para el éxito de las terapias con base genómica, aunque esta estrategia tiene muchas implicaciones técnicas y, a veces, incluso éticas. En primer lugar, es necesario contar con pruebas diagnósticas sólidas y válidas. Además, hay que evaluar las propiedades farmacológicas del fármaco antes de probarlo clínicamente. Para ello, es necesario realizar un perfil farmacocinético y farmacodinámico, definir los biomarcadores de inhibición de la vía y seleccionar el fármaco más adecuado para obtener los efectos biológicos deseados. Y lo que es más importante, hay que validar funcionalmente las variantes genómicas concretas cuya sensibilidad se espera predecir.

Tal como se ha mencionado anteriormente, los ensayos de fase I son el escenario perfecto para probar hipótesis. Además de estar investigándose la seguridad y toxicidad de múltiples biomarcadores, se pueden realizar estudios para determinar su interacción con la diana y comprobar la respuesta biológica (prueba de mecanismo), establecer la dosis biológica óptima del agente dirigido (determinada mediante los marcadores farmacodinámicos) e identificar la población de pacientes más apropiada para una terapia dada (denominado prueba de concepto, que viene determinada por la información facilitada por los marcadores predictivos). Los biomarcadores moleculares que identifican a aquellos pacientes con mayor

probabilidad de responder a un tratamiento (biomarcadores de selección) pueden pasar a ser biomarcadores predictivos una vez haya sido demostrada su eficacia clínica mediante rigurosas evaluaciones confirmatorias en ensayos de fase II y III (44). Que se defina una población de pacientes seleccionados en estudios precoces no impide que posteriormente se desarrolle un fármaco concreto solo para esa subpoblación, ya que un fármaco puede ser efectivo en varios contextos moleculares, y que ésto puede que no sea evidente en los ensayos iniciales.

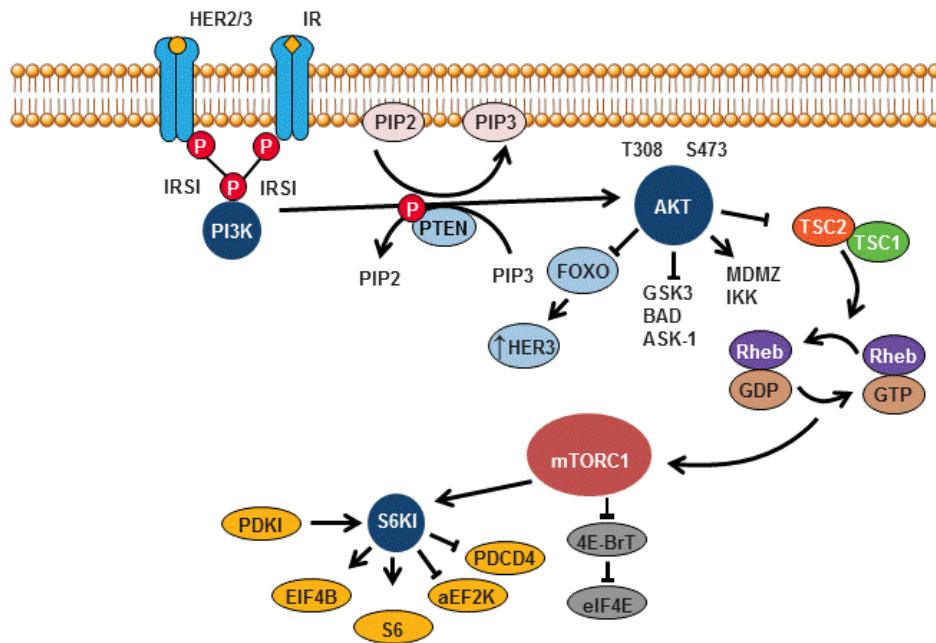
Desde esta perspectiva, la prueba de hipótesis en ensayos de fase I mediante el uso de biomarcadores de selección de pacientes (aberraciones moleculares concretas que pueden predecir la respuesta tumoral) puede demostrar la prueba de concepto y avalar la continuación de las investigaciones con un fármaco o diana concretos(47). Por otro lado, si no se obtiene un efecto antitumoral suficiente en el considerado mejor de los escenarios posibles, y siempre que los marcadores farmacodinámicos indiquen que se ha logrado una inhibición suficiente de la diana, se redefinirán radicalmente las estrategias de desarrollo del fármaco(48). Por lo tanto, la selección de pacientes basada en el uso de biomarcadores podría ofrecer ventajas con respecto a la estrategia basada en poblaciones no seleccionadas, permitiendo que los pacientes se beneficien antes de la terapia, ayudando a las agencias reguladoras y a los organismos responsables del reembolso de fármacos a la hora de tomar decisiones y modificar el proceso de registro de aprobación de agentes prometedores. Otras posibles ventajas de la selección de pacientes basada en el uso de biomarcadores moleculares en los ensayos clínicos de fase I son: a) aceleran el proceso de confirmación del potencial clínico de los biomarcadores predictivos para agentes dirigidos molecularmente; b) aportan información valiosa sobre la biología del cáncer; y c) proporcionan una oportunidad terapéutica importante en términos clínicos para los pacientes con cáncer en fase avanzada(49). No obstante, hay que añadir que el hecho de hallar una posible diana en el tumor de un paciente no implica necesariamente que éste vaya a responder a un agente terapéutico dirigido contra dicha diana.

Administrar una terapia dirigida molecularmente a una población seleccionada de pacientes tiene implicaciones económicas y logísticas, así como implicaciones para los propios pacientes a la hora de diseñar un programa de desarrollo clínico precoz de fármacos. Por ejemplo, si los inhibidores de PI3K son más efectivos en los tumores con alteraciones como las mutaciones de *PIK3CA* (y suponiendo una tasa de respuesta del 30% en los tumores con mutaciones en *PIK3CA* y una baja actividad en los tumores con *PIK3CA* salvaje o nativo), se podrían aplicar las siguientes dos estrategias: por un lado, realizar un ensayo clínico en el que se incluyeran pacientes con cualquier tipo de cáncer de mama para probar un inhibidor de PI3K (dado que el 30% de las pacientes con cáncer de mama presentan alteraciones de *PIK3CA*(50), habría que incluir 300 pacientes durante un periodo de reclutamiento de 36 meses para obtener un índice estimado de respuesta del 5%; o bien realizar un ensayo en el que se incluyeran 100 pacientes con cáncer de mama con mutaciones de *PIK3CA* y 12 meses de reclutamiento con un índice estimado de respuesta del 30%. Incluso realizando un cribado de alteraciones moleculares, se reducirían los costes en un 67% (B. Weber, Novartis, comunicación personal).

La validación clínica temprana del valor predictivo de algunos biomarcadores es un factor clave en el proceso de desarrollo de fármacos. En estudios recientes se ha investigado la viabilidad de obtener el perfil molecular en tiempo real de los tumores de los pacientes y asociar la aberración identificada con los tratamientos dirigidos. Von Hoff y col.(51) y Tsimberidou y col. (52) demostraron que los pacientes que recibieron agentes dirigidos combinados mostraron mejores índices de respuesta y períodos de tiempo más cortos hasta el fracaso del tratamiento.

#### 1.4 Biología molecular de Fosfoinositol 3 quinasa en el cáncer

El eje PI3K/AKT/mTOR regula las principales funciones celulares, esto es, el metabolismo, el crecimiento, la migración, la supervivencia celular y la angiogénesis (Figura 2).



**Figura 2.** Señalización de la vía PI3K/AKT/mTOR

Existen tres subtipos de quinasas PI3K según su estructura, regulación y especificidad del sustrato lipídico. De estas, hay cuatro parálogos catalíticos diferentes ( $\alpha$ ,  $\beta$ ,  $\delta$ , y  $\gamma$ ) relacionados con el cáncer, que componen las quinasas PI3K de clase I(53). Las isoformas de clase IA son proteínas heterodiméricas formadas por una subunidad catalítica (p110) y una subunidad regulatoria (p85) implicadas principalmente en la patogénesis del cáncer en humanos. En la clase IA se dan tres genes, *PIK3CA*, *PIK3CB* y *PIK3CD* que codifican las isoenzimas homólogas p110 $\alpha$ , p110 $\beta$  y p110 $\delta$ . La clase IB está compuesta por el gen *PIK3CG*, que codifica la subunidad p110 $\gamma$ . De éstas, p110 $\alpha$  y p110 $\beta$  se expresan de forma ubicua, mientras que p110 $\delta$  y p110 $\gamma$  se encuentran principalmente en células inmunes y hematopoyéticas (54).

Las quinasas PI3K generan el mensajero lipídico fosfatidilinositol trifosfato 3,4,5 (PIP3), que media la activación de varias proteínas quinasas, incluyendo la AKT. La quinasa AKT estimula la glicólisis activando las enzimas glicolíticas a través de la GSK3 $\beta$  y regulando los transportadores de glucosa(55). Este importante mecanismo molecular hace que las células tumorales

consuman gran cantidad de glucosa como fuente de ATP, lo que se conoce también como “efecto Warburg”(56). La quinasa AKT también regula la supervivencia celular, principalmente inhibiendo la transcripción de los genes antiapoptóticos BIM y FASLG a través de la inactivación de las proteínas Bad y del factor de transcripción FoxO. La quinasa AKT también promueve el avance del ciclo celular regulando los inhibidores de quinasas dependientes de ciclinas CDKN1A y CDKN1B (también conocidos como p21 y p27), y mediante la activación de las ciclinas D1 y E1 y los factores de transcripción cJUN y cMYC. La síntesis de proteínas, el crecimiento y la proliferación celular, así como las funciones *downstream* de AKT las regula el eje mTORC1/p70S6K y efectores como 4EBP1E, que inhibe el complejo IF4E–EIF4G3(57). La angiogénesis autocrina y paracrina es regulada por la expresión de mTORC1/HIF1 $\alpha$ /VEGF. Además, la regulación negativa de esta vía la realizan la fosfatasa PTEN y el polifosfato-4-fosfatasa de tipo II, una proteína codificada por INPP4B, que divide un grupo de fosfatos en PIP3 y PIP2, respectivamente(58).

Basándonos en lo que se conoce actualmente sobre la vía, se podrían utilizar como marcadores de su actividad: la fosforilación de AKT en los residuos T308 (marcador de la actividad de la quinasa PI3K) y S473 (fosfo-epítopo dependiente de mTORC2); la fosforilación del sustrato de la quinasa AKT, AKT1S1 (también conocido como PRAS40) en T246 (marcador de la actividad de la quinasa AKT), la proteína de unión al factor de iniciación eucariótico 4E (4EBP1) en S65 y T70 (marcador de la actividad del complejo de señalización mTORC1), y la fosforilación de la proteína ribosómica s6 (RPS6) en S240 y S244 (marcador de la actividad de mTORC1/S6K). La mejor opción sería analizar la actividad de la quinasa AKT, aunque, por nuestra experiencia, la toma y manejo de muestras para medir la AKT en el tejido se debe realizar en condiciones muy rigurosas, poco factibles en el contexto de los ensayos clínicos multicéntricos. Por esta razón, es preferible analizar la fosforilación de RPS6, 4EBP1 o pPRAS40 para el desarrollo de biomarcadores(59-61).

El potencial oncogénico de la vía PI3K está basado en dos factores: En primer lugar, las alteraciones en la vía PI3K/AKT/mTOR pueden inducir la transformación de la línea celular y la formación del tumor en ratones transgénicos(62-64). En segundo lugar, la activación de la señalización suele ocurrir como consecuencia de la presencia de múltiples alteraciones moleculares en algunos componentes de la vía, tales como mutaciones (*PIK3CA*, *AKT1*, *PTEN*), amplificaciones génicas (*PIK3CA*, y *AKT1* y *AKT2*), pérdida de expresión de los supresores tumorales *PTEN* (21)e inositol polifosfato-4-fosfatasa de tipo II y otros mecanismos (*INPP4B*) que se suelen observar en numerosos tumores humanos(53) (tabla 2).

Nodo/Alteración	Enfermedad	Frecuencia
<b><i>PIK3CA</i></b>		
Mutación en <i>PIK3CA</i>	Mama Endometrial Tracto urinario Colon Ovario	25% 26% 21% 12% 10%
Amplificación de <i>PIK3CA</i>	Cabeza/Cuello CPNM escamoso Gastrico Colon Mama	42% 66% 36% 37.9% 9%
<b><i>PTEN</i></b>		
Pérdida monoalélica de <i>PTEN</i>	Glioblastoma Colon Mama CPNM Próstata Gastrico	75% 20% 40 – 50% 37% 42% 47%
Pérdida bialélica de <i>PTEN</i>	Endometrial Glioblastoma Próstata Mama Colorectal	50% 30% 10% 5% 7%
Pérdida de expression de <i>PTEN</i> *	Endometrial Próstata Mama Ovario Glioblastoma Melanoma	NA
<b>Otros reguladores de PI3K</b>		

\* Pérdida de expression de *PTEN* puede deberse a mutación, perdida de heterozigosidad, o factores epigenéticos como hipermetilación del promotor o expresión anómala de microRNAs (miR-21 entre otros)

Pérdida de expression de INPP4B	Prótata Mama (TN) Ovario	NA
Mutación en PIK3R1	Glioma Colon Ovario	4 % 3 % <5%
<b>AKT</b>		
Mutación en AKT1	Colon Mama Ovario Endometrial	1 % 4 % 1 % NA
Amplificación de AKT1	Gástrico Mama	20% 1%
Mutación en AKT2	Colon	1%
Mutación en AKT2	Páncreas Ovario Mama	20% 14.1% 3%
Mutación en AKT3	Melanoma	1.5%
Amplificación de AKT3	Mama	9.9%
PTEN, phosphate and tensin homologue deleted from chromosome 10; INPP4B inositol polyphosphate-4-phosphatase type II; PIK3R1, PI3K regulatory subunit alpha. CPNM Cáncer de pulmón no microcítico		

**Tabla 2.** Alteraciones moleculares frecuentes en la vía PI3K en tumores en humanos

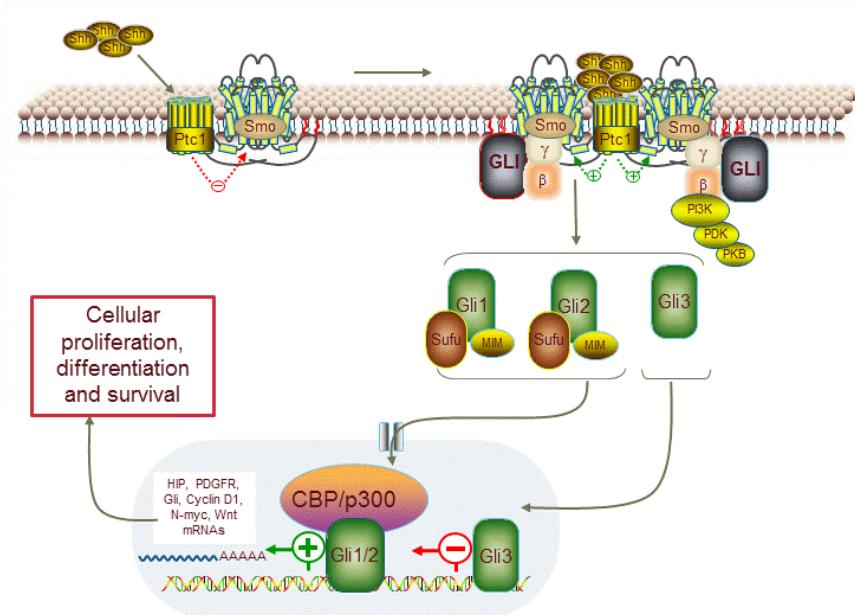
Estos mecanismos ofrecen una miríada de oportunidades para el desarrollo de fármacos para distintos tipos de cáncer, dirigidos contra aberraciones genéticas en la vía PI3K-AKT-mTOR susceptibles de tratamiento. Se han desarrollado numerosos compuestos para inhibir diferentes nodos en la señalización de PI3K/AKT/mTOR. Entre los inhibidores actuales de PI3K se encuentran inhibidores reversibles y competitivos con el ATP de las cuatro isoformas de PI3K de clase I (conocidas como inhibidores selectivos de PI3K de clase I o pan-inhibidores de PI3K, como Buparlisib -BKM120- o Pilaralisib -XL147); los inhibidores específicos de isoformas (como el inhibidor selectivo de p110 $\alpha$ , Alpelisib o BYL719); y los pan-inhibidores de PI3K/mTOR duales (NVPBEZ235, XL765, o GSK1059615). Otros agentes que actúan a niveles más profundos de la señalización de la vía PI3K son los inhibidores de mTOR (que se pueden dividir en

inhibidores rapálogos e inhibidores catalíticos de mTOR) y los inhibidores de Akt (inhibidores alostéricos y catalíticos).

Los ensayos de fase I con inhibidores de la vía PI3K son el escenario ideal para probar estrategias de selección molecular. Las mutaciones en el gen *PIK3CA* son muy frecuentes en el cáncer en humanos (65, 66) y los estudios preclínicos han demostrado que las mutaciones del gen *PIK3CA* y la activación de la vía PI3K están asociadas a la formación, crecimiento y mantenimiento tumoral y una actividad de los inhibidores de PI3K-AKT-mTOR sobre todo en los modelos preclínicos que albergaban una activación de la vía PI3K, como las mutaciones de *PIK3CA* y la pérdida de PTEN(67-69).

## 1.5 Biología molecular de la vía Sonic Hedgehog en el cáncer

La vía de señalización Hedgehog desempeña un papel esencial en el desarrollo cerebral, óseo y muscular embrionario y fetal(70). Durante el período neonatal y la edad adulta, la actividad de la vía Hh participa en la regulación del desarrollo óseo, el mantenimiento y reparación tisular y el mantenimiento de las poblaciones de células madre (como por ejemplo en los folículos pilosos)(70, 71). Ante la ausencia de proteínas Hedgehog (Hh) (Sonic Hedgehog SHH, Indian Hedgehog y Desert Hedgehog) los receptores PTCH (*Patched*) inhiben de manera constante SMO (*Smoothened*). Cuando la proteína Hh está presente y se acopla a su receptor (PTCH), se suprime la inhibición de SMO, activándolo. Un SMO activo (un receptor acoplado a proteínas G) interactúa con el complejo citoplasmático que regula las proteínas GLI<sub>1,2</sub> y 3. Esto permite a las proteínas GLI entrar en el núcleo y actuar como factores de transcripción, alterando la transcripción génica de los genes diana. La vía SHH regula la transcripción de genes que controlan la proliferación, diferenciación y supervivencia celular y orquestan un bucle de retroalimentación mediante la inducción de los genes *PTCH*.



**Figura 3.** La señalización de la vía Sonic Hedgehog

La activación aberrante de la vía Hh ha sido asociada a la patogénesis de muchos tipos de cáncer en humanos a través de mecanismos ligando dependientes y ligando independientes de Hh. Alteraciones genéticas como las mutaciones de pérdida de función en los reguladores negativos Patched 1 (*PTCH1*) y/o el receptor supresor de Fused (*SUFU*), o con menor frecuencia, las mutaciones de ganancia de función en el regulador positivo Smoothened (*SMO*), causan la activación de la vía ligando-independiente y se han asociado al carcinoma celular basal (BCC), el meduloblastoma y el rhabdomiosarcoma. Se ha observado una sobreexpresión del ligando Hh en los tumores de páncreas, pulmón, mama, próstata, esófago, colorrectales y gástricos. De ahí que la vía Hh sea una diana terapéutica tan atractiva(72).

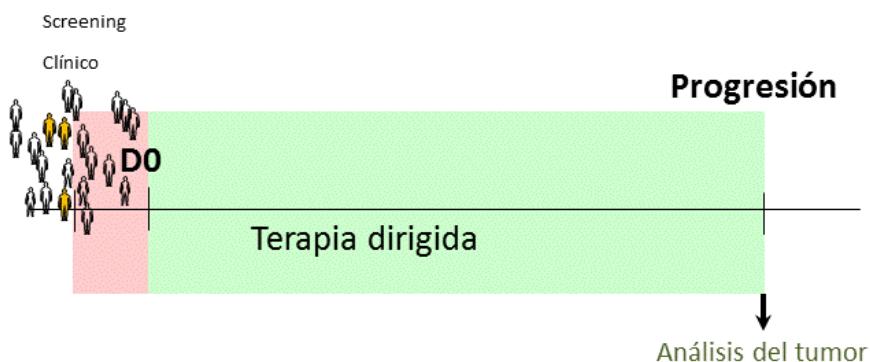
Sonidegib (LDE225) es un nuevo inhibidor selectivo de SMO, y ha demostrado tener actividad antitumoral en estudios preclínicos en modelos de ratones con meduloblastoma *Ptch<sup>+-/-</sup> p53<sup>-/-</sup>* y *Ptch<sup>+-/-</sup> Hic1<sup>+-/-</sup>* (hipermetilado en cáncer 1), lo que sugiere la inhibición dirigida de la vía de señalización Hh(73)

## **1.6 Establecimiento de un programa de preselección en el contexto de un programa de desarrollo clínico precoz de fármacos.**

En el contexto de un programa de desarrollo clínico precoz de fármacos, se pueden aplicar las siguientes estrategias para determinar el momento oportuno en el que realizar el análisis molecular de los tumores, con el fin de determinar su vulnerabilidad:

### ***1.6.1 Estrategia 1: análisis retrospectivo de biomarcadores predictivos***

En general, se considera que las aberraciones genéticas y moleculares en algunos tipos de cáncer son lo suficientemente frecuentes como para, aun sin preselección, poder realizar análisis retrospectivos con suficiente poder estadístico de un subconjunto de tumores con mutaciones. Partiendo de esa premisa, se puede seleccionar únicamente a pacientes con un tipo de tumor concreto, por ejemplo, el cáncer pancreático, que suele presentar mutaciones de *KRAS*(74), en los ensayos con inhibidores de MEK, o los liposarcomas, que presentan amplificaciones de *HDM2*(75), en los ensayos con inhibidores de HDM2. A continuación, se podría llevar a cabo un análisis retrospectivo empleando muestras de tejido tumoral conservadas de los pacientes del estudio para confirmar la hipótesis sobre la actividad del fármaco ante la presencia de alteraciones moleculares. En resumen, la selección se realizaría según el tipo de tumor, no según el análisis molecular (que se haría retrospectivamente). Al utilizar esta estrategia de selección de pacientes se expondría a varios pacientes al fármaco en estudio a pesar de no presentar la diana de interés.

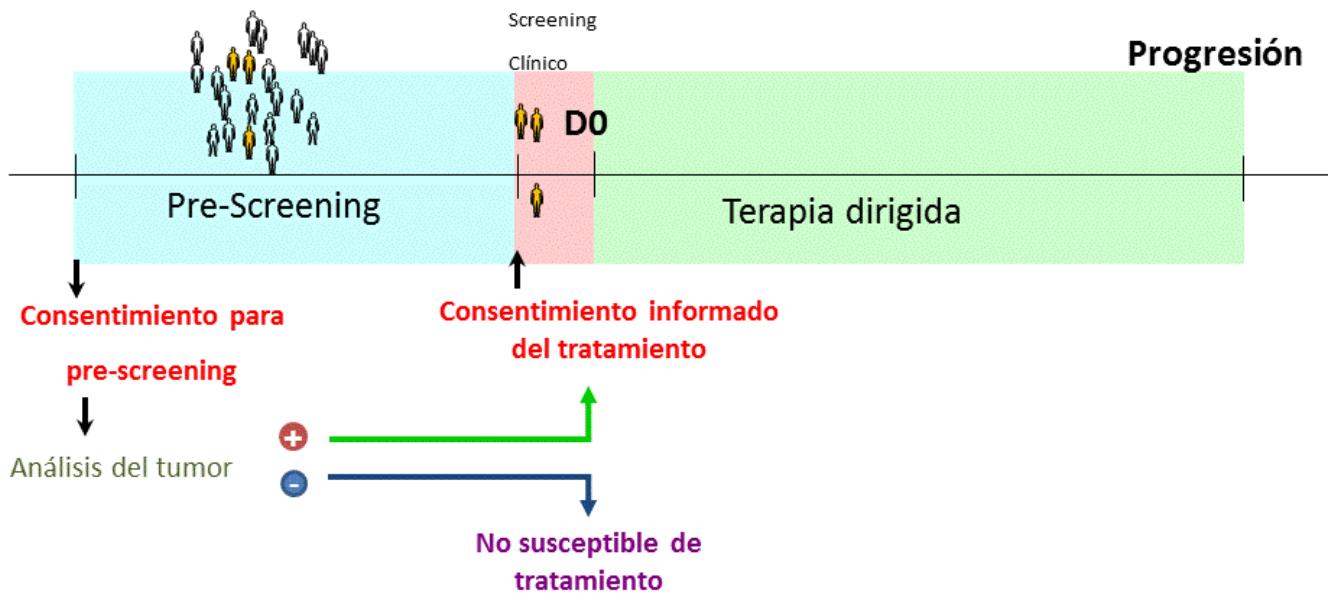


**Figura 4.** En la estrategia numero uno no hay preselección. Esta estrategia se basa en la asunción de que las alteraciones moleculares en algunos tipos tumorales particulares ocurren con una frecuencia elevada, por lo que se confía en que los pacientes incluidos en ensayos clínicos fase I tendrán estas alteraciones y se podrá analizar de forma retrospectiva.

### 1.6.2 Estrategia 2: análisis predictivo, central y enfocado de marcadores predictivos

La segunda estrategia consistiría en realizar una preselección de una población de pacientes enviando su tejido tumoral a un laboratorio central (ya sea del promotor del ensayo o de un proveedor) para su análisis, justo antes de considerar su inclusión en un ensayo con un agente dirigido. Por ejemplo, se podría realizar una preselección de todos los candidatos a recibir un inhibidor de PI3K para buscar alteraciones en la vía de señalización (pérdida de PTEN, mutaciones de PIK3CA(76, 77)) antes de su inclusión en el ensayo. Mediante esta estrategia se garantiza la aplicación de análisis de última generación, así como el apoyo financiero del promotor. Sin embargo, tiene también desventajas. En primer lugar, y para realizar el cribado de distintas alteraciones en el marco de varios ensayos clínicos, una vez se hayan firmado múltiples consentimientos informados puede que no se disponga de suficiente tejido tumoral como para enviar muestras a diferentes laboratorios. Habría que tener en cuenta previamente que hay que obtener bastante tejido tumoral (lo que entraría en conflicto con otras condiciones del protocolo del ensayo y de los protocolos de investigación del centro). La

segunda limitación importante de esta estrategia es el dilatado tiempo de respuesta entre la realización del análisis molecular y el inicio del tratamiento en estudio, lo cual podría comprometer el beneficio del paciente debido a un retraso poco ético en el inicio de la terapia.

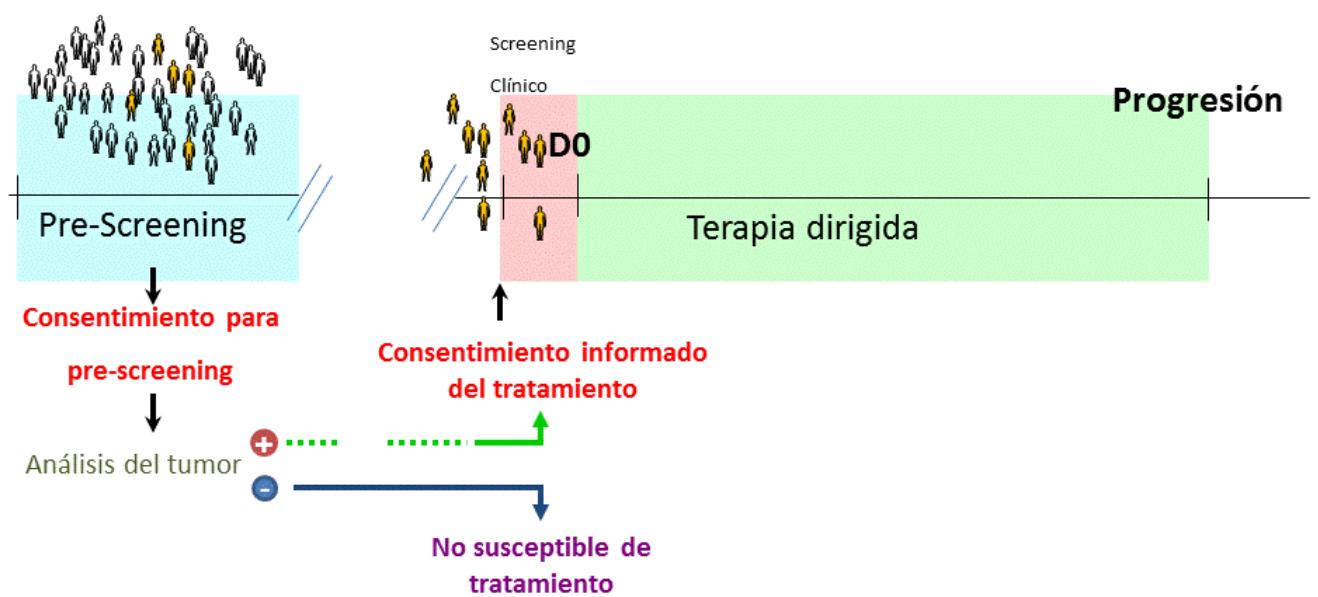


**Figura 5** Según la segunda estrategia, las muestras de pacientes referidos a ensayos clínicos son analizados mediante el uso de un laboratorio central, antes de considerar el tratamiento con un inhibidor específico.

#### 1.6.3 Estrategia 3: análisis avanzado, local y multiplexado de los marcadores predictivos

Esta estrategia consistiría en realizar análisis precoz en una institución académica, en pacientes con enfermedad avanzada que ya estén recibiendo tratamiento convencional, en vistas a una futura consideración de un ensayo con un agente dirigido. La selección molecular se podría realizar en cualquier momento durante el curso de la enfermedad. Aplicando esta estrategia, y con la ayuda de técnicas de alto rendimiento, solo habría que analizar unas pocas cantidades de muestra tumoral y obtener un único consentimiento informado (con un proyecto aprobado por el comité de revisión institucional local) y no habría demora entre la detección de

progresión de la enfermedad con tratamiento convencional y el reclutamiento para participar en un ensayo clínico con un agente dirigido. Con esto se podrían conseguir intervalos sin quimioterapia(78) durante los cuales los pacientes podrían recibir atención además de tratamiento con una terapia biológica en un ensayo clínico precoz. Las desventajas de esta estrategia son que se cribarían pacientes que puede que jamás llegaran a participar en un ensayo clínico (debido a su deterioro clínico en el momento de progresión de la enfermedad o por negarse a participar en el ensayo), unido a la necesidad de financiación adicional, ya que puede que las entidades aseguradoras o el presupuesto disponible para el estudio en las instituciones académicas no cubrieran el análisis de muestras.



**Figura 6** En base a la tercera estrategia se realiza un análisis local y multiplexado de muestras de pacientes con enfermedad metastásica. La información del análisis molecular es usada en procesos de decisión terapéutica en el momento de la progresión.

El tiempo necesario para obtener los resultados de los biomarcadores, especialmente en la secuenciación de próxima generación, es algo a tener muy en cuenta a la hora de determinar el perfil molecular de los pacientes, sobre todo en contextos metastásicos, cuando se disponga de poco tiempo para elegir un tratamiento. Como alternativa a la estrategia tradicional de

realizar un análisis de biomarcadores centralizado justo antes de plantear la inclusión de un paciente en un ensayo, nosotros nos decantamos por la estrategia de realizar una preselección local en instituciones académicas, cuando los pacientes con enfermedad avanzada aún están recibiendo tratamiento convencional, a pesar del coste adicional para el grupo de investigación(43). Con esta estrategia se ahorra tiempo y tejidos y se aumentan las probabilidades de reclutar pacientes para participar en ensayos clínicos precoces.

Todos estos esfuerzos no serían fructíferos si los médicos de la institución en cuestión no se implican y se comprometen con el proceso. Dado que son ellos los que les presentan los ensayos clínicos a los pacientes como una buena opción de tratamiento, se les debería facilitar los resultados de todos los análisis moleculares, así como una lista con los ensayos clínicos que se están realizando en dicha institución con combinaciones de terapias dirigidas. Del mismo modo, habría que tener en cuenta aspectos éticos como el estado de validación del biomarcador, la naturaleza exploratoria del valor predictivo de cada biomarcador para un fármaco concreto y la necesidad de contar con biopsias frescas para el análisis de biomarcadores específicos.

Es evidente que aún hay que resolver algunas dificultades técnicas, clínicas y de laboratorio antes de poder difundir la implementación de la selección de pacientes basada en el uso de biomarcadores en los ensayos de primera fase. En primer lugar, la presencia del biomarcador puede no ser representativa de la enfermedad (heterogeneidad tumoral), o de su biología (alteraciones genéticas en "inductores" frente a "pasajeros" -*drivers* vs. *Passengers*-) y evolución debido a la presión selectiva de tratamientos anteriores (evolución clonal(79)). En segundo lugar, existen limitaciones metodológicas, como que aún no se haya determinado un valor de corte para definir el estado de un posible biomarcador. Por otro lado, el rápido desarrollo de resistencia a los agentes únicos es un problema, debido a la retroalimentación negativa adaptativa y a estimulación compensatoria de receptores tirosina quinasa. También

cabría señalar que la estrategia de combinar un biomarcador de predicción a un agente dirigido no siempre será aplicable a todas las terapias nuevas como, por ejemplo, aquellas en las que se emplean inhibidores de amplio espectro dirigidos contra múltiples vías de señalización o que usan agentes antiangiogénicos(80).

En el Programa de desarrollo clínico precoz de fármacos del Instituto de Oncología Vall d'Hebron (VHIO) dentro del Hospital Universitari Vall d'Hebron, consideramos que esta última estrategia, es decir, la determinación del perfil molecular local y el uso de plataformas multiplexadas en las instituciones académicas está más enfocada al paciente, ofreciendo importantes ventajas, ya que el Comité de Ética solo tiene que aprobar un formulario de consentimiento informado, así como un único protocolo de preselección, lo que agiliza mucho los trámites; además, basta con contar con un poco de tejido tumoral para su análisis, y se puede determinar el perfil molecular de aquellos pacientes con enfermedad avanzada que estén recibiendo tratamiento convencional en la institución, lo que reduce el tiempo transcurrido entre el fracaso del tratamiento y la inclusión en un ensayo clínico con un agente dirigido.

Aquí describiremos nuestros esfuerzo en la Unidad de ensayos de fase I por desarrollar una plataforma para el análisis de mutaciones, que pueda ayudar en la toma de decisiones terapéuticas indicando, por ejemplo, la presencia de mutaciones de *PIK3CA*, así como en la realización de ensayos clínicos con inhibidores de PI3K. Así mismo, se describirá el desarrollo de un test para seleccionar pacientes candidatos a recibir un inhibidor de Sonic Hedgehog.

## **2. Hipótesis y objetivos**

### ***2.1 Hipótesis***

El uso terapéutico de los pan-inhibidores de PI3K, Pilaralisib y BKM120 y del inhibidor de Sonic Hedgehog es seguro en dosis que inhiban significativamente las vías diana, y su empleo es eficaz en poblaciones de pacientes seleccionados definidos mediante los factores predictivos oportunos.

### ***2.2 Objetivos***

- Evaluar el perfil de seguridad y la dosis máxima tolerada (MTD) de Pilaralisib, BKM120 y Sonidegib en monoterapia en pacientes con tumores sólidos avanzados.
- Determinar el perfil farmacocinético y los efectos farmacodinámicos de los biomarcadores de la vía Pilaralisib y BKM120 sobre los biomarcadores de la vía PI3K, así como los efectos de Sonidegib sobre los marcadores de SHH.
- Evaluar la eficacia de los inhibidores de PI3K, como Pilaralisib y BKM120 en pacientes con tumores sólidos mixtos con el gen *PIK3CA* mutado.
- Desarrollar y evaluar la capacidad de predicción de una prueba de firma genética apta para uso clínico en la selección de pacientes con alteraciones en la vía Sonic Hedgehog.



### **3. Primera parte: Inhibidores de PI3K y mutaciones en PIK3CA.**

Tal como se menciona en la Introducción, la realización simultánea de ensayos de fase I y de programas de preselección permite la prueba de hipótesis en el contexto del desarrollo clínico precoz de fármacos. Los hallazgos preclínicos sugieren que los tumores con mutaciones en *PIK3CA* o con una baja expresión de PTEN son más sensibles a los inhibidores de la vía PI3K, lo que subraya la necesidad de evaluar dichos agentes en aquellos pacientes con tumores que presenten estas alteraciones(76, 77, 81).

En la fase de escalada de dosis de los ensayos de fase I en los que se investigan el SAR245408 y BKM120 no se seleccionó previamente a los pacientes, sino que se realizó un análisis retrospectivo de tejido tumoral fijado (véase Estrategia 1 en la Introducción) con el fin de establecer una correlación entre genotipo y respuesta (fenotipo). En la parte de expansión del ensayo de fase I en el que se evaluaba el pan-inhibidor de PI3K, BKM120, solo se consideró elegibles a aquellos pacientes cuyos tumores presentaban mutaciones de *PIK3CA* o una baja expresión de PTEN. Con este fin, tres grandes instituciones oncológicas participantes en el estudio enviaron las muestras de sus pacientes a un laboratorio central (Estrategia 2). En el Hospital Universitari Vall d'Hebron, sin embargo, se realizó un análisis molecular local de las pacientes con cáncer de mama avanzado que estuvieran recibiendo tratamiento convencional en busca de alteraciones molculares (incluyendo la vía PI3K) (Estrategia 3). El objetivo era comprobar las posibles oportunidades terapéuticas relacionadas con la dependencia oncogénica para estas pacientes, en caso de recaída.

En el presente estudio presentamos los resultados de dos estudios en los que se investigó la seguridad, tolerabilidad y propiedades farmacocinéticas y farmacodinámicas de SAR245408 y CBKM120 administradas por vía oral en monoterapia a pacientes con tumores sólidos refractarios. Analizamos también los resultados de la estrategia de prescreening molecular

implementada en nuestra Institución, diseñada para enriquecer la población de ensayos clínicos con aquellos que más se puede beneficiar, impactando el reclutamiento y permitiendo probar hipótesis de forma precoz.

Contraviniendo las conclusiones de multitud de investigaciones preclínicas, descubrimos que las mutaciones de *PIK3CA* no son un factor predictivo claro. En el presente estudio analizaremos las posibles razones de este hallazgo, así como su impacto en el desarrollo posterior de inhibidores de PI3K y detallaremos las investigaciones clínicas, preclínicas y traslacionales que arrojaron estos resultados.

### **3.1 Implementación de un programa de preselección para identificar alteraciones de PI3K que permitan reclutar pacientes para ensayos clínicos de fase I con tumores con alteraciones de PI3K.**

\*Nota: aunque los datos preclínicos preliminares indicaban que las alteraciones de PTEN podrían ser sensibles a tratamiento con inhibidores de PI3K(67, 82-84), pronto descubrimos que la evaluación del estado del gen PTEN no tenía aplicación clínica, ya que éste puede verse alterado por muchos otros mecanismos, como complejos patrones de mutaciones, alteraciones en el número de copias y el silenciamiento epigenético de la expresión de PTEN, todo lo cual provoca la activación de AKT(85). Dado que la inmunohistoquímica no reflejaba todo el espectro de aberraciones de PTEN y presentaba importantes limitaciones técnicas (incluyendo la carencia de un método estándar de cuantificación y de un umbral validado para definir la pérdida de PTEN), esta determinación estaba incluida en los análisis preliminares, pero pronto dejamos de hacerlo. Por lo tanto, en nuestro análisis nos centraremos en la validez de las mutaciones de *PIK3CA* como predictores de la sensibilidad a los inhibidores de PI3K.

La mutaciones de *PIK3CA* se analizaron usando la plataforma multiplexada MassARRAY (Sequenom, Inc.) en VHIO(86). Para validar externamente los resultados de MassArray, se analizó la misma muestra tumoral con una plataforma similar en el MD Anderson Cancer Center (en adelante, MDACC). En ambos centros se utilizó un panel personalizado. Para la validación cruzada de ambas plataformas y centros (el VHIO y el MDACC) se emplearon muestras del cáncer primario de mama obtenidas en el VHIO, fijadas en formol y conservadas en parafina. Se utilizó el mismo bloque tumoral para la validación interna y externa, con el fin de minimizar errores. Las muestras fueron seleccionadas y preparadas por el mismo patólogo. El número de casos empleados para cada set de validación varió dependiendo de la

disponibilidad de tejido tumoral. El panel de MDACC, desarrollado en el MD Anderson Cancer Center(87), contiene ensayos a través de los cuales se puede realizar el genotipado de 86 mutaciones de puntos calientes (*hotspots*) en 15 oncogenes. Para la validación interna de los resultados se realizó la secuenciación de Sanger (usada como "gold standard") y la detección de mutaciones de *PIK3CA* mediante PCR cuantitativa en el VHIO (datos no mostrados). También se analizó el primer subconjunto de 17 muestras de cáncer de mama triple negativo para identificar alteraciones en el número de copias en todo el genoma y se examinó un panel ampliado de mutaciones que incluía el gen *PIK3CA* mediante el ensayo SNP de sondeo de inversión molecular (MIP) (OncoScan™ FFPE Express 2, este último realizado en Affymetrix). El ADN genómico fue aislado manualmente (QIAamp DNA FFPE Tissue Kit; Qiagen, Hilden, Germany). Se eluyó el ADN en 30–100 µL de tampón de elución y se cuantificó con PicoGreen (Invitrogen, Carlsbad, CA). Se sometieron 75 ng a análisis exprés OncoScan FFPE (Affymetrix, Santa Clara, CA) siguiendo el método descrito por Wang y col(88).

Al comparar los resultados del genotipado con MassARRAY de 21 muestras de tumor primario de cáncer de mama, observamos que había un 100% de concordancia entre el VHIO y MDACC, aunque se revisaron manualmente dos ensayos (2/21=10%) al obtener una baja frecuencia de alelos mutados (<10%), inicialmente clasificada como salvaje o nativo por uno de los laboratorios. Por el contrario, al comparar los resultados de Sanger con los de Mass ARRAY realizados en el VHIO, se obtuvo mayor grado de discordancia (4/20=20%). Esta discrepancia probablemente se debió a la baja sensibilidad de la secuenciación de Sanger, especialmente porque dichos casos presentaban una baja frecuencia de alelos mutados (<30% según MassARRAY). También se analizaron las mutaciones de *PIK3CA* con la plataforma SNP de MIP en un panel de 17 muestras de tumor primario de cáncer de mama. Los resultados concordaban con los obtenidos mediante MassARRAY en 15 de las 17 muestras (88%). Se hallaron discrepancias del orden del 10-20% en la frecuencia de alelos mutados determinada por MassARRAY. Para más detalles, véase la sección AMPLIACIÓN DE DATOS

### **3.2 Evaluación clínica de la seguridad, farmacología (farmacocinética y farmacodinamia) y biomarcadores predictivos de dos inhibidores de PI3K: Pilaralisib ó XL147 y Buparlisib ó BKM120.**

\*Nota: En nuestro programa de ensayos de fase I, nos hemos centrado especialmente en el desarrollo de inhibidores de PI3K/AKT/mTOR (véase Tabla 10 del Anexo 1). Así mismo, hemos colaborado intensamente con varios laboratorios traslacionales (tanto académicos como comerciales) para analizar los mecanismos de eficacia y resistencia a estos inhibidores (véase la Tabla 11 del Anexo 1). Para realizar dichas investigaciones, desarrollamos un programa de preselección para detectar situaciones de dependencia oncogénica (como mutaciones de *PIK3CA* y sensibilidad a inhibidores de PI3K) para facilitar las decisiones terapéuticas relacionadas con nuestros pacientes e incluir en nuestros ensayos a aquellos pacientes con más probabilidad de responder a tratamiento. En el presente estudio se detallan los hallazgos realizados en dichos ensayos. En la Tabla 12 del Anexo 1 se enumeran otros artículos relacionados con la misma área de estudio en los que también ha participado nuestro grupo de investigación.

Pilaralisib, XL147 ó SAR245408 (,Sanofi, Bridgewater, NJ, USA) es un nuevo y potente inhibidor reversible altamente selectivo de las isoformas PI3K  $\alpha$ ,  $\beta$ ,  $\gamma$  y  $\delta$  de clase I (con IC<sub>50</sub> de 48, 617, 10 y 260 nmol/L, respectivamente)(89). En el contexto preclínico, SAR245408 también mostró buena biodisponibilidad oral, una exposición plasmática dependiente de la dosis y una potente y sostenida inhibición de la vía PI3K, lo que causa la inhibición del crecimiento de los xenoinjertos de diferentes tipos de tumores, incluyendo aquellos que albergan una mutación de *PIK3CA* y deficiencias del gen *PTEN*.

En el presente estudio se detallan los resultados obtenidos en el primer estudio de fase I en humanos en el que se evalúa la seguridad, tolerabilidad y propiedades farmacocinéticas y farmacodinámicas de SAR245408 administrado por vía oral

*3.2.1 Primer manuscrito: Estudio de fase I sobre la farmacocinética y farmacodinamia de SAR245408 (XL147), un pan-inhibidor de PI3K de Clase I, en pacientes con tumores sólidos avanzados.*

# Clinical Cancer Research



## Phase I Safety, Pharmacokinetic, and Pharmacodynamic Study of SAR245408 (XL147), an Oral Pan-Class I PI3K Inhibitor, in Patients with Advanced Solid Tumors

Geoffrey I. Shapiro, Jordi Rodon, Cynthia Bedell, et al.

*Clin Cancer Res* 2014;20:233-245. Published OnlineFirst October 28, 2013.

**Updated version** Access the most recent version of this article at:  
doi: [10.1158/1078-0432.CCR-13-1777](https://doi.org/10.1158/1078-0432.CCR-13-1777)

**Supplementary Material** Access the most recent supplemental material at:  
<http://clincancerres.aacrjournals.org/content/suppl/2013/10/28/1078-0432.CCR-13-1777.DC1.html>

**Cited Articles** This article cites by 24 articles, 11 of which you can access for free at:  
<http://clincancerres.aacrjournals.org/content/20/1/233.full.html#ref-list-1>

**Citing articles** This article has been cited by 1 HighWire-hosted articles. Access the articles at:  
<http://clincancerres.aacrjournals.org/content/20/1/233.full.html#related-urls>

## Phase I Safety, Pharmacokinetic, and Pharmacodynamic Study of SAR245408 (XL147), an Oral Pan-Class I PI3K Inhibitor, in Patients with Advanced Solid Tumors

Geoffrey I. Shapiro<sup>1</sup>, Jordi Rodon<sup>4</sup>, Cynthia Bedell<sup>5</sup>, Eunice L. Kwak<sup>2</sup>, Jose Baselga<sup>4</sup>, Irene Brana<sup>~</sup>, Shuchi S. Pandya<sup>3</sup>, Christian Scheffold<sup>6</sup>, A. Douglas Laird<sup>6</sup>, Linh T. Nguyen<sup>6</sup>, Yi Xu<sup>7</sup>, Coumaran Egile<sup>7</sup>, and Gerald Edelman<sup>5</sup>

### Abstract

**Purpose:** SAR245408 is a pan-class I phosphoinositide 3-kinase (PI3K) inhibitor. This phase I study determined the maximum tolerated dose (MTD) of two dosing schedules [first 21 days of a 28-day period (21/7) and continuous once-daily dosing (CDD)], pharmacokinetic and pharmacodynamic profiles, and preliminary efficacy.

**Experimental Design:** Patients with refractory advanced solid malignancies were treated with SAR245408 using a 3  $\times$  3 design. Pharmacokinetic parameters were determined after single and repeated doses. Pharmacodynamic effects were evaluated in plasma, hair sheath cells, and skin and tumor biopsies.

**Results:** Sixty-nine patients were enrolled. The MTD of both schedules was 600 mg; dose-limiting toxicities were maculopapular rash and hypersensitivity reaction. The most frequent drug-related adverse events included dermatologic toxicities, diarrhea, nausea, and decreased appetite. Plasma pharmacokinetics showed a median time to maximum concentration of 8 to 22 hours, mean terminal elimination half-life of 70 to 88 hours, and 5- to 13-fold accumulation after daily dosing (first cycle). Steady-state concentration was reached between days 15 and 21, and exposure was dose-proportional with doses up to 400 mg. SAR245408 inhibited the PI3K pathway ( $\geq 40\%-80\%$  reduction in phosphorylation of AKT, PRAS40, 4EBP1, and S6 in tumor and surrogate tissues) and, unexpectedly, also inhibited the MEK/ERK pathway. A partial response was seen in one patient with advanced non–small cell lung cancer. Eight patients were progression-free at 6 months. Pharmacodynamic and clinical activity were observed irrespective of tumor PI3K pathway molecular alterations.

**Conclusions:** SAR245408 was tolerable at doses associated with PI3K pathway inhibition. The recommended phase II dose of the capsule formulation is 600 mg administered orally with CDD. *Clin Cancer Res*; 20(1); 233–45. © 2013 AACR.

**Authors' Affiliations:** <sup>1</sup>Dana-Farber Cancer Institute; <sup>2</sup>Massachusetts General Hospital; <sup>3</sup>Beth Israel Deaconess Medical Center, Boston, Massachusetts; <sup>4</sup>Vall d'Hebron Institute of Oncology (VHIO), Barcelona, Spain; <sup>5</sup>Mary Crowley Cancer Research Centers, Dallas, Texas; <sup>6</sup>Elexixis Inc., South San Francisco, California; and <sup>7</sup>Sanofi, Cambridge, Massachusetts, and Vitry-sur-Seine, France

**Present addresses:** Dr. Baselga: Memorial Sloan-Kettering Cancer Center, Memorial Hospital, New York, New York; Dr. Pandya: Acceleron Pharma, Cambridge, Massachusetts

**Note:** Supplementary data for this article are available at Clinical Cancer Research Online (<http://clincancerres.aacrjournals.org/>).

Presented in part at Annual Meetings of the American Society of Clinical Oncology (ASCO), June 2009 and June 2010, Chicago, IL, USA, and at the AACR-NCI-EORTC International Conference on Molecular Targets and Cancer Therapeutics, October 2008, Geneva, Switzerland.

**Corresponding Author:** Geoffrey I. Shapiro, Early Drug Development Center, Dana-Farber Cancer Institute, 450 Brookline Avenue, Boston, MA 02115. Phone: 617-632-4942; Fax: 617-632-1977; E-mail: [geoffrey\\_shapiro@dfci.harvard.edu](mailto:geoffrey_shapiro@dfci.harvard.edu)

doi: 10.1158/1078-0432.CCR-13-1777

© 2013 American Association for Cancer Research.

### Introduction

The phosphoinositide 3-kinase (PI3K) pathway regulates essential cellular functions, including proliferation, apoptosis, protein synthesis, and metabolism (1, 2). Class I PI3K enzymes convert phosphatidylinositol 4,5-bisphosphate (PIP<sub>2</sub>) into phosphatidylinositol 3,4,5-trisphosphate (PIP<sub>3</sub>) in response to external cell stimuli (3, 4). Activation of class IA PI3Ks (PI3K $\alpha$ , - $\beta$ , and - $\gamma$ ) is mediated by receptor tyrosine kinases (RTKs). In addition, PI3K $\beta$  and PI3K $\gamma$  are activated by G-protein-coupled receptors

(5). PIP<sub>3</sub> production promotes membrane localization and activation of several downstream effectors, such as phosphoinositide-dependent kinase-1 (PDK1) and AKT, leading to cell-cycle progression and inhibition of apoptosis through phosphorylation of their respective substrates (3).

Hyperactivation of PI3K signaling in cancer cells occurs through molecular alterations of PI3K pathway components and RTKs. The PIK3CA gene, encoding the

### Translational Relevance

The phosphoinositide 3-kinase (PI3K) pathway is heavily implicated in tumor cell growth, proliferation, and survival and contributes to resistance to chemotherapy and targeted agents. SAR245408 (XL147) is a novel orally bioavailable pan-class I PI3K inhibitor with potent antitumor activity in xenograft models. This first-in-human study established the maximum tolerated dose of the capsule formulation (administered daily either for the first 21 days of a 28-day period or continuously for 28 days), and showed a manageable toxicity profile and favorable pharmacokinetic parameters. SAR245408 demonstrated a pharmacodynamic effect on fasting insulin levels, as well as PI3K pathway modulation in hair, skin, and tumor tissue. Pathway inhibition and clinical activity were observed in tumors with and without apparent molecular alterations of PI3K pathway components. Overall, 25 (43.9%) patients had stable disease as the best response; eight were progression-free at 6 months. These results lay the groundwork for additional studies of SAR245408 either as monotherapy or in combination regimens.

catalytic subunit of PI3K $\alpha$  (p110 $\alpha$ ), is often mutated or amplified in solid tumors or lymphomas (3, 4, 6, 7). The tumor suppressor gene PTEN, a critical negative regulator of PIP<sub>3</sub> production, is frequently mutated, deleted, or downregulated in tumors (3, 8). Furthermore, PI3K activation via activating mutations of RAS contributes to RAS-mediated cellular transformation (9, 10). In addition to promoting cancer cell proliferation and survival, activation of the PI3K pathway mediates resistance to both RTK inhibitors and genotoxic agents (11, 12), such that PI3K inhibition increases cancer cell sensitivity to targeted agents, as well as platinum-based drugs and taxanes (13–15). Selective inhibition of key nodes of the PI3K pathway represents an attractive therapeutic intervention for the treatment of solid and hematologic cancers, and both pan-class I and isoform-selective PI3K inhibitors have entered clinical testing (3, 16).

SAR245408 (XL147) is a novel, ATP-competitive, highly selective, and reversible inhibitor of the PI3K p110 $\alpha$ , - $\beta$ , - $\gamma$ , and - $\delta$  isoforms, with half-maximal inhibitory concentration (IC<sub>50</sub>) values of 48, 617, 10, and 260 nmol/L, respectively, measured in biochemical assays (17, 18). SAR245408 shows high selectivity in a broad panel of kinases, with no inhibitory activity against RAF, MEK, and extracellular signal-regulated kinase (ERK; ref. 17). In xenografted tumor models, SAR245408 exhibited good oral bioavailability, dose-dependent plasma exposure, and potent and sustained PI3K pathway inhibition, leading to growth inhibition of xenografts representing various histologies, including those harboring PIK3CA or KRAS mutations and PTEN deficiency (17, 18). Here, we report results from a phase I study investigating safety, tolerability, and

pharmacokinetic (PK) and pharmacodynamic properties of SAR245408 administered orally as monotherapy to patients with refractory solid tumors.

### Materials and Methods

#### Patient population

Eligible patients were aged  $\geq$ 18 years, with an Eastern Cooperative Oncology Group (ECOG) performance status (PS)  $\leq$ 2 and histologically confirmed metastatic or unresectable solid tumors for which standard curative or palliative measures were unavailable or ineffective. Adequate organ and bone marrow function, fasting plasma glucose  $<$  160 mg/dL, HbA1c  $<$  8%, and the presence of disease evaluable by tumor markers or physical or radiologic means, were required. Patients who had previously received PI3K inhibitor treatment or had received chemotherapy, a biologic agent, or an investigational agent within 4 weeks, a small-molecule kinase inhibitor or radiotherapy within 2 weeks or 5 half-lives, or hormonal therapy within 1 week of the first dose of SAR245408, were excluded. Furthermore, patients with ongoing toxicity (grade  $\geq$  1) due to prior therapy or uncontrolled intercurrent illness (e.g., infection or heart disease) were excluded. *In vitro* inhibition and induction studies with SAR245408 using human liver material showed the potential of SAR245408 to inhibit cytochrome P450 (CYP)3A4 and CYP2C9 and induce CYP1A2 and CYP3A4. Therefore, concomitant use of drugs with narrow therapeutic indices that are substrates for CYP1A2, CYP2C9, and CYP3A4 were avoided unless considered clinically necessary.

Study populations included the safety, pharmacokinetic, pharmacodynamic, and efficacy (exploratory) populations. The safety and pharmacokinetic populations were defined as all patients who received at least one dose of study drug; the pharmacodynamic population was defined as patients receiving at least one dose of study drug and from whom tumor or non-tumor tissue samples were collected. The efficacy population encompassed all patients in the safety population evaluable for response (i.e., patients who had a baseline tumor assessment and at least one post-baseline tumor assessment).

#### Study design and dose escalation

This was a phase I, open-label, single-arm, dose-escalation study (NCT00486135). Patients received SAR245408 (capsule formulation) using 1 of 2 regimens: either once daily for the first 21 of every 28 days (21/7) or continuous once-daily dosing (CDD) in each 28-day cycle. For each schedule, a standard 3  $\times$  3 design was used for dose escalation, with dose-limiting toxicities (DLT) defined during the first 28-day cycle. Starting doses were 30 mg for the 21/7 regimen, calculated on the basis of results from pre-clinical *in vivo* studies, and 100 mg for the CDD cohort, chosen once safety and PK data from the 21/7 regimen were available.

A DLT was defined as the occurrence during the study treatment period (cycle 1) of specific events considered

related to the study drug, including: grade 4 neutropenia lasting  $\geq$  4 days; grade 4 febrile neutropenia; grade 3 febrile neutropenia lasting  $\geq$  3 days, or any other grade 4 hematologic adverse event (AE). In addition, grade  $\geq$  3 nonhematologic events that occurred despite prophylaxis or were not easily managed or corrected by medical intervention, and grade  $\geq$  3 hyperglycemia not controlled with oral hypoglycemic agents at standard doses, were also considered dose limiting. Drug-related AEs that prevented the start of cycle 2 within 14 days of the planned start date, or prevented  $\geq$  75% of the planned dose being taken in cycle 1, were considered DLTs. Any toxicity-related dose delay of  $>$  21 days (21/7 regimen) or  $>$  28 days (CDD regimen) resulted in patient withdrawal from the study, unless the patient was deriving clinical benefit from study treatment per investigator judgment.

The preliminary maximum tolerated dose (MTD) was defined as the highest dose level below the maximum administered dose at which  $\geq$  1 of 6 patients experienced a DLT. After the MTD was identified, additional patients were enrolled in MTD expansion cohorts to further assess safety, pharmacokinetic and pharmacodynamic effects. For the CDD regimen, a proportion of the additional patients enrolled was required to have solid tumors amenable to biopsy, and the rest were required to have non-small cell lung cancer (NSCLC). Information collected beyond cycle 2 and in MTD expansion cohorts was used to determine the recommended phase 2 dose.

To explore the hypothesis that tumors with molecular alterations affecting PI3K pathway components would be more sensitive to SAR245408, a cohort of patients with tumors harboring molecular alterations affecting PI3K pathway components and modulators, such as PIK3CA mutation or PTEN deficiency, was enrolled to a dose level one below the MTD.

Approval was obtained from the ethics committees at the participating institutions and from regulatory authorities. All patients provided informed consent. The study followed the Declaration of Helsinki and good clinical practice guidelines.

#### Safety assessments

Safety was assessed using standard clinical findings, AEs, echocardiograms, ECOG PS, physical examination, vital signs, concomitant medications, and laboratory assessments. Safety evaluations were conducted during the screening period, at set times during each cycle and during follow-up. Drug-related AEs occurring within 30 days after the last dose were followed until resolution, stabilization, or initiation of new treatment. AEs were graded according to National Cancer Institute Common Terminology Criteria for AEs version 3.0. Safety findings were reviewed on an ongoing basis.

#### Pharmacokinetic assessments

Whole blood (for plasma) was collected pre-dose on days 1, 2, 8, 15, and 21 (day 28 for CDD regimen) during cycle 1, on day 1 of every cycle for cycles 2–4 (both regimens), and on day 1 of every fourth cycle thereafter (both regimens). During cycle 1, post-dosing blood samples were collected at

0.5, 1, 2, 4, and 8 hours on day 1 (both regimens), at 4 hours on day 8 (both regimens), and at 0.5, 1, 2, 4, and 8 hours on day 21, and on days 22 and 23 or 24 (21/7 regimen) or at 0.5, 1, 2, 4, and 8 hours on day 28 (CDD regimen). During cycles 2–4, post-dosing blood samples were collected at 4 hours on day 1 of every cycle and every 4 cycles thereafter (both regimens). Urine samples were collected within 2 hours pre-dose on day 1 of cycle 1, with an additional single sample collected during the 24-hour period following the cycle 1 day 20 dose (21/7 regimen) or the cycle 1 day 28 dose (CDD regimen).

Pharmacokinetic parameters assessed included: terminal elimination half-life ( $t_{1/2,z}$ ), time to maximum concentration ( $t_{max}$ ), maximum concentration ( $C_{max}$ ), area under the concentration–time curve up to the last measurable concentration ( $AUC_{last}$ ), area under the concentration–time curve up to 24 hours ( $AUC_{0-24}$ ), area under the concentration–time curve from time 0 to infinity ( $AUC_{inf}$ ), accumulation ratio (AR), and apparent clearance (Cl/F). The amount and percentage of SAR245408 excreted unchanged in urine were also assessed.

#### Pharmacokinetic bioanalytical assays

Bioanalysis of human plasma (separated from whole blood by centrifugation) and urine samples was conducted by liquid chromatography-tandem mass spectrometry (LC-MS/MS) following a solid-phase extraction (SPE). K<sub>2</sub>EDTA anticoagulant was removed from plasma samples using an SPE cartridge (Waters Oasis HLB, 30 mg). SAR245408 was eluted from the SPE cartridge using methanol/acetonitrile solution (1:1, v/v), and 10 mL of solution was injected into the LC-MS/MS system. The standard curve covered a linear range of 1.00 to 2,000 ng/mL in human plasma and urine with 100 mL of matrix. The lowest detection limit of the method for plasma and urine was 1 ng/mL. Extracted samples were analyzed using a Shimadzu LC-20AD integrated HPLC system and an Applied Biosystems/MDS Sciex API 4000 mass spectrometer with an APCI interface. An isocratic mobile phase containing acetonitrile/water (65/35, v/v) with 1% formic acid at a flow rate of 0.40 mL/min and an HPLC analytical column (Thermo Electron Betasil CN, 2.1  $\times$  50 mm<sup>2</sup>, 5  $\mu$ m) were used. A positive ion multiple-reaction-monitoring mode was used to detect analyte ( $m/z$  541  $\pm$  456) and internal standard (D<sub>6</sub>-SAR245408,  $m/z$  546  $\pm$  456).

#### Pharmacodynamic and molecular profiling assessments

Detailed biomarker procedures are provided in the Supplementary section. Briefly, the pharmacodynamic effects of SAR245408 were evaluated in plasma, peripheral blood mononuclear cells (PBMCs), hair sheath cells, buccal mucosa, and skin biopsies from patients in the dose escalation cohort, and in paired tumor biopsies (and skin biopsies in several instances) from patients in the expanded MTD and the tumor molecular alteration cohorts. In addition, molecular profiling was conducted in archival and/or base-line fresh tumor tissue to identify molecular alterations of

PI3K pathway components and/or modulators that could affect the efficacy of or resistance to SAR245408.

#### Efficacy assessments

Tumor response was assessed using Response Evaluation Criteria in Solid Tumors (RECIST) version 1.0 (19). Patients with measurable disease were assessed using computed tomographic (CT) scans conducted within 21 days before the initial dose and approximately every 8 weeks thereafter. In patients with nonmeasurable lesions, tumor response was assessed (as feasible) using physical examination, radiographic methods, or tumor markers.

#### Statistical analyses

The study used a conventional 3  $\times$  3 dose-escalation design and had no formal sample size calculation or hypothesis testing. The total sample size was dependent on the number of dose levels required to determine the MTD. Safety assessment was the primary objective and efficacy assessment was an exploratory objective. All data were

summarized using descriptive statistics within each dose level and/or dosing schedule, and overall in all treated patients unless stated otherwise. For efficacy data, 90% confidence intervals (CI) were constructed on the basis of an exact binary distribution. Pharmacokinetic parameters ( $t_{1/2,z}$ ,  $t_{max}$ ,  $C_{max}$ ,  $AUC_{last}$ ,  $AUC_{0-24}$ ,  $AUC_{inf}$ , AR, CI/F) and the amount and percentage of SAR245408 excreted unchanged in urine were calculated from individual concentration–time data using a noncompartmental method. Further details on the calculation of each of these parameters are provided in the Supplementary Methods.

## Results

### Study population

Between July 2007 and February 2011, 68 patients with advanced solid tumors and 1 patient with small lympho-cytic lymphoma (SLL) were enrolled and treated ( $n = 41$ , 21/7 and  $n = 28$ , CDD). Fifty-seven patients ( $n = 34$ , 21/7 and  $n = 23$ , CDD) were evaluable for response. Patient characteristics are summarized in Table 1. Forty-three

**Table 1. Patient baseline and disease characteristics**

	All 21/7 combined (n = 41)	All CDD combined (n = 28)	Total (N = 69)
Age, y			
Mean (SD)	57.5 (13.68)	60.8 (9.03)	58.8 (12.05)
Median (range)	62.0 (25–86)	60.0 (43–84)	60.0 (25–86)
Sex, n (%)			
Male	23 (56.1)	13 (46.4)	36 (52.2)
Female	18 (43.9)	15 (53.6)	33 (47.8)
ECOG performance status, n (%)			
0	15 (36.6)	8 (28.6)	23 (33.3)
1	24 (58.5)	20 (71.4)	44 (63.8)
2	2 (4.9)	0	2 (2.9)
Race, n (%)			
American Indian or Alaska Native	0	1 (3.6)	1 (1.4)
Black or African American	2 (4.9)	2 (7.1)	4 (5.8)
White	39 (95.1)	25 (89.3)	64 (92.8)
Weight, kg			
Mean (SD)	74.45 (17.3)	77.35 (23.4)	75.62 (19.9)
Median (range)	72.40 (44.7–109.3)	73.85 (46.3–163.5)	72.40 (44.7–163.5)
Primary tumor sites, n (%)			
NSCLC	12 (29.3)	12 (42.9)	24 (34.8)
Breast	5 (12.2)	3 (10.7)	8 (11.6)
Colon	5 (12.2)	3 (10.7)	8 (11.6)
Other	19 (46.3)	10 (35.7)	29 (42.0)
Years since diagnosis			
Mean (SD)	4.04 (3.8)	4.07 (4.5)	4.05 (4.1)
Median (range)	2.78 (0.4–17.1)	2.46 (0.6–22.4)	2.62 (0.4–22.4)
Prior therapy, n (%)			
Prior systemic cancer therapy only	14 (34.1)	10 (35.7)	24 (34.8)
Prior radiation therapy only	1 (2.4)	0	1 (1.4)
Prior systemic therapy and radiation	25 (63.4)	18 (64.3)	43 (63.2)
No prior therapy reported	1 (2.4)	0	1 (1.4)

Abbreviations: 21/7  $\times$  21 consecutive days on treatment followed by 7 days off treatment; CDD, continuous once-daily dosing; NSCLC, non-small cell lung cancer; SD, standard deviation.

patients (62.3%) had received both radiation and a systemic anticancer therapy before the trial; the median number of prior regimens was 4 (maximum 11).

#### Dose-escalation and MTD

Seven dose levels were evaluated in the 21/7 administration schedule (30, 60, 120, 225, 400, 600, and 900 mg) and 3 were evaluated in the CDD administration schedule (100, 400, and 600 mg). The median overall duration of exposure was 50 days [range, 8–721 days; 21/7 regimen: 49 days (range, 8–616 days); CDD regimen: 56 days (range, 10–721 days)]. Twenty-eight patients (41%) completed 2 treatment cycles; 13 patients (18.8%) received more than 5 cycles.

In the 21/7 group, dose-escalation proceeded to 600 mg without DLT. In the first 600 mg cohort, one patient experienced drug-related grade 3 rash, but no other dose-limiting events occurred in another cohort of 3 patients. At 900 mg, 2 of 3 patients experienced grade 3 rash during the first cycle, defining this dose level as the maximum administered dose and 600 mg as the preliminary MTD. Another 10 patients were enrolled at 600 mg in an expanded cohort without further DLT, so that only 1 of 16 patients enrolled at the 600 mg dose level of the 21/7 schedule experienced a DLT. Six additional patients with PI3K pathway molecular alterations were enrolled at 400 mg. One of the additional patients experienced grade 2 rash (Supplementary Table S1).

With the CDD schedule, no DLTs occurred in the 100 and 400 mg cohorts. At 600 mg, there was one potential DLT of a grade 3 hypersensitivity reaction. A patient with penicillin allergy and an ongoing mild rash developed a diffuse blanching patchy maculopapular erythematous rash over the entire skin surface on day 8. Intravenous fluids, steroids, and antihistamines were administered and the patient discontinued the study on day 11. The rash resolved by day 18. The event was considered to be unlikely related to SAR245408 and more likely related to the patient's history of allergy and ongoing rash at baseline. No further DLTs were observed in 20 additional patients treated at 600 mg. Further escalation was not pursued because of the intolerability of 900 mg on the 21/7 schedule. Therefore, for SAR245408 capsules, 600 mg was the formally defined MTD on the 21/7 dosing schedule and the recommended dose for the CDD schedule (Supplementary Table S1).

#### Safety findings

As shown in Table 2, 44 (63.8%) patients experienced a drug-related AE (all grades), most commonly (all cohorts) skin toxicities (26%; including events such as macular or generalized rash, erythema, dry skin, and pruritus), nausea (21.7%), diarrhea (20.3%), and decreased appetite (11.6%). Drug-related laboratory abnormalities were uncommon and included 2 events of anemia and 5 instances of hyperglycemia, for which the highest severity was grade 2. Nine patients (13.0%) reported grade 3/4 drug-related AEs, the most frequent being rash and diarrhea. Drug-

**Table 2. All-grade and grade \_3 treatment-related adverse events occurring in at least 2 patients (21/7 and CDD capsule regimens, n ¼ 69)**

Adverse event	All grades, n (%)	Grade _ 3, n (%)
Patients with at least one AE	44(63.8)	9 (13)
Constitutional		
Fatigue	6(8.7)	0
Asthenia	5(7.2)	0
Peripheral edema	3(4.3)	1 (1.4)
Pyrexia	2(2.9)	0
Decreased appetite	8(11.6)	0
Weight loss	2(2.9)	0
Dermatologic		
Rash-related skin toxicities <sup>a</sup>	18(26.1)	5 (7.2)
Dry skin	5(7.2)	0
Pruritus	5(7.2)	0
Gastrointestinal		
Nausea	15(21.7)	0
Diarrhea	14(20.3)	2 (2.9)
Vomiting	7(10.1)	0
Constipation	4(5.8)	0
Dry mouth	3(4.3)	0
Hematologic		
Anemia	2(2.9)	0
Eosinophilia	2(2.9)	0
Metabolic/Biochemical		
Hyperglycemia	5(7.2)	0
Hypomagnesemia	3(4.3)	0
Musculoskeletal		
Muscle spasms	2(2.9)	0
Neurologic		
Dizziness	2(2.9)	0
Headache	2(2.9)	0
Vascular		
Hypotension	3(4.3)	0
Visual/Eye disorders		
Visual impairment <sup>b</sup>	2(2.9)	0

<sup>a</sup>Includes events coded as rash, macular rash, follicular rash, generalized rash, erythema, skin exfoliation, photosensitivity, eczema, and fissuring.

<sup>b</sup>Includes one event of chromatopsia and one self-limited event of visual disturbance.

related AEs (all grades) appeared to be more frequent in the CDD cohort than in the 21/7 cohort (75.0% vs. 56.1%). One (1.4%) occurrence of grade 3/4 g-glutamyltransferase was considered treatment-related.

Of the 18 patients with drug-related skin toxicities (rash group), 5 patients reported events of grade 3 severity, 2 of which occurred outside of the DLT observation period. Severe rashes were typically generalized, macular, erythematous, and pruritic, with nonspecific biopsy findings

Table 3. Pharmacokinetic results from single-dose analysis

Dose, mg	n	Median (range) T <sub>max</sub> , h	Mean (SD) (CV%)				
			C <sub>max</sub> , ng/mL	C <sub>max</sub> /D, ng/mL/mg	T <sub>last</sub> , h	AUC <sub>last</sub> , h ng/mL	AUC <sub>last</sub> /D, h ng/mL/mg
21/7	30	3 22.2 (8.0–23.8)	1,500 (615) (40.9)	50.1 (20.5) (40.9)	23.2 (0.853) (3.7)	27,600 (12,800) (46.5)	919 (427) (46)
	60	3 8.0 (8.0–24.1)	1,910 (1,320) (69.2)	31.8 (22.0) (69.2)	24.2 (0.347) (1.4)	39,000 (24,500) (62.8)	649 (409) (63.0)
	120	4 4.1 (2.0–8.0)	8,960 (3,230) (36.1)	74.7 (26.9) (36.1)	24.1 (0.199) (0.8)	1,79,000 (53,200) (29.7)	1,494 (443) (30.0)
	225	3 8.0 (8.0–8.1)	8,260 (4,610) (55.9)	36.7 (20.5) (55.9)	23.3 (1.29) (5.5)	1,55,000 (84,700) (54.8)	687 (377) (55.0)
	400	9 6.5 (4.0–25.4)	17,300 (14,000) (80.9)	43.3 (35.1) (80.9)	23.7 (0.748) (3.2)	3,01,000 (2,50,000) (83.3)	752 (414) (55.0)
	600	19 8.0 (4.0–23.9)	15,600 (8,550) (54.7)	26.1 (14.2) (54.7)	21.7 (6.09) (28.1)	2,74,000 (1,86,000) (67.9)	456 (309) (68.0)
	900	3 8.0 (4.0–23.7)	28,100 (19,100) (68.0)	31.2 (21.2) (68.0)	24.0 (0.376) (1.56)	5,90,000 (4,20,000) (71.2)	656 (467) (71.0)
CDD	100	4 4.1 (4.0–8.0)	5,200 (1,730) (33.2)	52.0 (17.3) (33.2)	24.4 (0.692) (2.8)	1,03,000 (35,500) (34.4)	1,030 (353) (34.0)
	400	3 4.0 (2.0–24.0)	12,500 (4,340) (34.8)	31.1 (10.8) (34.8)	24.2 (0.337) (1.4)	2,50,000 (77,000) (30.1)	816 (713) (31.0)
	600	21 8.0 (2.0–24.4)	13,900 (10,800) (77.9)	23.2 (18.1) (77.9)	23.3 (3.52) (15.1)	2,51,000 (1,93,000) (76.9)	418 (322) (77.0)

Abbreviations: 21/7, 21 consecutive days on treatment followed by 7 days off treatment; AUC<sub>last</sub>, area under the concentration–time curve up to the last measurable concentration; C<sub>max</sub>, maximum concentration; CDD, continuous once-daily dosing; CV, coefficient of variation; D, dose normalized; t<sub>max</sub>, time to maximum concentration; t<sub>1/2</sub>, z, terminal elimination half-life.

suggestive of either folliculitis or perivascular dermatitis. SAR245408 was discontinued for grade 3 events, and patients received oral steroids, with or without topical steroids. Resolution was variable and was documented within 4 to 50 days. After resolution, several patients resumed with dose reduction (e.g., 600 mg reduced to 400 mg) without recurrence of rash. However, one patient on the 600 mg CDD dose level, who also had mild eosinophilia (13.6%) at the time of the initial grade 3 presentation, developed a recurrent rash in areas of sun exposure when he resumed at 400 mg. SAR245408 was discontinued with gradual improvement, although leathery, cracking skin persisted. Grade 1 and 2 events often resolved with topical steroids, antihistamines, or, in some cases, no intervention and variably required dose interruption and reduction. On the 21/7 schedule, the onset of most events was during dosing, although in some instances, the onset was documented during the rest week of one of the first 2 cycles.

Overall, 22 patients (31.9%) experienced serious AEs (SAEs); of these, 3 (4.3%) patients reported an SAE possibly related to SAR245408 (grade 1 pyrexia; grade 3 full-body rash; and grade 4 arterial thrombosis). No notable trends in SAE types were apparent. No deaths due to SAR245408-related toxicity were reported. Eight patients died within 30 days of the last dose of SAR245408; all deaths were due to disease progression.

Dose reductions were reported in 10 patients (14.5%), most commonly at the 600 mg dose with either schedule (21/7 cohort: n = 3, CDD cohort: n = 2) and at the 900 mg dose with the 21/7 schedule (n = 3). Overall, 6 dose reductions occurred because of an AE, most commonly rash (n = 4) and/or diarrhea (n = 2). Seventeen dose delays were recorded, 16 of which were due to AEs, most commonly rash (n = 1 grade 1, n = 3 grade 2 and n = 2 grade 3) and diarrhea (n = 4 grade 3 and n = 1 grade 2).

The most common reason for treatment discontinuation was disease progression (RECIST: n = 41, 59.4%; clinical

deterioration: n = 9, 13.0%). Seven patients (10.1%) discontinued because of AEs, of which 2 AEs (grade 3 rash and grade 4 arterial thrombosis) were considered related to SAR245408. There were no apparent differences in reasons for discontinuation between treatment cohorts.

#### Pharmacokinetic analysis

Single-dose PK data are shown in Table 3. Single-dose exposure (C<sub>max</sub> and AUC<sub>last</sub>) for the 21/7 regimen showed high interpatient variability across cohorts. C<sub>max</sub> and AUC<sub>0–last</sub> appeared to increase dose proportionally in the dose range of 30 to 400 mg, but values at 400 and 600 mg appeared similar. C<sub>max</sub> and AUC<sub>0–last</sub> values were approximately 2-fold higher in the 900 mg cohort than in the 400 and 600 mg dose cohorts. Within the CDD cohort, C<sub>max</sub> and AUC<sub>0–last</sub> values appeared to increase with dose for the 100 and 400 mg dose levels; consistent with the 21/7 schedule cohorts, exposure values for the 400 and 600 mg dose levels were comparable. The median T<sub>max</sub> occurred at 4 to 22 hours post-dose across all cohorts.

Repeated-dose PK data at steady state were analyzed at cycle 1, day 21 for the 21/7 schedule and at cycle 1, day 28 for the CDD schedule (Table 4 and Supplementary Fig. S1). For the 21/7 schedule, the mean terminal half-life (t<sub>1/2</sub>) of SAR245408 ranged from 70 to 103 hours across the dose cohorts. Steady-state plasma levels were achieved after 15 to 21 days of drug administration. Interpatient variability was observed, with the AUC coefficient of variation percentage ranging 5% to 89%. On both schedules, C<sub>max</sub> and AUC<sub>0–24</sub> values increased approximately dose proportionately up to the 400 mg dose levels, whereas values were similar for the 400 and 600 mg dose level cohorts. Mean total Cl/F values were 2-fold higher for the 600 mg dose than for doses ranging 30 to 400 mg (Supplementary Fig. S1). For all dose levels, high accumulation of SAR245408 was observed; the mean AR

Table 4. Pharmacokinetic results from repeated-dose analysis

Dose, mg	Mean (SD) (CV%)									
	$t_{1/2z}^{\text{last}}$ , n	$T_{\text{max}}$ , h	$C_{\text{max}}$ , ng/ml	$C_{\text{max}}/\text{D}$ , ng/ ml/mg	$t_{\text{last}}$ , n	$AUC_{0-24}/\text{D}$ , h ng/ml/mg	$\text{Cl}/\text{F}$ , ml/h	$\text{AR } C_{\text{max}}$	$\text{AR AUC}$	$AUC_{\text{last}}$ , h ng/ml
21/7										
30	n $\frac{1}{4}$ 1 69.8 (NC) (NC)	n $\frac{1}{4}$ 3 2.69 (1.13) (42.0)	n $\frac{1}{4}$ 3 12,300 (5,510) (44.7)	n $\frac{1}{4}$ 3 411 (184) (44.7)	n $\frac{1}{4}$ 3 143 (82.7) (57.7)	n $\frac{1}{4}$ 3 2,58,000 (1,12,000) (43.6)	n $\frac{1}{4}$ 3 8,589 (3,743) (44.0)	n $\frac{1}{4}$ 3 140 (82) (58.0)	n $\frac{1}{4}$ 3 10.34 (7.98) (77.2)	n $\frac{1}{4}$ 3 11.91 (8.31) (69.8)
60	n $\frac{1}{4}$ 0 NC	n $\frac{1}{4}$ 2 4.04 (0.0589) (1.46)	n $\frac{1}{4}$ 2 10,200 (5,140) (50.6)	n $\frac{1}{4}$ 2 169 (85.7) (50.6)	n $\frac{1}{4}$ 2 192 (1.77) (0.92)	n $\frac{1}{4}$ 2 2,29,000 (1,22,000) (53.3)	n $\frac{1}{4}$ 2 3,808 (2,039) (54.0)	n $\frac{1}{4}$ 2 307 (164) (54.0)	n $\frac{1}{4}$ 2 4.76 (1.04) (21.8)	n $\frac{1}{4}$ 2 5.01 (0.46) (9.2)
120	n $\frac{1}{4}$ 1 103 (NC) (NC)	n $\frac{1}{4}$ 2 14.0 (14.2) (2.1)	n $\frac{1}{4}$ 2 44,000 (919) (2.1)	n $\frac{1}{4}$ 2 366 (7.66) (2.1)	n $\frac{1}{4}$ 2 179 (14.5) (8.1)	n $\frac{1}{4}$ 2 9,82,000 (50,200) (5.1)	n $\frac{1}{4}$ 2 8,192 (436) (5.3)	n $\frac{1}{4}$ 2 122 (7) (5.3)	n $\frac{1}{4}$ 2 6.02 (0.14) (2.4)	n $\frac{1}{4}$ 2 6.42 (0.28) (4.4)
225	n $\frac{1}{4}$ 1 79.8 (NC) (NC)	n $\frac{1}{4}$ 3 6.69 (2.29) (34.2)	n $\frac{1}{4}$ 3 45,700 (39,800) (87)	n $\frac{1}{4}$ 3 203 (177) (87)	n $\frac{1}{4}$ 3 137 (77.8) (56.8)	n $\frac{1}{4}$ 3 10,60,000 (9,39,000) (89.0)	n $\frac{1}{4}$ 3 4,699 (4,185) (89.0)	n $\frac{1}{4}$ 3 334 (213) (64.0)	n $\frac{1}{4}$ 3 5.25 (2.26) (43.0)	n $\frac{1}{4}$ 3 6.49 (3.00) (46.2)
400	n $\frac{1}{4}$ 1 79.5 (NC) (NC)	n $\frac{1}{4}$ 4 3.63 (3.25) (89.7)	n $\frac{1}{4}$ 4 99,700 (36,600) (36.7)	n $\frac{1}{4}$ 4 249 (91.4) (36.7)	n $\frac{1}{4}$ 4 109 (97.8) (89.5)	n $\frac{1}{4}$ 3 22,60,000 (2,35,000) (10.4)	n $\frac{1}{4}$ 3 5,642 (586) (10)	n $\frac{1}{4}$ 3 178 (18) (10)	n $\frac{1}{4}$ 4 10.98 (6.62) (60.3)	n $\frac{1}{4}$ 3 10.64 (5.80) (54.5)
600	n $\frac{1}{4}$ 2 88.0 (21.3) (24.2)	n $\frac{1}{4}$ 9 4.22 (7.54) (179)	n $\frac{1}{4}$ 9 95,200 (43,600) (45.8)	n $\frac{1}{4}$ 9 159 (72.7) (45.8)	n $\frac{1}{4}$ 9 156 (62.3) (39.9)	n $\frac{1}{4}$ 9 20,60,000 (9,38,000) (45.6)	n $\frac{1}{4}$ 9 3,429 (1,562) (46.0)	n $\frac{1}{4}$ 9 462 (484) (105.0)	n $\frac{1}{4}$ 9 8.05 (6.49) (80.6)	n $\frac{1}{4}$ 9 8.98 (7.07) (78.7)
900	NC NC	n $\frac{1}{4}$ 1 0.5.9 (NC) (NA)	n $\frac{1}{4}$ 1 1,36,000 (NC) (NC)	n $\frac{1}{4}$ 1 151 (NC) (NC)	n $\frac{1}{4}$ 1 217 (NC) (NC)	n $\frac{1}{4}$ 1 29,40,000 (NC) (NC)	n $\frac{1}{4}$ 1 3,267 (NC) (NC)	n $\frac{1}{4}$ 1 306 (NC) (NC)	n $\frac{1}{4}$ 1 12 (NC) (NC)	n $\frac{1}{4}$ 1 13.0 (NC) (NC)
CDD	n $\frac{1}{4}$ 0 NC	n $\frac{1}{4}$ 3 6.67 (2.31) (NC)	n $\frac{1}{4}$ 3 23,800 (11,500) (34.6)	n $\frac{1}{4}$ 3 238 (115) (48.2)	n $\frac{1}{4}$ 3 23.9 (0.173) (0.7)	n $\frac{1}{4}$ 3 54,1000 (2,76,132) (51)	n $\frac{1}{4}$ 3 5,410 (2,761) (51.0)	n $\frac{1}{4}$ 3 219 (103) (47.0)	n $\frac{1}{4}$ 3 5.15 (1.38) (26.9)	n $\frac{1}{4}$ 3 5.88 (1.62) (27.5)
400	n $\frac{1}{4}$ 0 NC	n $\frac{1}{4}$ 3 4.00 (3.46) (86.6)	n $\frac{1}{4}$ 3 1,18,000 (51,900) (44.1)	n $\frac{1}{4}$ 3 294 (130) (44.1)	n $\frac{1}{4}$ 3 24.4 (1.65) (6.8)	n $\frac{1}{4}$ 3 26,53,333 (11,66,462) (44.0)	n $\frac{1}{4}$ 3 6,633 (2,916) (44.0)	n $\frac{1}{4}$ 3 182 (107) (59.0)	n $\frac{1}{4}$ 3 10.79 (6.88) (63.7)	n $\frac{1}{4}$ 3 11.95 (7.74) (64.8)
600	n $\frac{1}{4}$ 0 NC	n $\frac{1}{4}$ 14 3.36 (3.03) (90.2)	n $\frac{1}{4}$ 14 87,200 (40,300) (46.3)	n $\frac{1}{4}$ 14 145 (67.2) (46.3)	n $\frac{1}{4}$ 14 24.4 (1.1) (4.51)	n $\frac{1}{4}$ 14 19,31,286 (91,3057) (47.3)	n $\frac{1}{4}$ 14 3,219 (1,522) (47.3)	n $\frac{1}{4}$ 14 439 (329) (75.0)	n $\frac{1}{4}$ 14 10.5 (4.3) (40.7)	n $\frac{1}{4}$ 14 13.0 (6.8) (52.4)

Abbreviations: 21/7, 21 consecutive days on treatment followed by 7 days off treatment;  $AUC_{\text{last}}$ , area under the concentration–time curve up to the last measurable concentration;  $AUC_{0-24}$ , area under the concentration–time curve up to 24 hours; AR, accumulation ratio;  $C_{\text{max}}$ , maximum concentration; CDD, continuous once-daily dosing; CL, apparent clearance; CV, coefficient of variation; D, dose normalized; NC, not calculated;  $t_{\text{last}}$ , time to last measurable concentration;  $t_{\text{max}}$ , time to maximum concentration;  $t_{1/2z}$ , terminal elimination half-life.

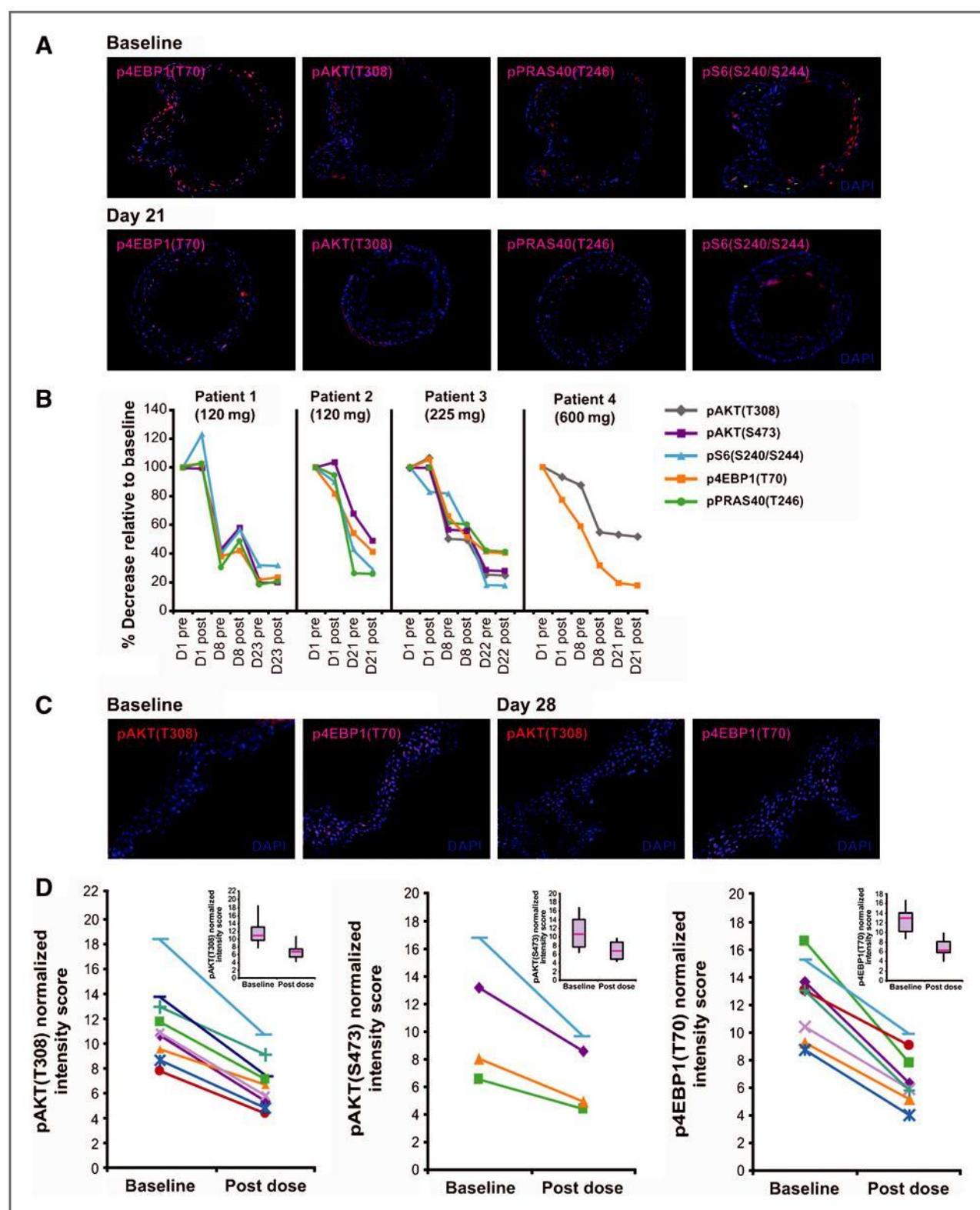


Figure 1. Reduction of PI3K signaling by SAR245408 in serial hair cells and in skin biopsies. Pharmacodynamic inhibition by SAR245408 on the PI3K pathway was documented by immunofluorescence in hair sheath cells and skin biopsies. A, effect of SAR245408 on the PI3K/mTOR pathway in hair sheath cells, assessed by immunofluorescence staining of phosphorylated eIF4E-binding protein-1 ( $p4EBP1^{T70}$ ),  $pAKT^{T308}$ , phosphorylated proline-rich AKT1 substrate ( $pPRAS40^{T246}$ ), and  $pS6^{S240/S244}$  in cross-sections of baseline and post-dose hair collected from a patient who received SAR245408 225 mg daily on the 21/7 schedule. A representative field was captured per readout at  $\times 400$  magnification. (Continued on the following page.)

based on AUC<sub>0-24</sub> ranged 5- to 12-fold (21/7 cohort) and 6- to 13-fold (CDD cohort). The percentage of drug excreted unchanged in urine was low (<0.1%) and independent of dose.

#### Pharmacodynamic analysis

Repeated dosing of SAR245408 caused a minor increase in fasting plasma insulin 2 hours post-dose on days 8 and 21, and a significant food-induced insulin increase at 4 hours post-dose on days 8 and 21, indicative of hyperinsulinemia (Supplementary Fig. S2) and consistent with the known role of PI3K in insulin signaling. In contrast, minimal to no effect on plasma glucose concentrations was evident. No consistent modulation of VEGF-A, insulin-like growth factor binding protein-2 (IGFBP-2, or regulated and normal T-cell expressed and secreted (RANTES) levels was observed (data not shown).

The impact of SAR245408 on the PI3K pathway was assessed in paired surrogate tissues (hair sheath cells and skin) from a limited number of patients during dose escalation and in tumor biopsies (and in some instances skin biopsies) from patients treated at the MTD. Novel immunofluorescence staining methods providing more quantitative readout than traditional immunohistochemistry were developed to investigate PI3K pathway modulation (20). Analysis of hair sheath sets collected from four patients treated with 120, 225, and the 600 mg MTD dose in the 21/7 cohort showed a time-dependent pathway inhibition, with pronounced effects at 120 and 225 mg (Fig. 1A and B; Supplementary Table S2) for PI3K.

Proximal biomarkers (pAKT<sup>S473</sup>, pPRAS40<sup>T246</sup> and pAKT<sup>T308</sup> with 51%–80%, 59%–80%, and 48%–75% reduction, respectively) and downstream biomarkers (p4EBP1<sup>T70</sup> and pS6<sup>S240/S244</sup> with 59%–82% and 68%–82% reduction, respectively). No obvious dose-response relationship was observed but pathway inhibition was more pronounced at higher plasma concentrations of SAR245408. Analysis of paired skin biopsies collected from 9 patients treated with doses ranging from 30 to 900 mg showed moderate pathway inhibition, with maximum inhibition ranging from 30% to 55% for proximal and distal biomarkers (Fig. 1C and D and Supplementary Table S2). Pharmacodynamic effects were also explored in PBMC lysates and buccal mucosal smears; however, because of technical challenges, limited data were obtained (data not shown).

Analysis of paired tumor biopsies collected from 9 patients enrolled in the 600 mg MTD cohort (including a patient enrolled in the 900 mg cohort who underwent dose reduc-

tion to 600 mg) showed robust but partial PI3K pathway inhibition, with >60% pAKT<sup>T308</sup> reduction in 5 of the 9 tumor sets (range, 41%–82%; Fig. 2A–C and Supplementary Table S3). Downstream inhibition of the PI3K pathway was evident with reduction of TORC1 biomarkers (p4EBP1<sup>T70</sup> and pS6<sup>S240/S244</sup>) of 39%–73% and 68%–70% and TORC2 biomarkers (pAKT<sup>S473</sup> and pPRAS40<sup>T246</sup> of 55%–61% and 50%–68%). Interestingly, SAR245408 also had an effect on the RAS/MEK/ERK pathway in tumor tissue, with pERK<sup>T202/Y204</sup> and pMEK<sup>S217/S221</sup> reductions of 42% to 70% and 46% to 59%, respectively (Fig. 2 and Supplementary Fig. S3). The interpatient variability in exposure observed did not account for observed differences in pathway inhibition. The impact on cell proliferation was modest (15%–49% reduction of Ki67) and induction of apoptosis was minimal or not apparent (Fig. 2C). Inhibition of the PI3K pathway occurred in tumors with and without molecular alterations in PI3K pathway components/modulators (Fig. 2 and Supplementary Table S3).

Pathway modulation by SAR245408 was more pronounced in tumor tissue compared with normal skin in 3 patients receiving the 600 mg MTD dose for whom both tumor and normal skin samples were available (Figs. 1C and 2B; Supplementary Tables S2 and S3). Similarly, when tumor and adjacent normal skin were collected in the same biopsy from either a patient with hamartoma (Cowden syndrome) or one with Merkel cell carcinoma, pathway modulation was more pronounced in tumor tissue than in normal skin adjacent to tumor tissue (Fig. 2D and Supplementary Table S3).

#### Antitumor activity

Twenty-five patients (43.9%) had stable disease as the best response, and 8 patients were progression-free at 6 months, including patients with NSCLC, prostate, and head and neck cancer, as well as the patient with SLL (Supplementary Table S4). Three patients were on study for ≥12 months. Prolonged stable disease was observed among patients regardless of the mutational status of components of the PI3K pathway. Three of 33 patients evaluated had tumors with PIK3CA mutation, 6 of 33 patients had tumors harboring PTEN deletion or mutation, and 5 of 14 patients had tumors with TP53 mutation, all consistent with frequencies expected for this phase I population. In the one patient with NSCLC with a partial response, no mutations in PIK3CA, PTEN, KRAS, MET, EGFR, or LKB1 genes were detected in archival tumor tissue (Supplementary Table S4). There were also no apparent differences in efficacy between the 2 dosing regimens.

(Continued.) B, progressive reduction in immunofluorescence staining of pAKT<sup>T308</sup>, pAKT<sup>S473</sup>, pPRAS40<sup>T246</sup>, p4EBP1<sup>T70</sup>, and pS6<sup>S240/S244</sup> in hair sheath cells collected from 4 patients receiving SAR245408 120 mg (n = 2), 225 mg and 600 mg daily on the 21/7 schedule. C, effect of SAR245408 on the PI3K/mTOR signaling pathway in skin biopsies assessed by immunofluorescence staining for pAKT<sup>S473</sup> and p4EBP1<sup>T70</sup> in samples collected at baseline and day 28 from a patient receiving SAR245408 100 mg daily on the CDD schedule. D, quantification of SAR245408 modulation of pAKT<sup>T308</sup>, pAKT<sup>S473</sup> and p4EBP1<sup>T70</sup> in cross-sections of skin biopsied at baseline and post-dose (on cycle 1, day 20 or day 29) in 9 patients who received SAR245408 on the 21/7 schedule (30, 60, 225, 600, and 900 mg reduced to 600 mg) and CDD schedule (100 mg). Individual patient data (each line representing an individual patient) and box plot representations (inset plot) are shown.

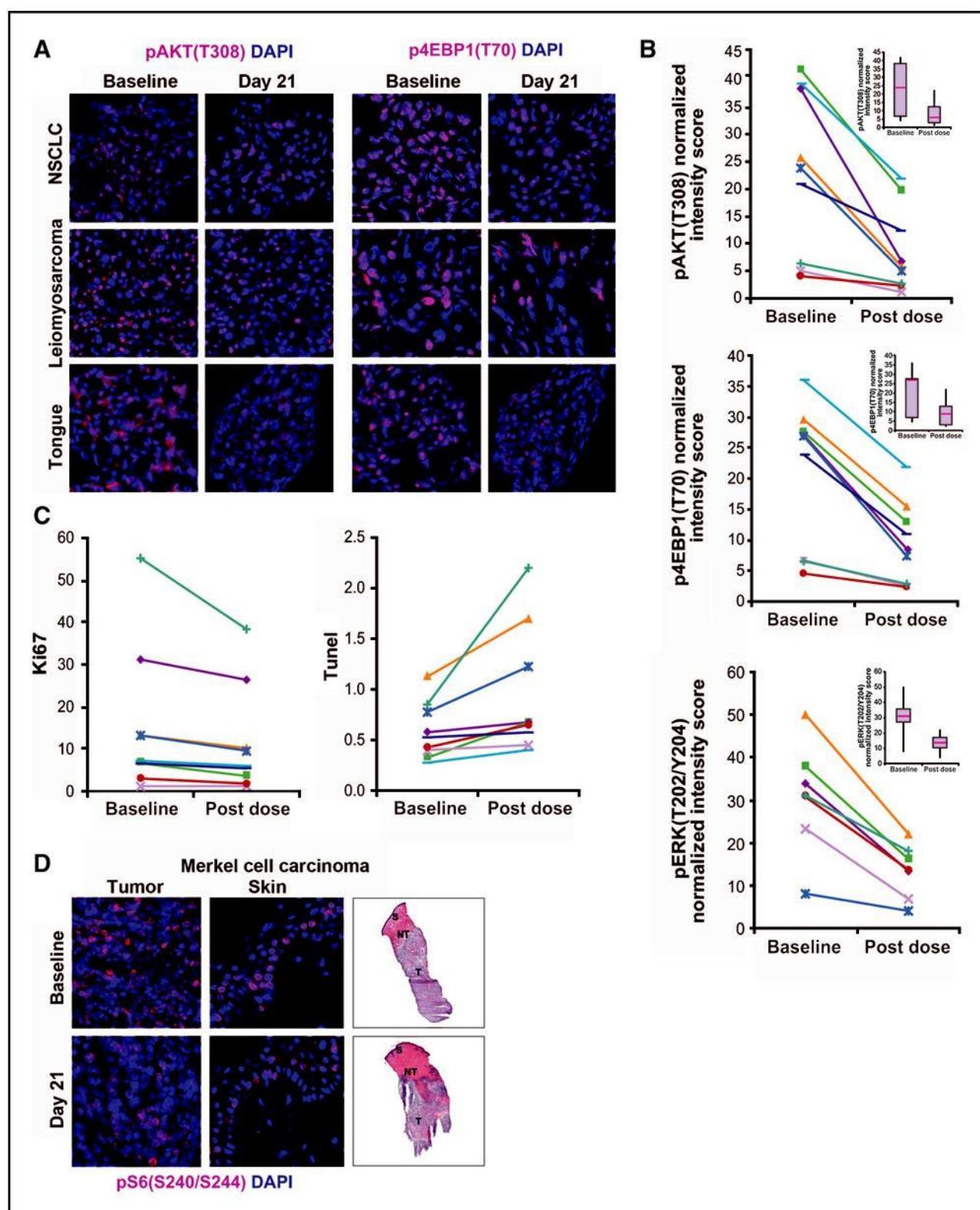


Figure 2. Reduction in PI3K signaling by SAR245408 in paired tumor biopsies. Pharmacodynamic inhibition by SAR245408 of the PI3K and ERK pathways, documented by immunofluorescence. A, effect of SAR245408 on the PI3K pathway and tumor proliferation in 3 patients treated with SAR245408 at 600 mg daily. Inhibition of the PI3K pathway was assessed by immunofluorescence staining of cryopreserved biopsy samples collected from patients with NSCLC or Leiomyosarcoma treated with the 21/7 schedule, and a patient with tongue SCC treated with the CDD schedule. (Continued on the following page.)

## Discussion

We report here the safety, tolerability, and preliminary efficacy results of the pan-class I PI3K inhibitor SAR245408. The MTD in the capsule formulation was determined to be 600 mg, both for the 21/7 and for the CDD schedules. Although the number of dose reductions was higher in the 21/7 cohort and treatment-related AEs were slightly more common in the CDD cohort, differences between the schedules appeared to be of limited importance, such that SAR245408 had a manageable toxicity profile, with drug-related severe AEs being infrequent. Of note is the relatively low incidence of hyperglycemia, a secondary effect of PI3K pathway inhibition observed with some PI3K pathway inhibitors. In this study, 5 patients (7.2%) presented with drug-related hyperglycemia (all grades). In a recent study of BKM120, a pan-class I PI3K inhibitor, 37% of patients experienced drug-related hyperglycemia, including 9% with grade 2–3 severity (21). In contrast, minimal and/or transient hyperglycemia was observed with the irreversible pan-class I PI3K inhibitor PX-866 (22) and the AKT inhibitor MK-2206 (23), probably due to compensatory mechanisms.

The drug exposure parameters presented here show that pharmacokinetic variables were similar between the 21/7 and CDD cohorts and did not change with duration of dosing. The plasma concentrations associated with robust pharmacodynamic impact and antitumor efficacy in mice (17) are comparable to plasma concentrations associated with PI3K pathway inhibition in this clinical study. Pharmacodynamic analyses showed evidence of PI3K pathway inhibition in tumor tissue and hair sheath cells and, to a lesser extent, in skin tissue.

PI3K pathway modulation was observed in all 9 tumor biopsy sets evaluated (Supplementary Table S3). Furthermore, PI3K pathway inhibition was still apparent at day 28 in a patient with ovarian leiomyosarcoma enrolled to the 21/7 schedule, suggesting that pathway modulation persisted through the 7-day break in dosing. This observation may be attributed to the long half-life and high accumulation of SAR245408.

PI3K pathway inhibition in tumor was partial (pAKT<sup>T308</sup> range in reduction of 41%–82%, including four tumor sets with >60% reduction). The degree of inhibition required for antitumor activity in patients is unknown and may vary depending on tumor addiction to the pathway and/or the presence of molecular alterations causing resistance. PI3K pathway modulation has also been documented for MK-2206 (45%–96% pAKT<sup>S473</sup> reduction in tumor tissue;

ref. 23) and BKM120 (40%–85% pS6<sup>S240/244</sup> reduction in skin tissue; ref. 21). Pathway inhibition in skin tissue was less pronounced than in adjacent tumor tissue, suggesting some degree of tumor-cell selectivity and hence the potential to minimize AEs in clinical practice. Such selectivity may stem from differences in pathway activation state or local exposure to SAR245408 due to altered vascular integrity in tumors.

Because mTOR inhibition by rapalogs has been associated with ERK pathway activation in both preclinical models and patients (24), we examined the impact of SAR245408 on the RAF/MEK/ERK pathway in tumor tissue. A clear inhibition of the MEK/ERK pathway was observed. SAR245408 is highly selective for class I PI3K isoforms, with IC<sub>50</sub> values for BRAF, CRAF, and MEK >10 mmol/L, and no effect on the MAPK pathway was observed in preclinical models (18, 19). The mechanisms underlying SAR245408 inhibition of the ERK pathway in tumor tissue are unclear; PI3K inhibition may disrupt positive crosstalk between the PI3K and ERK pathways, or alternatively, the effect may be indirect and mediated by changes in tumor biology caused by PI3K inhibition that are not yet elucidated. This observation is of substantial interest given the potential importance of simultaneously inhibiting the PI3K and MAPK pathways in tumor cells. These data may explain the efficacy of SAR245408 against KRAS-mutant xenograft models (18).

Modest effects on proliferation and apoptosis were observed, suggesting that with the exposure achieved in the study population, SAR245408 effects are primarily cytostatic and not pro-apoptotic. As such, it is not surprising that stable disease was the best response seen in 44% of patients, of which 8 maintained their response for >6 months. These results are comparable to those reported for the pan-class I PI3K inhibitor BKM120 (21), the irreversible pan-class I PI3K inhibitor PX-866 (22), and the AKT inhibitor MK-2206 (23). Several PI3K isoform-specific inhibitors are in development, and these agents may have the potential to achieve greater pathway inhibition at doses associated with minimal AEs. The data here show that the pan-PI3K inhibitor SAR245408 administrated at biologically active doses is associated with a manageable safety profile that compares favorably with other pan-PI3K inhibitors (21).

Frequencies of molecular alterations in PIK3CA, PTEN, and TP53 were consistent with those expected for this phase I population. Inhibition of the PI3K pathway occurred in tumors with and without alterations in PI3K pathway components/modulators, and no correlation between efficacy and molecular alterations was identified. Some

(Continued.) Representative fields were captured per readout at  $\times 400$  magnification [colors: red for pAKT<sup>T308</sup> or phosphorylated eIF4E-binding protein-1 (p4EBP1)<sup>T70</sup>, blue for 4',6-diamidino-2-phenylindole (DAPI) and magenta for Ki67]. The NSCLC tumor had an about 2-fold PIK3CA amplification; the tongue SCC had a PIK3CA E545K mutation and no mutation was detected in NSCLCs and leiomyosarcoma tumors. In the leiomyosarcoma tumor, reductions of 82% in pAKT<sup>T308</sup> level and 68% in p4EBP1<sup>T70</sup> level were observed. In the NSCLC tumor, reductions of 79% in pAKT<sup>T308</sup> level and 73% in p4EBP1<sup>T70</sup> level were observed. In the tongue SCC tumor, reductions of 59% in pAKT<sup>T308</sup> level and 56% in p4EBP1<sup>T70</sup> level were observed. B and C, quantification of immunofluorescence staining in tumor biopsies taken at baseline and on cycle 1 day 21 or 28 in 9 patients receiving the MTD of SAR245408 (600 mg daily). Individual patient data (each line representing an individual patient) and box plot representations (inset plot) for pAKT<sup>T308</sup>, p4EBP1<sup>T70</sup>, pERK<sup>T202/Y204</sup>, Ki67 and TUNEL levels are shown. D, effects of SAR245408 on the PI3K pathway and tumor proliferation in tumor and adjacent skin. Immunofluorescence and H&E staining of Merkel cell carcinoma and adjacent skin tissue biopsies taken at baseline and day 21 from a patient treated with SAR245408 600mg daily. pS6<sup>S240/244</sup> reduction of 70% in tumor and 35% in skin were observed. NT, non-tumor stroma; S, skin; T, tumor cell.

preclinical studies have suggested that PIK3CA mutations might predict sensitivity to pan-PI3K and dual PI3K/mTOR inhibitors (25); however, these results have not yet been clinically validated. In contrast, studies with  $\alpha$ -isoform-selective and  $\beta$ -isoform-sparing PI3K inhibitors have produced responses among patients with tumors harboring PIK3CA mutations (26, 27); in addition, the presence of a PIK3CA mutation-related gene signature may identify patients with breast cancer who may benefit from the addition of everolimus to letrozole (28), although clinical benefit from everolimus is not restricted to patients with PIK3CA-mutant tumors. The molecular profiling conducted here was limited to analysis of a few candidate genes and evaluation of PTEN protein levels in a heterogeneous population. Further extensive next-generation molecular profiling analyses in homogenous patient populations with agents targeting the PI3K/mTOR pathway will be required to better define signatures of response, clinical benefit, and resistance.

In summary, this first-in-human phase I study showed a favorable safety profile, demonstrable pharmacodynamic effects and preliminary antitumor activity of SAR245408 in patients with advanced solid tumors, supporting its further development. Evaluation of SAR245408 as monotherapy is ongoing in endometrial cancer, glioblastoma, and lymphoma. In addition, a tablet formulation of SAR245408 is being evaluated in patients with advanced solid tumors. Rational combination studies of SAR245408 with other targeted or cytotoxic agents are ongoing or completed, which should help define the role of PI3K inhibition in the anticancer armamentarium.

#### Disclosure of Potential Conflicts of Interest

A.D. Laird, L.T. Nguyen, and C. Scheffold are employees of Exelixis Inc. as Senior/Executive Directors (A.D.L., L.T.N.) and Medical Director (C.S.). A.D.

Laird and C. Scheffold hold stocks and shares in Exelixis Inc. C. Egile and Y. Xu are employees of Sanofi as Directors. E.L. Kwak declares financial support from Sanofi (paid to the place of employment) related to the study reported here. C. Bedell has Honoraria from Speakers Bureau of Celgene and is a Consultant/Advisory Board member of Teva. No potential conflicts of interest were disclosed by the other authors.

#### Authors' Contributions

Conception and design: G.I. Shapiro, J. Baselga, C. Scheffold, A.D. Laird  
Development of methodology: J. Baselga, C. Scheffold, Y. Xu  
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): G.I. Shapiro, J. Rodon, C. Bedell, E.L. Kwak, I. Brana, S.S. Pandya, A.D. Laird, G. Edelman  
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): G.I. Shapiro, J. Rodon, J. Baselga, C. Scheffold, A.D. Laird, L.T. Nguyen, Y. Xu, C. Egile, G. Edelman  
Writing, review, and/or revision of the manuscript: G.I. Shapiro, J. Rodon, E.L. Kwak, I. Brana, S.S. Pandya, C. Scheffold, A.D. Laird, C. Egile  
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): G.I. Shapiro, E.L. Kwak, A.D. Laird, C. Egile, G. Edelman  
Study supervision: G.I. Shapiro, J. Rodon, E.L. Kwak, C. Scheffold, C. Egile, G. Edelman

#### Acknowledgments

The authors thank the patients and their families, as well as Dr. Art DeCillis of Exelixis and Celine Lefranc and Dr. Joanne Lager of Sanofi for their contributions to the study. They also thank Valentina Vysotskaia, Bih Hsu, Belinda Cancilla, and Frauke Bentzien (Exelixis Inc.) for tumor molecular profiling, bioanalytics, and pharmacodynamic analyses. Additional pharmacokinetic analysis was provided by Gary Emmons of Sanofi. The authors received editorial support from Dr. Melissa Purves of MediTech Media Ltd.

#### Grant Support

This study was supported by Exelixis Inc. and Sanofi.  
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received July 9, 2013; revised October 2, 2013; accepted October 7, 2013; published OnlineFirst October 28, 2013.

#### References

- Cantley LC. The phosphoinositide 3-kinase pathway. *Science* 2002; 296:1655–7.
- Engelman JA, Luo J, Cantley LC. The evolution of phosphatidylinositol 3-kinases as regulators of growth and metabolism. *Nat Rev Genet* 2006;7:606–19.
- Liu P, Cheng H, Roberts TM, Zhao JJ. Targeting the phosphoinositide 3-kinase pathway in cancer. *Nat Rev Drug Discov* 2009;8:627–44.
- Zhao L, Vogt PK. Class I PI3K in oncogenic cellular transformation. *Oncogene* 2008;27:5486–96.
- Guillermet-Guibert J, Bjorklof K, Salpekar A, Gonella C, Ramadani F, Bilancio A, et al. The p110beta isoform of phosphoinositide 3-kinase signals downstream of G protein-coupled receptors and is functionally redundant with p110gamma. *Proc Natl Acad Sci U S A* 2008;105: 8292–7.
- Samuels Y, Wang Z, Bardelli A, Silliman N, Ptak J, Szabo S, et al. High frequency of mutations of the PIK3CA gene in human cancers. *Science* 2004;304:554.
- Brown JR, Hanna M, Tesar B, Werner L, Pochet N, Asara JM, et al. Integrative genomic analysis implicates gain of PIK3CA at 3q26 and MYC at 8q24 in chronic lymphocytic leukemia. *Clin Cancer Res* 2012;18:3791–802.
- Hollander MC, Blumenthal GM, Dennis PA. PTEN loss in the continuum of common cancers, rare syndromes and mouse models. *Nat Rev Cancer* 2011;11:289–301.
- Rodriguez-Viciano P, Warne PH, Dhand R, Vanhaesebroeck B, Gout I, Fry MJ, et al. Phosphatidylinositol-3-OH kinase as a direct target of Ras. *Nature* 1994;370:527–32.
- Rodriguez-Viciano P, Warne PH, Khwaja A, Marte BM, Pappin D, Das P, et al. Role of phosphoinositide 3-OH kinase in cell transformation and control of the actin cytoskeleton by Ras. *Cell* 1997;89:457–67.
- Braccini L, Ciraolo E, Martini M, Pirali T, Germena G, Rolfo K, et al. PI3K keeps the balance between metabolism and cancer. *Adv Biol Regul* 2012;52:389–405.
- McCubrey JA, Steelman LS, Chappell WH, Abrams SL, Montalvo G, Cervello M, et al. Mutations and deregulation of Ras/Raf/MEK/ERK and PI3K/PTEN/Akt/mTOR cascades which alter therapy response. *Oncotarget* 2012;3:954–87.
- Klein S, Levitzki A. Targeting the EGFR and the PI3K pathway in cancer. *Curr Opin Cell Biol* 2009;21:185–93.
- Priulla M, Calastretti A, Bruno P, Azzariti A, Paradiso A, Canti G, et al. Preferential chemosensitization of PTEN-mutated prostate cells by silencing the Akt kinase. *Prostate* 2007;67:782–9.
- Qi J, McTigue MA, Rogers A, Lifshits E, Christensen JG, Janne PA, et al. Multiple mutations and bypass mechanisms can contribute to development of acquired resistance to MET inhibitors. *Cancer Res* 2011;71:1081–91.
- Courtney KD, Corcoran RB, Engelman JA. The PI3K pathway as drug target in human cancer. *J Clin Oncol* 2010;28:1075–83.

17. Edelman G, Bedell C, Shapiro GI, Pandya SS, Kwak EL, Scheffold C, et al. A phase 1 dose-escalation study of XL147 (SAR245408), a PI3K inhibitor administered orally to patients with advanced malignancies. *J Clin Oncol* 28, 2010 (suppl; abstr A3004).
18. Sidhu SS, Egile C, Malfilatre M, Lefranc C, Ruffin Y, Ma J, et al. Antitumor activity of pimasertib in combination with SAR245409 or SAR245408 in human primary colorectal cancer xenograft models bearing PI3K/KRAS and KRAS mutations [abstract]. In: Proceedings of the 104th Annual Meeting of the American Association for Cancer Research; 2013 Apr 6–10; Washington, DC. Philadelphia (PA): AACR; 2013. Abstract nr A4638.
19. Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, et al. New guidelines to evaluate the response to treatment in solid tumors. *J Natl Cancer Inst* 2000;92:205–16.
20. Li C, Takahashi C, Zhang L, Huseni M, Stankovich B, Mashhedi H, et al. Development of a robust flow cytometry-based pharmacodynamic assay to detect phospho-protein signals for phosphatidylinositol 3-kinase inhibitors in multiple myeloma. *J Transl Med* 2013;11:76.
21. Bendell JC, Rodon J, Burris HA, de Jonge M, Verweij J, Birle D, et al. Phase I, dose-escalation study of BKM120, an oral pan-Class I PI3K inhibitor, in patients with advanced solid tumors. *J Clin Oncol* 2012; 30:282–90.
22. Hong DS, Bowles DW, Falchook GS, Messersmith WA, George GC, O'Bryant CL, et al. A multicenter phase I trial of PX-866, an oral irreversible phosphatidylinositol 3-kinase inhibitor, in patients with advanced solid tumors. *Clin Cancer Res* 2012;18:4173–82.
23. Yap TA, Yan L, Patnaik A, Fearen I, Olmos D, Papadopoulos K, et al. First-in-man clinical trial of the oral pan-AKT inhibitor MK-2206 in patients with advanced solid tumors. *J Clin Oncol* 2011;29: 4688–95.
24. Carracedo A, Ma L, Teruya-Feldstein J, Rojo F, Salmena L, Alimonti A, et al. Inhibition of mTORC1 leads to MAPK pathway activation through a PI3K-dependent feedback loop in human cancer. *J Clin Invest* 2008; 118:3065–74.
25. Maira SM, Pecchi S, Huang A, Burger M, Knapp M, Sterker D, et al. Identification and characterization of NVP-BKM120, an orally available pan-class I PI3-kinase inhibitor. *Mol Cancer Ther* 2012; 11:317–28.
26. Gonzalez-Angulo AM, Juric D, aArgiles G, Schellens JHM, Burris HA, Berlin J, et al. Safety, pharmacokinetics, and preliminary activity of the alpha-specific PI3K inhibitor BYL719: results from the first-in-human study. *J Clin Oncol* 31, 2013 (suppl; abstr A2531).
27. Juric D, Krop I, Ramanathan RK, Xiao J, Sanabria S, Wilson TR, et al. GDC-0032, a beta isoform-sparing PI3K inhibitor: results of a first-in-human phase Ia dose escalation study [abstract]. In: Proceedings of the 104th Annual Meeting of the American Association for Cancer Research; 2013 Apr 6–10; Washington, DC. Philadelphia (PA): AACR; 2013. Abstract nr LB-64.
28. Loi S, Michiels S, Baselga J, Bartlett JM, Singhal SK, Sabine VS, et al. PIK3CA genotype and a PIK3CA mutation-related gene signature and response to everolimus and letrozole in estrogen receptor positive breast cancer. *PLoS One* 2013;8:e53292.

Buparlisib, o BKM120 (Novartis Pharma AG, Basel, Suiza) es un pan-inhibidor de PI3K derivado de la pirimidina con potente actividad específica contra las isoformas de PI3K de clase I. Buparlisib inhibe las isoformas PI3K $\alpha$  tanto mutado como salvaje o nativo, así como las isoformas  $\beta$ ,  $\delta$  y  $\gamma$  de PI3K en concentraciones nanomolares (de 52 nM/166 nM/116 nM/262 nM, respectivamente). Buparlisib no tiene actividad inhibidora contra las isoformas de PI3K de clase III, o diana de rapamicina en células de mamífero (mTOR)(90). Los experimentos *in vitro* muestran que Buparlisib posee un potente efecto antiproliferativo en las líneas celulares de cáncer en humanos con alteraciones en la vía PI3K. In vivo, Buparlisib mostró una significativa actividad antitumoral en modelos de xenoinjertos tumorales en humanos, con una buena correlación entre la exposición a Buparlisib y la inhibición de la señalización de PI3K.

En el presente estudio detallamos un análisis completo del primer ensayo de fase I en humanos del agente único buparlisib para evaluar su seguridad, sus biomarcadores farmacocinéticos y farmacodinámicos, así como los factores predictores de eficacia.

*3.2.2 Segundo manuscrito: Estudio de fase I de aumento de dosis y expansión de Buparlisib (BKM120), un pan-inhibidor oral de PI3K de Clase I, en pacientes con tumores sólidos avanzados.*

# Phase I dose-escalation and -expansion study of buparlisib (BKM120), an oral pan-Class I PI3K inhibitor, in patients with advanced solid tumors

Jordi Rodon & Irene Braña & Lillian L Siu & Maja J De Jonge & Natasha Homji & David Mills & Emmanuelle Di Tomaso & Celine Sarr & Lucia Trandafir & Cristian Massacesi & Ferry Eskens & Johanna C Bendell

Received: 17 December 2013 / Accepted: 28 February 2014 / Published online: 21 March 2014  
© Springer Science+Business Media New York 2014

**Purpose** The pan-Class I PI3K inhibitor buparlisib (BKM120) has shown activity in a range of pre-clinical cancer models. This first-in-man study was initiated to identify the maximum tolerated dose (MTD) of buparlisib (100 mg/day) and to assess safety and preliminary efficacy. **Methods** Patients with advanced solid tumors (N=83) enrolled in a Phase I dose-escalation and -expansion study of single-agent buparlisib. Patients in the dose-expansion arm (n=43) had tumor samples with PIK3CA and/or PTEN alterations. **Results** The most common cancers were colorectal (n=31) and breast cancer (n=21). Median number of prior antineo-plastic regimens was four (range: 1–12). Grade 3/4 adverse events (AEs) included asthenia (12.0 %) and performance status decrease (9.6 %). Treatment-related AEs (all grades) included decreased appetite, diarrhea, nausea (each in 33 % of

patients), hyperglycemia (31 %) and rash (29 %). One confirmed partial response (PR; triple-negative breast cancer) and three unconfirmed PRs (parotid gland carcinoma, epithelioid hemangiothelioma, ER + breast cancer) were reported. Tumor molecular status did not predict clinical benefit in the full study cohort, or among the colorectal or breast cancer sub-populations. Pharmacodynamic biomarkers (<sup>18</sup>F-FDG-PET, C-peptide, pS6) demonstrated dose-dependent changes; however, tumor heterogeneity precluded a clear correlation with clinical benefit. **Conclusion** Buparlisib was well tolerated up to the 100 mg/day dose and showed preliminary activity in patients with advanced cancers. Future studies in more homogeneous patient populations will evaluate buparlisib in combination with other agents and further investigate the use of predictive biomarkers.

**Electronic supplementary material** The online version of this article (doi:10.1007/s10637-014-0082-9) contains supplementary material, which is available to authorized users.

J. Rodon I. Braña  
Vall d'Hebron University Hospital, Barcelona, Spain

J. Rodon I. Braña  
Universitat Autònoma de Barcelona, Barcelona, Spain

L. L. Siu  
Princess Margaret Cancer Centre, Toronto, Canada

M. J. De Jonge F. Eskens  
Erasmus MC Cancer Institute, Rotterdam, The Netherlands

N. Homji  
Novartis Pharmaceuticals, Florham Park, NJ, USA

D. Mills  
Novartis Pharma AG, Basel, Switzerland

E. Di Tomaso  
Novartis Institutes for BioMedical Research, Inc., Cambridge, MA, USA

C. Sarr  
Novartis Pharmaceuticals, East Hanover, NJ, USA

L. Trandafir C. Massacesi  
Novartis Oncology, Paris, France

J. C. Bendell  
Sarah Cannon Research Institute, Nashville, TN, USA

J. Rodon (\*)  
Medical Oncology Department, Vall d'Hebrón University Hospital, Barcelona, Spain  
e-mail: [jrodon@vhio.net](mailto:jrodon@vhio.net)

**Keywords** Buparlisib · BKM120 · Oncology · PI3K inhibitor · Targeted therapy · Solid tumors

## Introduction

The phosphatidylinositol 3-kinase (PI3K) pathway is one of the most frequently dysregulated signaling pathways in cancer and has been associated with resistance to chemotherapy and targeted agents [1–5]. Class IA PI3Ks, the type most commonly implicated in cancer, are heterodimers comprising a regulatory (p85) and a catalytic (p110) subunit. Various iso-forms of these subunits exist, including five regulatory sub-units (p85 $\alpha$ , p85 $\beta$ , p55 $\alpha$ , p55 $\gamma$ , and p50 $\alpha$ ) and three catalytic subunits (p110 $\alpha$ , p110 $\beta$ , and p110 $\delta$ ) [6]. PI3Ks transduce extracellular signals received by receptor tyrosine kinases and G protein-coupled receptors, among others, and initiate a signaling cascade that activates several important down-stream effectors that promote cancer cell growth, proliferation, survival, motility and metabolism [6].

Mutation or amplification of PIK3CA, which encodes the p110 $\alpha$  isoform of the PI3K catalytic subunit, is frequently observed in cancer. Similarly, the gene encoding phosphatase and tensin homolog (PTEN), a phosphatidylinositol-3,4,5-trisphosphate 3-phosphatase, is also found to be deleted, mutated, or silenced in a wide range of malignancies [1, 7–9]. Given the prevalence of PI3K pathway alterations, and the critical role that PI3K plays in oncogenic signal transduction, targeted inhibition of PI3K has emerged as a promising therapeutic target for cancer.

Buparlisib (BKM120; Novartis Pharma AG, Basel, Switzerland) is an oral pan-Class I PI3K inhibitor that selectively targets all four isoforms of Class I PI3K ( $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$ ), with at least 50-fold selectivity against several other lipid or protein kinases [10]. Single-agent buparlisib has demonstrated anti-proliferative, pro-apoptotic, and antitumor activity in a variety of cell lines and xenograft models from cancers with and without aberrant PI3K pathway activation [10–14].

Preliminary results from the dose-escalation portion of this first-in-human, Phase I dose-escalation study of single-agent buparlisib, which began on November 24, 2008, were reported for 40 patients with advanced solid tumors (NCT01068483) [15]. In the preliminary report, the maximum tolerated dose (MTD) of single-agent buparlisib was established at 100 mg/day [15]. Here, we report the full analysis of this Phase I trial, which includes additional data on 5 patients who were enrolled in the dose-escalation phase of the trial (n=40), and an additional 43 patients who were enrolled in the dose-expansion phase (N= 83 in total). In addition to this updated analysis, we also present updated pharmacodynamic biomarker data.

## Patients and methods

### Patient population

Adult patients with histologically confirmed, advanced unresectable breast, colorectal, ovarian, endometrial or other solid cancer, who had progressed on (or not been able to tolerate) standard therapy, or for whom no standard anticancer therapy was available, were eligible for enrollment in this two-part study [15]. Seventeen patients in the dose-escalation arm of the study were treated at the MTD [15], with an additional 43 patients treated as part of the dose-expansion arm.

To further investigate how PI3K pathway alterations might influence the effect of buparlisib treatment, the dose-expansion arm of the trial was enriched for patients with common PI3K pathway alterations. Additional patients were eligible for enrollment in the dose-expansion arm if they demonstrated the criteria described above for the dose-escalation phase, and provided fresh or archival tumor biopsies that showed alterations in the PI3K pathway, defined as mutated or amplified PIK3CA and/or mutated PTEN and/or null/low PTEN protein expression (H Score  $\leq 50$  by immuno-histochemistry). PI3K pathway alterations were not compulsory for enrollment in the dose-escalation arm but the PI3K activation markers were still evaluated using archival tumor tissue.

Based on clinical observations, from January 14, 2010, patients with the following psychiatric disorders were excluded from enrollment: medically documented history of or active major depressive episode, bipolar disorder (I or II), obsessive-compulsive disorder, schizophrenia, a history of suicidal attempt or ideation, or homicidal ideation (immediate risk of doing harm to others). The presence of these disorders was assessed by the investigator or a psychiatrist, or from the results of self-rating patient mood assessment questionnaires. Two patient questionnaires were used: the 7-item generalized anxiety disorder scale (GAD-7), and the 9-item patient health questionnaire (PHQ-9) for the assessment of depression [16, 17].

Approval was obtained from the ethics committees of participating institutions and regulatory authorities. All participating patients provided written informed consent and agreed to comply with the protocol. The study was conducted in accordance with the Declaration of Helsinki and guidelines for Good Clinical Practice as defined by the International Conference on Harmonization.

### Study design and treatment

This was a Phase I, multicenter, open-label, single-agent study, which consisted of two parts: a dose-escalation part [15] and a dose-expansion part (presented here as an updated and extended analysis alongside additional data from the

dose-escalation cohorts). The primary objective of the study was to establish the MTD of buparlisib on a once-daily continuous schedule in adults with advanced solid tumors. Secondary objectives included the assessment of pharmacokinetics, the safety and tolerability of buparlisib, preliminary anti-tumor activity (according to Response Evaluation Criteria In Solid Tumors [RECIST] version 1.0), and pharmacodynamic assessments, including markers of glucose metabolism, cell proliferation, angiogenesis and cell death, and the use of <sup>18</sup>F-FDG-PET as a potential marker of early efficacy.

Patients received oral buparlisib as once-daily hard gelatin capsules in a continuous schedule of 28-day cycles. In the dose-escalation part of the study, successive cohorts of patients received buparlisib starting at 12.5 mg/day, until disease progression, unacceptable toxicity, or withdrawal of consent. Clinical judgment on the overall safety profile together with an adaptive Bayesian logistic regression model for dose escalation with overdose control [18] guided the dose escalation based on the incidence of dose-limiting toxicities (DLTs) evaluated during the first treatment cycle. The MTD was defined as the highest dose of buparlisib not causing DLT in more than 33 % of patients in the first treatment cycle [15]. Patients enrolling in the dose-expansion arm received buparlisib at the MTD (100 mg/day).

### Safety and efficacy assessments

Routine clinical and laboratory assessments were conducted at baseline, weekly until Day 22 of Cycle 2, and then on Days 1 and 15 of subsequent cycles. Other safety assessments included, fasting plasma glucose, insulin and C-peptide tests; assessment of fructosamine and hemoglobin A1c levels; and electrocardiography. Following the January 2010 amendment, patients were also assessed for psychiatric disorders using patient self-rating mood questionnaires for anxiety and depression (PHQ-9 and GAD-7).

Adverse events (AEs) were collected continuously and graded using the National Cancer Institute's Common Terminology Criteria for Adverse Events (NCI-CTCAE) v3.0. Radiologic response was assessed by computed tomography (CT) scan according RECIST version 1.0 at baseline and at approximately Day 28 of Cycle 2 and every 8 weeks thereafter.

### Biomarker and pharmacodynamic assessments

Archival or fresh tumor biopsies were collected from all patients as part of prescreening for enrollment into the dose-expansion portion of the trial, and were used for central assessment of the presence of PIK3CA mutation in exons 9 or 20 as determined by SNaPshot genotyping assay, and low or null PTEN expression as defined by an immunohistochemistry H-Score <50. KRAS mutation was also assessed by genomic sequencing as part of exploratory analysis. Pre- and

post-treatment tumor biopsies were obtained where possible, and used to assess the pharmacodynamic markers pAKT, pS6, and p4EBP1, and the cell proliferation marker Ki-67. Changes in pS6 (baseline to Day 1 of Cycle 2) were also assessed in skin biopsies, which were used as a surrogate tissue for assessing PI3K inhibition.

Blood samples (8 mL) for glucose metabolism markers (fasting plasma glucose, insulin and C-peptide) were collected at baseline, and predose and 0.5 h, 1 h, 2 h, 4 h and 24 h post-dose on Days 1, 8, and 28 of Cycle 1. Regular blood samples were also used to assess circulating markers of angiogenesis (BFGF, PLGF, SVEGFR1, SVEGFR2, and VEGF; 4 mL samples taken pre-dose at baseline, weekly during Cycle 1, and on Day 1 of every odd cycle thereafter) and cell death (M30 and M65; 3.5 mL samples taken at baseline, pre-dose at Day 1 Cycle 1, then 6 h post-dose at Day 8 and Day 28 of Cycle 1, and Day 1 of Cycle 3).

Whole-body <sup>18</sup>F-FDG-PET scans were performed at base-line and on Day 28 of Cycles 1 and 2 to assess tumor metabolic response to treatment. A significant biological response was defined as a change of ≥25 % in standardized uptake value (SUV) as described in EORTC guidelines [19].

## Results

### Patient characteristics

In total, 83 patients were enrolled in this Phase I trial between November 24, 2008 and August 9, 2012. Forty patients were previously reported as receiving daily buparlisib at six dose-levels, as part of the dose-escalation phase (12.5 mg, n=1; 25 mg, n=2; 50 mg, n=5; 80 mg, n=11; 100 mg, n=17, 150 mg, n=4) [15]. In the dose-expansion phase, 43 patients received buparlisib at 100 mg/day, including 5 patients who were enrolled within a separate terminal elimination half-life cohort (TEC), which was designed to further investigate the pharmacokinetic profile of buparlisib (Table 1).

Predominant primary tumor types were colorectal cancer (n=31) and breast cancer (n=21), among others. The study population had received multiple prior therapies: the median number of prior antineoplastic regimens was 4 (range 1–12). PIK3CA mutation and PTEN null/low expression assessments were available for 69 and 76 patients, respectively (Table 2). PTEN loss and PIK3CA mutation were observed in tumor samples from 21 to 15 patients, respectively, and 35 patients had a tumor sample with at least one of these alterations. KRAS mutation was assessed in 64 tumor samples, with 18 samples demonstrating this alteration.

Tumors from patients with colorectal cancer had high rates of molecular alterations in PIK3CA, PTEN, and KRAS. Of the 31 patients with colorectal cancer, 27 were evaluable for PIK3CA mutation and/or PTEN loss, and 25 were evaluable

Table 1 Baseline patient characteristics

Characteristic	12.5 mg N=1	25 mg N=2	50 mg N=5	80 mg N=11	100 mg N=55	150 mg N=4	TEC 100 mg N=5	All patients N=83
<b>Age, years</b>								
Median (range)	63 (63–63)	57.5 (49–66)	52 (49–60)	55 (40–72)	56 (37–78)	55 (37–76)	52 (30–73)	55 (30–78)
<b>Sex</b>								
Male (n, %)	1 (100)	1 (50.0)	2 (40.0)	2 (18.2)	22 (40.0)	2 (50.0)	3 (60.0)	33 (39.8)
<b>WHO performance status</b>								
0	1 (100)	2 (100)	3 (60.0)	6 (54.5)	20 (36.4)	4 (100)	0	36 (43.4)
1	0	0	2 (40.0)	5 (45.5)	34 (61.8)	0	5 (100)	46 (55.4)
2	0	0	0	0	1 (1.8)	0	0	1 (1.2)
Patients with prior antineoplastic regimens (n, %)	1 (100.0)	2 (100.0)	5 (100.0)	11 (100.0)	53 (96.4)	4 (100.0)	4 (80.0)	80 (96.4)
<b>No. of prior antineoplastic regimens</b>								
Median (range)	5 (5–5)	5 (5–5)	3 (2–5)	3 (1–12)	4 (1–10)	3 (1–12)	4 (2–4)	4 (1–12)
<b>Primary sites of tumor (n, %)</b>								
Colon	1 (100)	1 (50.0)	3 (60.0)	3 (27.3)	14	14 (25.5)	1 (25.0)	0
Breast	0	0	0	1 (9.1)	19 (34.5)	1 (25.0)	0	21 (25.3)
Rectum	0	0	0	0	6 (10.9)	0	2 (40.0)	8 (9.6)
Lung	0	0	0	1 (9.1)	3 (5.5)	0	0	4 (4.8)
Ovary	0	0	0	1 (9.1)	2 (3.6)	0	0	3 (3.6)
Head and neck	0	0	0	0	1 (1.8)	0	1 (20.0)	2 (2.4)
Pancreas	0	0	0	0	2 (3.6)	0	0	2 (2.4)
Prostate	0	0	1 (20.0)	0	1 (1.8)	0	0	2 (2.4)
Liver	0	0	0	0	1 (1.8)	0	0	1 (1.2)
Oral cavity	0	0	0	0	0	0	1 (20.0)	1 (1.2)
Stomach	0	0	0	0	0	0	1 (20.0)	1 (1.2)
Small intestine	0	0	0	0	1 (1.8)	0	0	1 (1.2)
Kidneys	0	0	0	0	0	1 (25.0)	0	1 (1.2)
Thyroid	0	0	0	1 (9.1)	0	0	0	1 (1.2)
Gall bladder	0	0	0	1 (9.1)	0	0	0	1 (1.2)
Other	0	1 (50.0)	1 (20.0)	3 (27.3)	5 (9.1)	1 (25.0)	0	11 (13.3)

TEC terminal elimination half-life assessment cohort, WHO world health organization

for KRAS mutation: 16 colorectal cancer samples demonstrated PIK3CA mutation or low/null PTEN expression; 12 samples had KRAS mutation, and 8 samples demonstrated alterations in both pathways (KRAS mutation in addition to PIK3CA mutation or low/null PTEN expression).

Of the 21 patients with breast cancer, 20 patients were evaluable for PIK3CA mutation, and 21 for null/low PTEN expression. Tumors from these patients showed high rates of PIK3CA mutation (5 samples) and PTEN loss (6 samples), with 1 sample (estrogen receptor [ER]-positive, pro-gesterone receptor [PgR]-positive, HER2-negative breast cancer) showing both alterations. KRAS mutation was evaluable in 18 patients and identified in two samples, with 1 sample also showing low/null PTEN expression. Among the 21 patients with breast cancer, 17 patients had tumors that were positive for ER, 12 had PgR-positive tumors, 4

demonstrated HER2-positive expression, and 3 had “triple-negative” breast cancer, with no expression of ER, PgR, or HER2.

#### Patient disposition

As of November 12, 2012, all 83 patients enrolled in the dose-escalation and -expansion phases had discontinued study treatment: 54 discontinued due to disease progression, 4 due to withdrawal of consent, 4 due to death, 1 due to protocol deviation, and 20 patients reported adverse event(s) as being the primary reason for study discontinuation.

The most frequent adverse events leading to study drug discontinuation were rash (3 patients), followed by asthenia, hyperbilirubinemia, hyperglycemia, transaminases increased and mood altered (2 patients each). Discontinuations due to

Table 2 Summary of tumor molecular status in patient samples at baseline

Tumor molecular status	All patients	
	N=83	
	n	%
<b>PIK3CA mutational status (n=69)</b>		
PIK3CA mutation	15	18.1
PIK3CA wild-type	54	65.1
<b>PTEN expression status (n=76)</b>		
PTEN null or low <sup>a</sup>	21	25.3
PTEN not low	55	66.3
<b>PIK3CA mutation OR PTEN expression status</b>		
PIK3CA mutation and/or PTEN null/low <sup>a</sup>	35	42.2
Neither PIK3CA mutation nor PTEN null/low <sup>a</sup>	37	44.6
Patients with at least one unknown status for PIK3CA mutation or PTEN loss <sup>b</sup>	11	13.3
<b>KRAS mutational status (n=64)</b>		
KRAS mutation	18	21.7
KRAS wild-type	46	55.4

9. Low PTEN expression defined as immunohistochemistry H-Score <50

10. Includes patients that have demonstrated a lack of one of the following: PIK3CA mutation or PTEN null/low

death occurred because of disease progression (2 patients), renal failure secondary to cancer, and sudden death (1 patient each, respectively). The patient who died of sudden death was a male patient with cholangiocarcinoma who had experienced disease progression (reported as “clinical worsening”) 3 days previously, which required hospitalization and was not suspected to be related to the study drug. No deaths were attributed to study treatment. Median exposure to buparlisib was 7.3 weeks (range: 0.1–160.3) in the full analysis set, and 7.6 weeks (range: 1.0–160.3) in the 55 patients treated at MTD.

#### Safety and tolerability

All 83 patients received at least one dose of buparlisib and at least one post-baseline safety assessment, and were included in the evaluation of safety and tolerability. Overall, the most common Grade 3/4 adverse events, regardless of causality, were asthenia (12.0 %) and performance status decrease (9.6 %). The most common Grade 3/4 adverse events observed at any point during treatment and suspected to be related to buparlisib were generally typical of those experienced with PI3K inhibitors, namely hyperglycemia, rash, and transaminase increases (Table 3). Onset of Grade 3/4 hyperglycemia typically occurred early on in treatment, with 4 out of 7 patients experiencing an event within the first cycle. In contrast, liver toxicities generally occurred further into treatment, with 55 out of 81 events occurring after the first treatment cycle.

The following Grade 3 psychiatric disorders suspected to be related to study treatment were described: mood alteration in 2 patients (1 patient treated at 100 mg/day, and another treated at

80 mg/day who also experienced Grade 3 restlessness), and anxiety and depression, which were experienced by 1 patient each (both treated with buparlisib 100 mg/day). Only one of the patients who experienced Grade 3 psychiatric events suspected to be related to study treatment (Grade 3 anxiety) had started on the study after the January 2010 amendment to exclude patients with active (or a documented history of) major psychiatric disorders. No Grade 4 psychiatric events suspected to be related to study treatment were reported.

Thirty-six patients (43.4 %) experienced a serious adverse event (SAE), regardless of causality. Among these patients, 11 experienced an SAE that was suspected to be related to study treatment. The most frequent SAEs suspected to be treatment related were hyperglycemia, reported in 3 patients, followed by diarrhea and fatigue, each reported in 2 patients. Fifteen patients experienced an adverse event suspected to be due to study drug treatment, which led to treatment discontinuation. Thirteen on-study deaths occurred, 4 during treatment (described above), and 9 following treatment discontinuation, but within 28 days of last administration of study drug, which were due to disease progression.

#### Clinical activity

Seventy-two patients were evaluable for response by investigator review of target lesion radiologic assessments. As described previously [15], one patient with triple-negative breast cancer and KRAS mutation (exon 1, G12V [detected in an archival specimen]) from the 100 mg/day cohort of the dose-escalation phase had a confirmed partial response (PR), and an overall exposure of 160 weeks. An additional three patients experienced unconfirmed PRs (demonstrating a best percent-age reduction in tumor size of at least 30 % in sum of longest diameters, without subsequent confirmation of the response). One patient with parotid gland ductal carcinoma (PIK3CA and KRAS wild-type, PTEN expression normal) and lung and bone metastases that had previously been treated with multiple antineoplastic therapies, including cyclophosphamide and doxorubicin, demonstrated a 33 % reduction in the size of a neck nodule following 8 weeks of treatment with 100 mg/day buparlisib. Another patient, who had liver, lung and mediastinal lymph node metastases arising from epithelioid hemangioendothelioma of the finger (PIK3CA and KRAS wild-type, PTEN expression normal) demonstrated a 34 % reduction in a liver metastasis after 80 weeks of treatment (initially with 150 mg/day buparlisib, but primarily at 100 mg/day), demonstrating an overall stable disease for a total duration of

Table 3 Summary of adverse events (AEs) suspected to be related to study drug (&gt;15 % of patients)

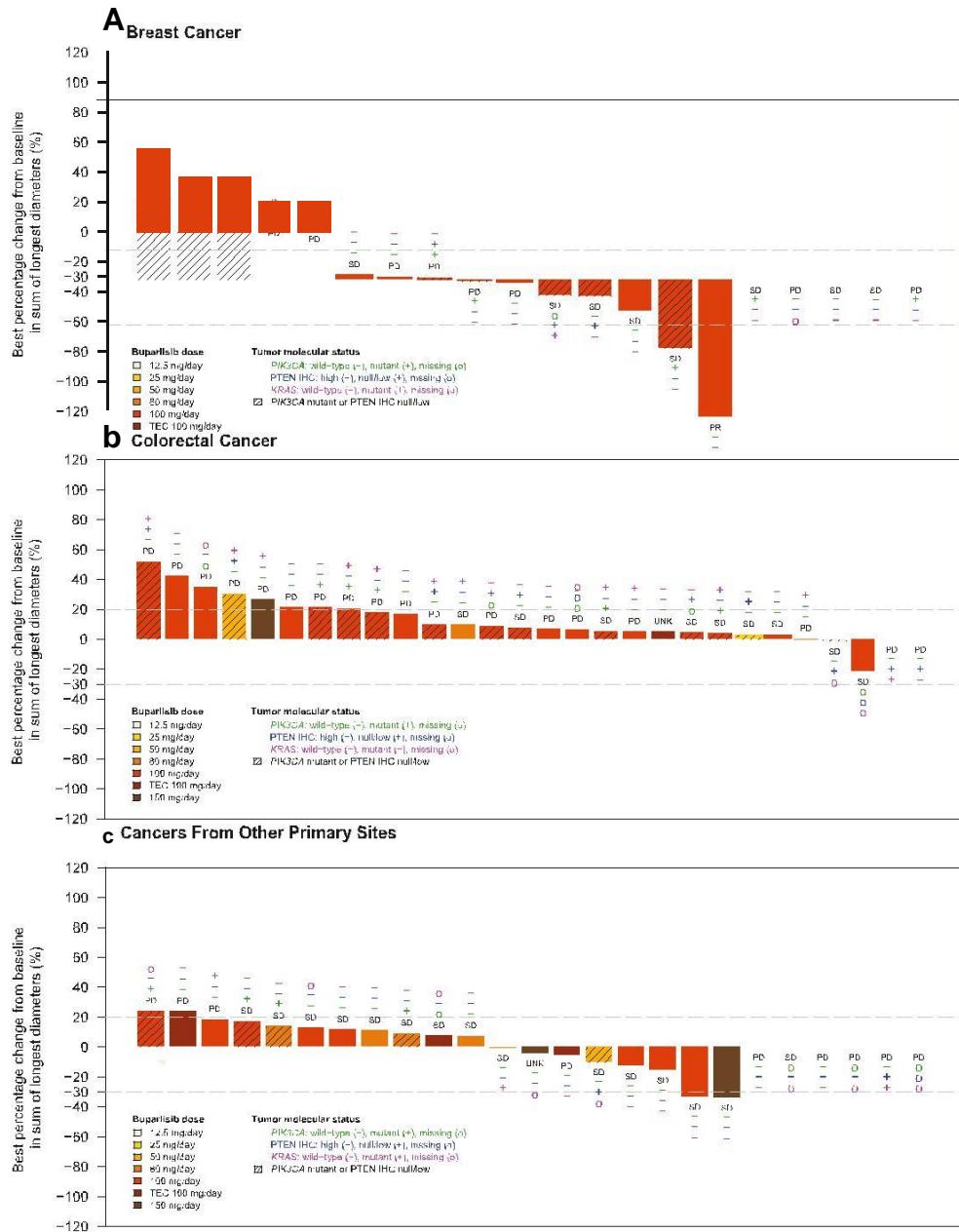
Preferred term	12.5 mg		25 mg		50 mg		80 mg		100 mg		150 mg		TEC 100 mg		All patients	
	N=1		N=2		N=5		N=11		N=55		N=4		N=5		N=83	
	All grade n (%)	Grade 3/4 n (%)														
Total patients experiencing AEs suspected to be related to buparlisib	1 (100)	0	2 (100)	1 (50.0)	4 (80.0)	0	9 (81.8)	3 (27.3)	54 (98.2)	25 (45.5)	4 (100)	3 (75.0)	3 (60.0)	1 (20.0)	77 (92.8)	33 (39.8)
AEs suspected to be related to buparlisib occurring in 15 % of patients																
Decreased Appetite	0	0	2 (100)	0	0	0	4 (36.4)	0	18 (32.7)	0	1 (25.0)	0	2 (40.0)	0	27 (32.5)	0
Diarrhea	0	0	0	0	0	0	2 (18.2)	0	23 (41.8)	3 (5.5)	1 (25.0)	0	1 (20.0)	0	27 (32.5)	3 (3.6)
Nausea	0	0	1 (50.0)	0	1 (20.0)	0	2 (18.2)	0	18 (32.7)	0	3 (75.0)	0	2 (40.0)	0	27 (32.5)	0
Hyperglycemia	1 (100)	0	0	0	1 (20.0)	0	3 (27.3)	1 (9.1)	18 (32.7)	4 (7.3)	3 (75.0)	2 (50.0)	0	0	26 (31.3)	7 (8.4)
Rash	0	0	1 (50.0)	0	0	0	3 (27.3)	1 (9.1)	18 (32.7)	4 (7.3)	1 (25.0)	1 (25.0)	1 (20.0)	0	24 (28.9)	6 (7.2)
Fatigue	1 (100)	0	0	0	1 (20.0)	0	4 (36.4)	0	13 (23.6)	2 (3.6)	0	0	1 (20.0)	0	20 (24.1)	2 (2.4)
Stomatitis	0	0	0	0	0	0	4 (36.4)	0	13 (23.6)	0	0	0	2 (40.0)	0	19 (22.9)	0
Asthenia	0	0	0	0	1 (20.0)	0	1 (9.1)	0	13 (23.6)	3 (5.5)	0	0	0	0	15 (18.1)	3 (3.6)
Pruritus	0	0	0	0	0	0	1 (9.1)	0	11 (20.0)	2 (3.6)	1 (25.0)	0	1 (20.0)	0	14 (16.9)	2 (2.4)
Anxiety	0	0	0	0	0	0	1 (9.1)	0	11 (20.0)	1 (1.8)	1 (25.0)	0	0	0	13 (15.7)	1 (1.2)
Depression	0	0	0	0	0	0	0	0	12 (21.8)	1 (1.8)	0	0	1 (20.0)	0	13 (15.7)	1 (1.2)

All AEs, with the exception of hyperglycemia, were defined according to NCI-CTCAE v3.0 criteria

147 weeks. An unconfirmed PR was also noted in a patient with ER-positive, HER2-negative breast cancer (PIK3CA mutation, KRAS wild-type, PTEN expression normal), who was multiply pretreated (four prior lines of chemotherapy), and yet demonstrated 53 % and 14 % size reductions in multiple lung and liver lesions, respectively, and a 28 % size reduction in a brain metastasis lesion, with 100 mg/ day buparlisib.

Thirty three patients (39.8 %) had stable disease for at least 6 weeks (Fig. 1), corresponding with a disease control rate (DCR; rate of complete response rate + PR rate+stable disease [SD] rate, reported as best response) of 41.0 % (95 % CI: 31.8–50.6). PI3K pathway activation (as defined by

Fig. 1 Radiologic response to buparlisib with corresponding tumor molecular status for patients with breast cancer (a), colorectal cancer (b), or cancers from other primary sites (c). IHC immunohistochemistry, PD progressive disease, PR partial response, SD stable disease, TEC terminal elimination half-life assessment cohort, UNK unknown response. Patients with a missing best percentage change from baseline and unknown response are not shown



alterations in PIK3CA or PTEN) was not correlated with improved disease control. Kaplan-Meier estimates were generated using a data cut-off date of September 22, 2011, at which point three of the patients were still continuing on study treatment. At the time of data cut-off, the 6-month progression-free survival (PFS) rate among patients treated at the MTD was 19 % (95 % CI: 1, 37) among patients with PI3K pathway activation (n=26), compared with 29 % (95 % CI: 7, 50) among patients without PI3K pathway activation (n= 23). Among patients treated at the MTD and above (n=59), the median PFS, regardless of PI3K pathway activation, was 57 days (95 % CI: 50, 106, n=59). Overall, tumor molecular status was not predictive of response among

patients with breast cancer, colon cancer, or collectively among patients with any other primary site of cancer; however, the small sample size and heterogeneous nature of the study population as a whole makes it difficult to confirm these observations.

### $^{18}\text{F}$ -FDG-PET assessments

Changes in  $^{18}\text{F}$ -FDG uptake (SUVmax) from baseline were evaluable for 52 patients at Day 28 of Cycle 1, and 30 patients at Day 28 of Cycle 2 (Fig. 2). At both time points, the majority of patients at Day 28 of Cycles 1 and 2 (45 and 21 patients, respectively) demonstrated decreases in  $^{18}\text{F}$ -FDG uptake demonstrating on-target inhibition of the PI3K pathway. The median change in SUVmax was  $-23.24\%$  (range:  $-63.8$  to  $14.6$ ) among patients treated at the MTD, and  $-19.93\%$  (range:  $-63.8$  to  $29.1$ ) overall at Day 28 of Cycle 1. Baseline changes in SUVmax at Day 28 of Cycle 2 were generally lower, with a median changes of  $-13.40\%$  (range:  $-60.9$  to  $40.0$ ) observed in patients treated at MTD, and  $-10.75\%$  (range:  $-60.9$  to  $40.0$ ) overall.

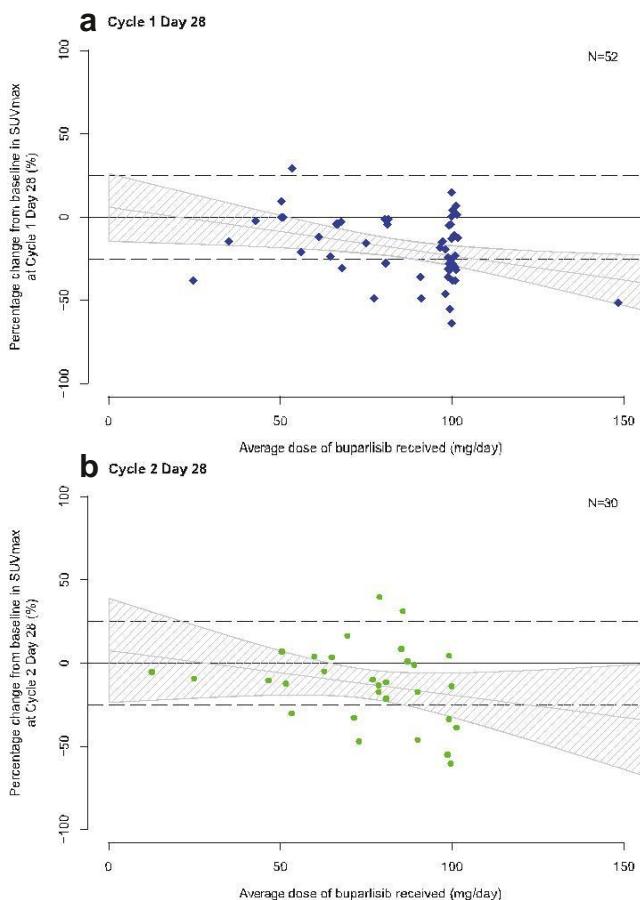


Fig. 2 Percentage change from baseline in  $^{18}\text{F}$ -FDG uptake (SUVmax) vs average dose of buparlisib received at Cycle 1 Day 28 (a) and Cycle 2 Day 28 (b). Shaded areas represent 95 % confidence intervals based on linear regression analysis (dotted lines)

Linear regression analysis suggested a relationship between the extent of  $^{18}\text{F}$ -FDG uptake and the average dose of buparlisib received (accounting for dose interruptions and dose reductions) up until the point of measurement at Day 28 of Cycles 1 and 2 (Fig. 2). Despite observations of a dose-dependent effect, a clear relationship between the presence of a partial metabolic response at 1 month or the percentage change in SUVmax and PFS, best response as per RECIST, or best percent change in CT scan (sum of longest diameters), was not apparent. Partial metabolic responses (defined as  $\geq 25\%$  decrease from baseline in SUVmax in the absence of new lesions) by central review were observed in 21 patients at Day 28 of Cycle 1, and in 9 patients at Day 28 of Cycle 2.

### Pharmacodynamic biomarker assessments demonstrating PI3K pathway inhibition

Mean plasma concentrations of glucose metabolism markers, such as C-peptide, increased as a function of the first dose of buparlisib administered (Fig. 3). Median area under the plasma glucose curve ( $\text{AUC}_{0-4\text{h}}$ ) was relatively stable at all measurement time points (21.53, 23.14, 23.84 mmol/L $\cdot$ hr, observed at C1D1, C1D8, and C1D28, respectively), whereas median exposure to C-peptide and insulin demonstrated increases between C1D1 and C1D8, stabilizing thereafter (4.38, 7.45, and 7.46 nmol/L $\cdot$ hr, and 271.63, 740.41, 732.75 pmol/L $\cdot$ hr, for the three timepoints, respectively). Systemic exposure ( $\text{AUC}_{0-4\text{h}}$ ) for all three plasma glucose metabolism markers increased with increasing buparlisib exposure ( $\text{AUC}_{0-4\text{h}}$ ).

Fifty four patients were evaluable for changes in pathway phosphorylation in skin at baseline and at C2D1, with the majority demonstrating a decrease in pS6 H-Score (see figure in Online Resource 1). Inhibition of pS6 in skin increased moderately with the mean administered dose of buparlisib over the same time period (mean inhibition of  $-22$ ,  $-29$ , and  $-40\%$ , for  $12.5$ – $60$  mg,  $>60$ – $90$  mg, and  $>90$  mg mean dose ranges,

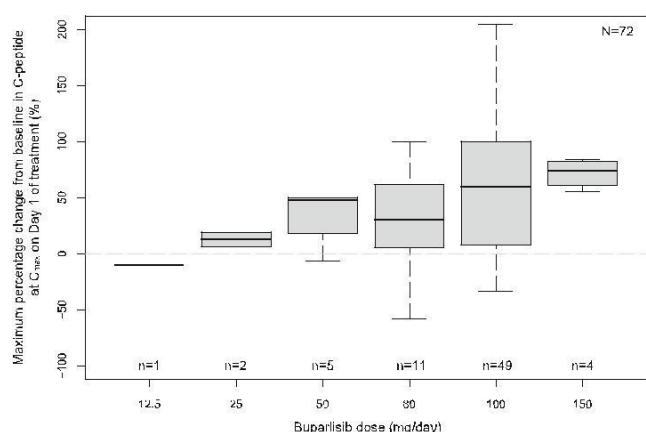


Fig. 3 Maximum percentage change in C-peptide as measured in blood at baseline and at  $C_{\text{max}}$  on Day 1 of treatment. Grey boxes represent interquartile range, black bars represent mean percentage change, whiskers represent minimum and maximum values

respectively) suggesting a relationship between treatment dose and the degree of PI3K pathway inhibition. A relationship between S6 phosphorylation in skin and clinical response was not identified; among the 23 patients who had skin biopsies showing >50 % decrease in pS6, 12 had SD or PR (including unconfirmed PR) as best overall response, whereas 11 had progressive disease. Twenty-two of 31 patients who had <50 % decrease in skin pS6, had SD or PR (including unconfirmed PR).

Pre- and post-treatment (C2D1) tumor biopsies were available for 10 patients, who were all treated at a dose of 100 mg/day (see table in Online Resource 2). A reduction in pS6 (range: -33 % to -64 %) was observed in tumor tissue from 6 patients, 5 of whom were also assessed for pAKT and p4EBP levels, with 3 patients demonstrating a decrease in either pAKT (range: -35 % to -70 %) or p4EBP1 (range: -10 to -35 %). One patient demonstrated a decrease of >30 % in all 3 markers. Collectively, these observations demonstrate that buparlisib is capable of inhibiting the PI3K pathway in tumor tissue; however, a clear association with clinical response was not evident in this small number of samples. Reductions in Ki-67 (range: -16 % to -87 %) were observed in 6 of the tumor samples analyzed, suggesting a possible impact on tumor cell proliferation. pS6 in skin generally showed good concordance with pS6 in tumor biopsies, with 7 out of 9 patients demonstrating <33 % difference between the percent-age change in H-score measured in the two tissues sampled.

Changes from baseline in the circulating markers of cell death, M30 and M65, were evaluable in 38 patients treated at the MTD at Cycle 1 Day 8, and in 30 and 29 patients at Cycle 1 Day 28, respectively; 10 patients were evaluable for both markers at Cycle 3 Day 1 (see table in Online Resource 3A). A slight trend was observed by which both markers tended to show greater increases from baseline as treatment progressed; however, intrapatient variability was high.

Changes from baseline in circulating markers of angiogenesis (BFGF, PLGF, SVEGFR1, SVEGFR2 and VEGF) were evaluable at Cycle 1 Day 8, Cycle 1 Day 15, and Cycle 1 Day 28, in 45, 31, and 34 patients treated at the MTD, respectively (see table in Online Resource 3B). By Cycle 1 Day 28, for each of the 5 biomarkers analyzed, at least half of the samples evaluable showed decreases from baseline measurements. However, as with the assessment of M30 and M65, intrapatient variability was high, and a statistical relationship between pre-and post-treatment levels of these angiogenesis markers was not confirmed.

## Discussion

This updated report provides further confirmation that buparlisib is well tolerated and has an acceptable safety profile at the estimated MTD of 100 mg/day. The most common

adverse events associated with buparlisib were generally typical of those experienced with PI3K inhibitors, namely hyper-

glycemia, gastrointestinal complications, rash, and fatigue/asthenia. Hyperglycemia is a known class effect of inhibitors of the PI3K pathway, and arises through the perturbation of insulin signaling, glucose transport and glycogen synthesis [20–22]. In this study, hyperglycemia was among the most frequent adverse events suspected to be related to treatment (reported in 31.3 % of patients), and was primarily managed with oral antidiabetes drugs, with insulin and dose interruptions when necessary. Observations of hyperglycemia are indicative of PI3K pathway inhibition in patients treated with buparlisib, and demonstrate a potential role for metabolites such as C-peptide as pharmacodynamic biomarkers. The analysis of glucose metabolism biomarkers presented here further supports the observation that buparlisib inhibits the PI3K pathway and perturbs glucose metabolism; however, due to the heterogeneous patient population and the limited numbers of patients treated at dose levels other than the MTD, a clear relationship between these biomarkers and the dose of buparlisib administered or the degree of pathway inhibition, was not established. Further work is ongoing to establish the utility of biomarkers such as C-peptide, as indicators of PI3K inhibitor activity.

Among the clinical trials with PI3K inhibitors that have been reported to date, buparlisib is the only agent found to be associated with psychiatric adverse events [23–26]. Buparlisib has been shown to cross the blood–brain barrier [27], and dysfunctional PI3K signaling in the CNS has been implicated with anxiety and altered serotonin levels [28–30]. In the present study, Grade 3 psychological events (which include Grade 3 anxiety) suspected to be related to the study drug were experienced by four patients (3 at 100 mg/day, and 1 at 80 mg/day). A range of interventions were used to limit or manage psychiatric events in this trial. This included excluding patients with major risk factors for psychological disorders, careful patient monitoring and self-assessment questionnaires, and the use of concomitant medications (e.g. benzodiazepine and selective serotonin reuptake inhibitors), with dose interruptions where necessary. The amendment to exclude patients with active or historical record of psychiatric disorders was implemented just over a year into the trial.

The ability of buparlisib to cross the blood–brain barrier presents the possibility of treating patients with brain metastases, one of the main causes of cancer-related death [27]. In this study, one patient with ER-positive/HER2-negative/PIK3CA-mutant breast cancer demonstrated a 28 % reduction in the size of a brain metastasis lesion. Buparlisib has also demonstrated anti-tumor activity in mice with xenografts of human HER2-positive brain metastases [27], and ongoing studies are investigating the pharmacodynamic effects and efficacy of buparlisib in patients with glioblastoma or brain metastases from HER2-positive breast cancer (NCT01473901 and NCT01132664) [31, 32].

The observations of reduced pAKT, p4EBP1, and pS6 expression in post-treatment tumor biopsies, demonstrate that buparlisib is capable of inhibiting the PI3K pathway in tumor tissue. Another pharmacodynamic biomarker, <sup>18</sup>F-FDG-PET, has previously demonstrated an ability to predict response to therapy in early-phase trials with other targeted agents in advanced cancers, such as advanced melanoma, metastatic breast cancer, and non-small cell lung cancer [33–35]. In vitro experiments with 3D tumor spheroids, and in vivo experiments in a mouse model of BRCA1-related breast cancer, suggest that <sup>18</sup>F-FDG-PET may have use as an early and predictive pharmacodynamic marker for response to PI3K inhibitor-based treatments [36, 37]. In the present study, buparlisib administration instigated partial metabolic responses in 21 of 54 of evaluable patients after 28 days of treatment; however, no association with PFS, best response as per RECIST, or best percent change in CT scan was identified.

The effect of PI3K inhibitors on glucose metabolism, the small number of patients and responses observed, and the highly heterogeneous range of patients and tumor types treated in this study may explain why <sup>18</sup>F-FDG-PET was unable to predict response to therapy in this study, despite demonstrating capability as a pharmacodynamic biomarker. Future studies in more homogeneous populations should continue to investigate whether the pharmacodynamic information provided by <sup>18</sup>F-FDG-PET can be interpreted as a predictive marker of response to PI3K inhibitor therapy. Differences in therapeutic history, tumor types, and molecular alterations may also explain the high intrapatient variability observed in biomarkers of cell death and angiogenesis following buparlisib treatment.

As with many early-phase studies with other PI3K inhibitors in advanced solid tumors, no association was identified between the extent of tumor shrinkage or best overall response as per investigator assessment, and the tumor molecular alterations analyzed. This lack of association could be due to several factors, such as the small sample size, the time lag between the archival sample used for pathway analysis and the time of patient entry in the trial, and the heterogeneous patient population, who possessed a wide variety of different tumor types. It is also possible that many of the patients who were defined as lacking PI3K pathway activation according to the criteria used in this study (PIK3CA mutation and/or reduced or null PTEN expression) may actually exhibit pathway activation driven by molecular alterations that were not evaluated in the present analysis. Such alterations might include the amplification or activation of upstream RTKs, the loss of tumor suppressors, such as INPP4B, or the activation of other pathway oncogenes, such as PDK1 or AKT. In addition, recent studies have shown that PIK3CA mutations often co-exist with other molecular alterations, which may impact the response to PI3K pathway inhibition [38, 39]. Therefore, it is possible that the single-agent design of this trial may not be

optimal to identify a relationship between tumor molecular status and response. For a true assessment of the predictive value of PIK3CA mutation, a prospective, sufficiently powered trial using an inhibitor of the PI3K pathway in combination with another pathway inhibitor (e.g. endocrine therapy, RTKs, or MEK inhibitor) may be required.

Pan-class I PI3K inhibitors, such as buparlisib, are part of a growing spectrum of agents that includes dual inhibitors of PI3K and mTOR, and isoform-specific PI3K inhibitors. One of the challenges associated with the clinical development of PI3K inhibitors is to identify the optimal tumor types and treatment contexts for each class of agent. BYL719 is a specific inhibitor of the p110 $\alpha$  isoform of the PI3K catalytic subunit, which has recently been investigated in a first-in-man Phase I study in patients with advanced solid tumors [40]. Preclinical investigations with BYL719 have demonstrated a pattern of in vitro sensitivity similar to that of buparlisib, with increased activity observed in cell lines with PIK3CA alterations and ERBB2 amplification, and decreased sensitivity in those with PTEN and BRAF mutations [10, 41]. Patients enrolling in the Phase I study of BYL719 were therefore required to have tumors with alterations in PIK3CA. Preliminary efficacy was observed, with three PRs reported in patients with ER-positive breast cancer, cervical cancer, and colon cancer, respectively [40]. It is currently unclear whether isoform-specific inhibition will only show activity in tumors with certain molecular aberrations (e.g. PIK3CA mutation) and whether treatment with isoform-specific inhibitors should be restricted to patients with those molecular aberrations only. In tumors driven by PTEN alterations, PI3K signaling has been shown to be dependent on p110 $\beta$ , as opposed to p110 $\alpha$  [42, 43]. Pan-Class I inhibitors, such as buparlisib, might therefore show greater benefit than p110 $\alpha$  inhibitors in tumors associated with a broader range of molecular alterations, including those with high frequencies of PTEN alteration. A range of studies involving buparlisib are currently underway in patients with alterations in the PI3K pathway, including PIK3CA mutation, loss of PTEN expression, and EGFR over-expression, amongst others. Data from these studies will help determine the anti-tumor activity of buparlisib in patients with heterogeneous tumor mutations and guide future treatment strategies with this agent.

Based on the results of this trial and other clinical studies with pan-Class I PI3K inhibitors reported to date, it remains difficult to determine whether there is a strong relationship between PI3K pathway alterations and sensitivity to pan-Class I PI3K inhibitors [44]. Future trials with buparlisib will continue to investigate the relationship between pathway alterations and clinical response in trials with more homogenous patient populations. An ongoing Phase II trial (NCT01297491) is investigating the efficacy of buparlisib in patients with metastatic non-small cell lung cancer with PI3K pathway activation. Two large Phase III studies

(NCT01610284 and NCT01633060) are investigating the combination of buparlisib plus fulvestrant in hormone receptor-positive/HER2-negative breast cancer, both in patients with PI3K pathway activation (PIK3CA mutation or loss of PTEN expression), and in the study population as a whole. PI3K inhibitors could be effective combination partners for a variety of anticancer agents [45], and so buparlisib is also being investigated in combination with several antineoplastic agents (i.e. endocrine agents, other targeted therapies, and cytotoxic chemotherapies) and with radiation therapy.

**Acknowledgments** The following members of the Vall d'Hebron study team are thanked for their contributions to the study: Dr Begoña Graña, Dr Cristina Cruz, Dr Javier Cortes, Dr Cristina Saura, Dr Rodrigo Dienstmann, and Adelaida Piera. From SCRI: Dr Howard Burris, Dr Jeffrey Infante, and Dr Suzanne Jones. Diane van der Biessen and Leni van Doorn of the Erasmus MC Cancer Institute are also thanked for their contributions. Karyn McKeever, Lindsay Carlsson, and Monika Wizemann of the Princess Margaret Cancer Centre are also thanked for their contributions. Douglas Robinson of the Novartis Institute for Biomedical Research is thanked for his statistical expertise. Lea Dutta, previously of Novartis Pharmaceuticals, is thanked for her contributions. Financial support for medical editorial assistance was provided by Novartis Pharmaceuticals. We thank Ben Holtom for medical editorial assistance with this manuscript.

**Conflict of interest** Jordi Rodon: Advisory board participation: Novartis (remunerated), Irene Braña, No conflicts of interest, Lillian L Siu: Research funding: Novartis, Maja J De Jonge: No conflicts of interest, Natasha Homji: Employed by Novartis Pharmaceuticals, David Mills: Employee of Novartis, Emmanuelle Di Tomaso: Employee of Novartis, Celine Sarr: Employee of Novartis, Lucia Trandafir: Employee of Novartis, Cristian Massacesi: Employee of Novartis, Ferry Eskens: No conflicts of interest, Johanna C Bendell: No conflicts of interest

## References

- Liu P, Cheng H, Roberts TM, Zhao JJ (2009) Targeting the phosphoinositide 3-kinase pathway in cancer. *Nat Rev Drug Discov* 8(8):627–644
- Huang WC, Hung MC (2009) Induction of Akt activity by chemo-therapy confers acquired resistance. *J Formos Med Assoc* 108(3): 180–194
- Nahta R, O'Regan RM (2010) Evolving strategies for overcoming resistance to HER2-directed therapy: targeting the PI3K/Akt/mTOR pathway. *Clin Breast Cancer* 10(Suppl 3):S72–S78
- Miller TW, Balko JM, Arteaga CL (2011) Phosphatidylinositol 3-kinase and antiestrogen resistance in breast cancer. *J Clin Oncol* 29(33):4452–4461
- Kolasa IK, Rembiszewska A, Felisiak A, Ziolkowska-Seta I, Murawska M, Moes J et al (2009) PIK3CA amplification associates with resistance to chemotherapy in ovarian cancer patients. *Cancer Biol Ther* 8(1):21–26
- Courtney KD, Corcoran RB, Engelman JA (2010) The PI3K pathway as drug target in human cancer. *J Clin Oncol* 28(6):1075–1083
- The Cancer Genome Atlas Research Network (2008) Comprehensive genomic characterization defines human glioblastoma genes and core pathways. *Nature* 455(7216):1061–1068
- Cancer Genome Atlas Research Network, Hammerman PS, Hayes DN, Wilkerson MD, Schultz N, Bose R et al (2012) Comprehensive genomic characterization of squamous cell lung cancers. *Nature* 489(7417):519–525
- Cancer Genome Atlas Network (2012) Comprehensive molecular portraits of human breast tumours. *Nature* 490(7418):61–70
- Maira SM, Pecchi S, Huang A, Burger M, Knapp M, Sterker D et al (2012) Identification and characterization of NVP-BKM120, an orally available pan-class I PI3-kinase inhibitor. *Mol Cancer Ther* 11(2): 317–328
- Koul D, Shen R, LaFortune TA, Tiao N, Kim YW, Liu JL et al (2010) NVP-BKM120: a selective pan-PI3 kinase inhibitor induces G2/M arrest in glioma cell lines via FOXO3a and GADD45a loop. American Association for Cancer Research Congress. [abstract 350]
- Schnell CR, Arnal S, Becquet M, Allegri P, Voliva C, Cozens R et al (2010) NVP-BKM120, a pan class I PI3K inhibitor impairs microvascular permeability and tumor growth as detected by DCE-MRI and IFP measurements via radio-telemetry: comparison with NVP-BEZ235. American Association for Cancer Research Congress. [abstract 4472]
- Maira M, Menezes D, Pecchi S, Shoemaker K, Burger M, Schnell C et al (2010) NVP-BKM120, a novel inhibitor of phosphoinositide 3-kinase in phase I/II clinical trials, shows significant antitumor activity in xenograft and primary tumor models. AACR Meeting Abstracts. 4497 (abstract)
- Voliva CF, Pecchi S, Burger M, Nagel T, Schnell C, Fritsch C et al (2010) Biological characterization of NVP-BKM120, a novel inhibitor of phosphoinositide 3-kinase in phase I/II clinical trials. AACR Meeting Abstracts 4498 (abstract)
- Bendell JC, Rodon J, Burris HA, de Jonge M, Verweij J, Birle D et al (2012) Phase I, dose-escalation study of BKM120, an oral pan-class I PI3K inhibitor, in patients with advanced solid tumors. *J Clin Oncol* 30(3):282–290
- Spitzer RL, Kroenke K, Williams JB, Lowe B (2006) A brief measure for assessing generalized anxiety disorder: the GAD-7. *Arch Intern Med* 166(10):1092–1097
- Kroenke K, Spitzer RL, Williams JB (2001) The PHQ-9: validity of a brief depression severity measure. *J Gen Intern Med* 16(9):606–613
- Babb J, Rogatko A, Zacks S (1998) Cancer Phase I clinical trials: efficient dose escalation with overdose control. *Stat Med* 17(10): 1103–1120
- Young H, Baum R, Cremerius U, Herholz K, Hoekstra O, Lammertsma AA et al (1999) Measurement of clinical and subclinical tumour response using [18F]-fluorodeoxyglucose and positron emission tomography: review and 1999 EORTC recommendations. European organization for research and treatment of cancer (EORTC) PET study group. *Eur J Cancer* 35(13):1773–1782
- Foukas LC, Claret M, Pearce W, Okkenhaug K, Meek S, Peskett E et al (2006) Critical role for the p110alpha phosphoinositide-3-OH kinase in growth and metabolic regulation. *Nature* 441(7091):366–370
- Taniguchi CM, Emanuelli B, Kahn CR (2006) Critical nodes in signalling pathways: insights into insulin action. *Nat Rev Mol Cell Biol* 7(2):85–96
- Knight ZA, Gonzalez B, Feldman ME, Zunder ER, Goldenberg DD, Williams O et al (2006) A pharmacological map of the PI3-K family defines a role for p110alpha in insulin signaling. *Cell* 125(4):733–747
- Von Hoff DD, LoRusso P, Tibes R, Shapiro G, Weiss GJ, Ware JA et al (2010) A first-in-human phase I study to evaluate the pan-PI3K inhibitor GDC-0941 administered QD or BID in patients with advanced solid tumors. ASCO Meeting Abstracts 28(15\_suppl):2541
- Peyton JD, Rodon Ahnert J, Burris H, Britten C, Chen LC, Tabernero J et al (2011) A dose-escalation study with the novel formulation of the oral pan-class I PI3K inhibitor BEZ235, solid dispersion system (SDS) sachet, in patients with advanced solid tumors. ASCO Meeting Abstracts 29(15\_suppl):3066

17. Wagner AJ, Bendell JC, Dolly S, Morgan JA, Ware JA, Fredrickson J et al (2011) A first-in-human phase I study to evaluate GDC-0980, an oral PI3K/mTOR inhibitor, administered QD in patients with advanced solid tumors. *J Clin Oncol* 29(suppl):abstract 3020 (poster presentation)
18. Munster PN, van der Noll R, Voest EE, Dees EC, Tan AR, Specht JM et al (2011) Phase I first-in-human study of the PI3 kinase inhibitor GSK2126458 (GSK458) in patients with advanced solid tumors (study P3K112826). ASCO Meeting Abstracts 29(15\_suppl):3018
19. Nanni P, Nicoletti G, Palladini A, Croci S, Murgo A, Ianzano ML et al (2012) Multiorgan metastasis of human HER-2(+) breast cancer in Rag2(−/−);Il2rg(−/−) mice and treatment with PI3K inhibitor. *PLoS One* 7(6):e39626
20. Tohda C, Nakanishi R, Kadowaki M (2009) Hyperactivity, memory deficit and anxiety-related behaviors in mice lacking the p85alpha subunit of phosphoinositide-3 kinase. *Brain Dev* 31(1):69–74
21. Ackermann TF, Hortnagl H, Wolfer DP, Colacicco G, Sohr R, Lang F et al (2008) Phosphatidylinositol dependent kinase deficiency increases anxiety and decreases GABA and serotonin abundance in the amygdala. *Cell Physiol Biochem* 22(5–6):735–744
22. Kalkman HO (2006) The role of the phosphatidylinositol 3-kinase-protein kinase B pathway in schizophrenia. *Pharmacol Ther* 110(1):117–134
23. A phase I dose escalation study of BKM120 with radiation therapy and temozolomide in patients with newly diagnosed glioblastoma - ClinicalTrials.gov (NCT01473901) [homepage on the Internet]. [cited 4/3/2013]
24. Safety and efficacy of BKM120 in combination with trastuzumab in patients with relapsing HER2 overexpressing breast cancer who have previously failed trastuzumab - ClinicalTrials.gov (NCT01132664) [homepage on the Internet]. [cited 4/3/2013]
25. Mortazavi-Jehanno N, Giraudet AL, Champion L, Lerebours F, Le Stanc E, Edeline V et al (2012) Assessment of response to endocrine therapy using FDG PET/CT in metastatic breast cancer: a pilot study. *Eur J Nucl Med Mol Imaging* 39(3):450–460
26. Mileshkin L, Hicks RJ, Hughes BG, Mitchell PL, Charu V, Gitlitz BJ et al (2011) Changes in 18F-fluorodeoxyglucose and 18F-fluorodeoxythymidine positron emission tomography imaging in patients with non-small cell lung cancer treated with erlotinib. *Clin Cancer Res* 17(10):3304–3315
27. McArthur GA, Puzanov I, Amaravadi R, Ribas A, Chapman P, Kim KB et al (2012) Marked, homogeneous, and early [18F]fluorodeoxyglucose-positron emission tomography responses to vemurafenib in BRAF-mutant advanced melanoma. *J Clin Oncol* 30(14):1628–1634
36. Juvekar A, Burga LN, Hu H, Lunsford EP, Ibrahim YH, Balmana J et al (2012) Combining a PI3K inhibitor with a PARP inhibitor provides an effective therapy for a mouse model of BRCA1-related breast cancer. *Cancer Discov* 2(11):1048–1063
37. Kelly CJ, Hussien K, Muschel RJ (2012) 3D tumour spheroids as a model to assess the suitability of [18F]FDG-PET as an early indicator of response to PI3K inhibition. *Nucl Med Biol* 39(7):986–992
38. Di Nicolantonio F, Arena S, Tabernero J, Grossi S, Molinari F, Macarulla T et al (2010) Deregulation of the PI3K and KRAS signalling pathways in human cancer cells determines their response to everolimus. *J Clin Invest* 120(8):2858–2866
39. Rexer BN, Chanthaphachith S, Dahlman KB, Arteaga CL (2014) Direct inhibition of PI3K in combination with dual HER2 inhibitors is required for optimal antitumor activity in HER2+ breast cancer cells. *Breast Cancer Res* 16(1):R9
40. Juric D, Rodon J, Gonzalez-Angulo AM, Burris HA, Bendell J, Berlin JD et al (2012) In: BYL719, a next generation PI3K alpha specific inhibitor: preliminary safety, PK, and efficacy results from the first-in-human study. <http://www.abstractsonline.com/Plan/ViewAbstract.aspx?sKey=d14acbf7-7602-45e9-a577-11db6325068&cKey=04a16b3b-4956-4cf9-833f-b223331f46b4&mKey={2D8C569E-B72C-4E7D-AB3B-070BEC7EB280}.p. Abstract nr CT-01>
41. Huang A, Fritsch C, Wilson C, Reddy A, Liu M, Lehar J et al (2012) Single agent activity of PI3KCA inhibitor BYL719 in a broad cancer cell line panel. In: Proceedings of the 103rd Annual Meeting of the American Association for Cancer Research; 2012 Mar 31-Apr 4; Chicago, Illinois. Philadelphia (PA): AACR Abstract nr 3749
42. Jia S, Liu Z, Zhang S, Liu P, Zhang L, Lee SH et al (2008) Essential roles of PI(3)K-p110beta in cell growth, metabolism and tumorigenesis. *Nature* 454(7205):776–779
43. Wee S, Wiederschain D, Maira SM, Loo A, Miller C, de Beaumont R et al (2008) PTEN-deficient cancers depend on PIK3CB. *Proc Natl Acad Sci U S A* 105(35):13057–62
44. Juric D, Baselga J (2012) Tumor genetic testing for patient selection in phase I clinical trials: the case of PI3K inhibitors. *J Clin Oncol* 30(8):765–766
45. Engelman JA (2009) Targeting PI3K signalling in cancer: opportunities, challenges and limitations. *Nat Rev Cancer* 9(8):550–562

## AMPLIACIÓN DE DATOS DE LA PRIMERA PARTE

### 3.2.3 Implementación de un programa de preselección para identificar alteraciones de PI3K que permitan reclutar pacientes para ensayos clínicos de fase I con tumores con alteraciones de PI3K.

#### 3.2.3.1 *Métodos de análisis central y local, comparación de diferentes estrategias de preselección y reclutamiento.*

Para comparar la viabilidad de reclutar pacientes con aberraciones de la vía PI3K en ensayos de fase I, analizamos las diferentes estrategias implementadas en los tres ensayos (A, B y C).

	Drug	Class	Study type	Identificador de ClinicalTrials.gov
Trial A	BGT226	PI3K/mTOR inh	First-in-human	NCT00600275
Trial B	BEZ235	PI3K/mTOR inh	First-in-human	NCT00620594
Trial C	BKM120	panPI3K inh	First-in-human	NCT01068483

**Tabla 3.** Ensayos incluidos en el análisis combinado de diferentes estrategias de preselección molecular y reclutamiento.

Los análisis centrales son aquellos que se realizaron en el laboratorio designado por el promotor. Los análisis locales son aquellos que se llevaron a cabo en el Laboratorio de Patología Molecular del VHI. En la parte escalada de la dosis de los tres ensayos mencionados anteriormente no se seleccionó a los pacientes según su perfil molecular. La disponibilidad de bloques tumorales era, sin embargo, obligatoria, para facilitar un análisis retrospectivo central al final del estudio. En cambio, en las cohortes de expansión con la dosis máxima tolerada (MTD) de dos estudios (ensayos A y B) se preseleccionaron las muestras tumorales de los posibles candidatos (ya sea en el laboratorio central o local) y solo se consideraron elegibles aquellos pacientes cuyos tumores albergaran aberraciones en la vía PI3K (mutación de *PIK3CA* y pérdida de *PTEN*). En el Ensayo A había dos cohortes, una de tumores sólidos (inhibidor de PI3K en monoterapia) y otra de cáncer de mama HER2+ (inhibidor de PI3K añadido a

trastuzumab). En el ensayo A se realizó un análisis central de muestras de 18 instituciones, mientras que en el ensayo B se analizaron las de 3 hospitales. En nuestra institución (centro VHI), optamos por el reclutamiento de pacientes tras realizar los análisis moleculares en nuestro laboratorio (análisis locales). En este caso, los pacientes firmaron un único formulario de consentimiento informado aprobado por el Comité Etico de Vall d'Hebrón, para que se realizara el análisis de su tumor. Comparamos el rendimiento de cada una de las estrategias en cuanto al número de pacientes incluidos por centro en el mismo periodo (de diciembre de 2009 a noviembre de 2011) y la tasa de renuncia, entendida como el número de pacientes elegibles de acuerdo con su perfil molecular que no recibieron tratamiento al no participar en el estudio.

### *3.2.3.2 Resultados del análisis central frente al análisis local, comparación de diferentes estrategias de preselección y reclutamiento.*

En la tabla 4 se resume la estrategia implementada en la parte de escalada de dosis de los tres ensayos de fase I (A, B y C), en las que se incluyeron pacientes independientemente de su perfil molecular, pero con análisis retrospectivo de las muestras de archivo. En estos ensayos se incluyeron 233 pacientes y se recogieron 196 bloques tumorales para realizar el análisis central de su perfil molecular. De todos los pacientes incluidos, solo se evaluaron con éxito las mutaciones de *PIK3CA* y *PTEN* y los niveles de expresión de la proteína PTEN en 182 pacientes (78.1%). Se observaron mutaciones de *PIK3CA* en 20 muestras (9 de mama, 5 colorrectales, 3 endometriales, 1 colangiocarcinoma, 1 prostático, 1 de origen desconocido), y mutaciones de *PTEN* en 22 muestras (7 de mama, 5 endometriales, 3 pulmonares, 2 prostático, 1 cervical, 1 adenoide quístico, 1 de tiroides, 1 síndrome de Cowden, 1 de origen desconocido), lo que representa el 10,9% y el 12,3% de los pacientes incluidos, respectivamente. Las aberraciones de PI3K, entendidas como cualquier mutación de *PIK3CA* o *PTEN* o niveles bajos de expresión de *PTEN* fueron las más frecuentes (43.4% de las muestras).

Se utilizaron dos estrategias diferentes para la preselección de pacientes: central (análisis ad hoc de los posibles candidatos) o locales (análisis en las primeras fases de la enfermedad). Para investigar el resultado del análisis central recopilamos los resultados del periodo de inclusión en las cohortes de expansión en los MTD de los ensayos A y B (véase Tabla 5). Un 39,1% de los 596 pacientes preseleccionados presentaba al menos una aberración de PI3K, y por lo tanto fueron considerados elegibles, según los resultados proporcionados por el laboratorio central del promotor. Sin embargo, solo 52 de los 233 pacientes elegibles (22,3%) fueron finalmente incluidos y tratados en alguno de los 21 centros. Solo una pequeña proporción de los pacientes (6.8%) no cumplía los criterios de inclusión/exclusión, y la tasa de renuncia (sujetos elegibles que no firmaron el consentimiento informado y no reciben tratamiento) fue del 77,7%. En el VHIO aplicamos una estrategia diferente, que consistía en el análisis precoz, local, con plataformas multiplexadas de pacientes con enfermedad avanzada. Esta estrategia se empleó para delinear las aberraciones susceptibles de tratamiento que pudieran ser utilizadas como dianas terapéuticas en el futuro. Entre estas aberraciones se encuentran las mutaciones de PIK3CA y la pérdida de PTEN para los ensayos con inhibidore de PI3K (llamada "Iniciativa del VHIO"). Como se muestra en la Tabla 5, un 44.8% de los 573 pacientes preseleccionados presentaba al menos una aberración de PI3K, y por lo tanto fueron considerados elegibles, según los resultados proporcionados por el laboratorio local. Durante el mismo periodo de tiempo, nuestro centro reclutó 80 pacientes para los ensayos con inhibidores de PI3K, concretamente en la cohorte de MTD del Ensayo B, reclutamos 29 pacientes, mientras que los otros tres centros que emplearon la preselección central solo reclutaron nueve pacientes.

	TRIAL A		TRIAL B		TRIAL C		Pooled analysis	
	N	%	N	%	N	%	N	%
Patients enrolled	136	100,0%	40	100,0%	57	100,0%	233	100,0%
Samples available	116	85,3%	36	90,0%	44	77,2%	196	84,1%
Enough tumor tissue for analysis	108/136	79,4%	36/40	90,0%	38/57	66,6%	182	78,1%
PIK3CA mutation	12/108	11,1%	3/36	8,3%	5/38	13,1%	20	10,9%
PTEN mutation	15/104	14,4%	5/36	13,9%	2/38	5,2%	22	12,3%
PTEN low	29/103	28,1%	9/36	25,0%	7/38	18,4%	45	25,4%
Any PI3K aberration	49/108	45,4%	17/36	47,2%	13/38	34,2%	79	43,4%

DE: dose escalation cohort

**Tabla 4.** Evaluación combinada y desglosada del análisis retrospectivo de los Ensayos A, B y C, en los que los pacientes no fueron preseleccionados según su perfil molecular.

December 2009 to November 2011	Central Analysis								VHIO Initiative	
	(18 sites)				(3 sites)		(21 sites)		(1 site)	
	TRIAL A (MTD of PI3Ki)		TRIAL A (PI3Ki plus trastuzumab)		TRIAL B MTD		Pooled analysis		Pooled analysis	
	N	%	N	%	N	%	N	%	N	%
Patients pre-screened	313	100,0%	181	100,0%	102	100,0%	596	100,0%	573	100,0%
Eligible patients (PI3K aberration)	133	42,5%	67	37,0%	33	32,4%	233	39,1%	257	44,8%
Signed main ICF but failed screening	2	1,5%	3	4,5%	3	9,1%	8	6,8%	11	4,3%
Enrolled	17	12,8%	26	38,8%	9	27,3%	52	22,3%	80*	31,1%*
Attrition (eligible but did not sign main ICF)	114	85,7%	38	56,7%	21	63,6%	181	77,7%	NA**	NA**

MTD: maximum tolerated dose, expansion cohort; NA: not available  
\* 4 in the TRIAL A Monotherapy at MTD and Pi3Ki with trastuzumab cohorts, 29 in the TRIAL B MTD cohort and the remaining in other trials with PI3Kpi.  
\*\*Patients continue to be followed and some are still eligible for future trials

**Tabla 5.** Evaluación combinada y desglosada de los programas de preselección central y local (Iniciativa del VHIO) para las cohortes de expansión en los Ensayos A y B.

### 3.2.3.3 Métodos para el análisis combinado de la eficacia de los inhibidores de la vía PI3K en tumores con aberraciones de PI3K.

Se consideró elegibles para el análisis a aquello pacientes con confirmación patológica de cáncer metastásico refractario con aberraciones de la vía PI3K que recibieron Inhibidores de PI3K como agente único en los ensayos clínicos de fase I en la Unidad de Investigación de Terapias Moleculares Oncológicas del VHIO entre enero de 2009 y mayo de 2012. Los agentes

eran pan-inhibidores de PI3K (n=1), PI3K-mTOR (n=3) o inhibidores selectivos de PI3K alfa (n=2) (tabla 6).

Fármaco	Clase	Tipo ensayo	Identificador ClinicalTrials.gov
BEZ235	PI3K/mTOR inh	First-in-human	NCT00620594
XL765	PI3K/mTOR inh	First-in-human	NCT00485719
PKI587	PI3K/mTOR inh	First-in-human	NCT00940498
BKM120	panPI3K inh	First-in-human	NCT01068483
XL147	panPI3K inh	First-in-human	NCT00486135
BYL719	selective PI3K $\alpha$ inh	First-in-human	NCT01219699
MLN1117	selective PI3K $\alpha$ inh	First-in-human	NCT01449370

**Tabla 6.** Ensayos incluidos en el análisis combinado de la eficacia de los inhibidores de la vía PI3K en tumores con aberraciones de PI3K.

Además, solo se emplearon los datos obtenidos de pacientes tratados con dosis 2/3 por encima de la dosis máxima tolerada (MTD) de cada agente, excluyendo así a los que hubieran recibido una dosis insuficiente. Los pacientes fueron incluidos en los ensayos de fase I aplicando criterios clínicos y logísticos como los criterios de elegibilidad y disponibilidad de vacantes. Se obtuvo consentimiento informado al inicio de todos los estudios, que se llevaron a cabo de conformidad con las directrices del Comité de Ética.

Se consideró que la muestra tumoral presentaba una aberración de la vía PI3K si tenía una mutación de *PIK3CA* (determinada mediante la secuenciación de Sanger, la prueba de mutación de PI3K DxS o el genotipado mediante MassARRAY multiplexado), una baja expresión de PTEN (IHQ con una puntuación H <50), o ambas. La amplificación de *PIK3CA* y las mutaciones de *PTEN* no se determinaron en el laboratorio local, por lo que no fueron incluidas en este análisis. Se consideró que había una aberración de la vía MAPK cuando el tumor presentaba una mutación de *KRAS* o *BRAF* (determinada mediante secuenciación de Sanger, la prueba de mutación de *KRAS* TheraScreen, la prueba de mutación de *BRAFV600* Cobas 4800 o genotipado MassARRAY multiplexado). Aquellos tumores en los que no se analizó el estado de mutación de *KRAS* y *BRAF* fueron clasificados como estado MAPK desconocido.

La formulación de la base de datos de los pacientes y la recopilación de datos para este estudio se realizaron de forma retrospectiva. Antes de iniciar el reclutamiento de pacientes para realizar un ensayo de fase I con Inhibidores de PI3K se revisaron las historias clínicas para comprobar las características demográficas y clínicas de la población; así mismo, se registró la actividad antitumoral (disminución máxima del tumor) y el tiempo transcurrido hasta el fracaso del tratamiento (*time to treatment failure*, TTF2) con el agente experimental, así como con la terapia sistémica inmediatamente anterior (TTF1). Se continuó con el tratamiento administrado en los estudios de fase I según cada protocolo hasta producirse progresión de la enfermedad, fallecimiento o toxicidad inaceptable. La eficacia del tratamiento se evaluó regularmente mediante TAC al inicio del estudio y cada dos ciclos (6-8 semanas) según el protocolo de cada uno de los estudios de fase I. De acuerdo con los criterios de evaluación de respuesta en tumores sólidos (RECIST) v1.0 o 1.1 (dependiendo de cada protocolo), las respuestas tumorales se clasificaron como respuesta completa (RC), respuesta parcial (RP), enfermedad estable (ES), o progresión de enfermedad (PE). El tiempo hasta el fracaso del tratamiento (TTF) se definió como el intervalo de tiempo transcurrido desde el inicio de la terapia hasta su interrupción por cualquier razón, ya sea por progresión de la enfermedad, toxicidad del tratamiento o fallecimiento, lo que ocurra en primer lugar. Se consideró que la terapia con Inhibidores de PI3K había sido beneficiosa para los pacientes cuando se observaba respuesta del tumor al agente (RC o RP) o cuando la enfermedad se mostraba estable durante una periodo prolongado de tiempo (TTF>16 semanas, esto es, >2 revisiones radiológicas aplicando los criterios RECIST. Como análisis secundario, calculamos la relación entre el TTF en el ensayo de fase I (TTF2) y el TTF en el tratamiento anterior (TTF1), y cuando esta relación era  $\geq 1,3$ , se consideraba que la terapia dirigida combinada con Inhibidores de PI3K había sido eficaz (un aumento de  $\geq 30\%$  en el TTF con respecto al tratamiento anterior). Todos los análisis estadísticos se realizaron empleando el programa SPSS v 12.0 (SPSS, Chicago, IL).

### **3.2.3.4 Resultados de eficacia de los inhibidores de la vía PI3K en tumores con aberraciones de PI3K**

Entre enero de 2009 y mayo de 2012 se incluyeron un total de 88 pacientes con tumores con aberraciones de PI3K en seis ensayos clínicos de fase I diferentes con Inhibidores de PI3K en monoterapia realizados en el VHHIO. En la tabla 7 se muestran las características demográficas y clínicas de los pacientes. En la Tabla 8 se detallan los perfiles moleculares de los tumores de los pacientes. En total, 31 presentaban mutaciones de *PIK3CA* (ocho de ellas con pérdida simultánea de PTEN) y 57 fueron incluidos por la pérdida de expresión de PTEN. Se evaluó el estado de MAPK en 53 pacientes (60,2%) y 19 (35,9%) presentaban mutaciones activadoras de *KRAS* o *BRAF*.

En la Tabla 9 y la Figura 7 se resumen las respuestas y beneficios clínicos registrados en nuestra base de datos. Se observaron respuestas parciales en seis pacientes (6,8%; 2 con cáncer de mama, 1 cáncer de ovario, 1 cáncer cervical, 1 cáncer de esófago y 1 cáncer colorrectal, todos con mutaciones de *PIK3CA*). La enfermedad se mostró estable durante más de 16 semanas en otros 14 pacientes (15,9%). También se observó una respuesta *minor* del tumor (una reducción de entre el 10 y el 29% en las lesiones diana) en otros cinco pacientes (cuatro de ellos mostró enfermedad estable durante más de 16 semanas). Las medianas de TTF en los pacientes que recibieron Inhibidores de PI3K en monoterapia fueron similares en pacientes con respuesta parcial y estable, esto es, >16 semanas (254 y 226 días, respectivamente). Se tuvo que interrumpir el tratamiento en nueve pacientes debido a la toxicidad (10,2%).

En total, el tratamiento con un agente de Inhibidores de PI3K combinado fue beneficioso para 20 pacientes (22,7%). Tal como se muestra en la Tabla 9, el análisis por tipos de tumores indica que el beneficio clínico fue superior en las pacientes con tumores ginecológicos (50%) y de mama (26,3%) en comparación con el cáncer colorrectal (11,9%). Aquellos pacientes que recibieron inhibidores específicos de PI3Kα parecieron obtener un mayor beneficio clínico

(46,7%), frente a los pacientes que recibieron pan-inhibidores de PI3K o PI3K-mTOR (17,8%).

Dado que los estudios con inhibidores selectivos de isoformas solo incluían tumores con *PIK3CA* mutado, se realizó un análisis exploratorio centrado únicamente en aquellos pacientes con *PIK3CA* mutado. Dicho análisis mostró que no había diferencias estadísticamente significativas entre el beneficio aportado por los inhibidores de PI3K $\alpha$  y los pan-inhibidores de PI3K o PI3K-mTOR (tasa de beneficio clínico: 46.7% frente al 31.3%, respectivamente; p=0,6) aunque se percibe una tendencia. Tanto los tumores con mutaciones en el dominio quinasa como en el helicoidal respondieron a estos agentes aunque se denotaba una tendencia a favor de los primeros (50% frente al 21,4% en los exones 9 y 20 respectivamente, p=0, 1). El análisis por subgrupos según el perfil molecular sugiere que los pacientes incluidos en los ensayos de fase I con baja expresión de PTEN (incluyendo la ausencia de PTEN) mostraron un menor beneficio clínico (14%) que los pacientes con *PIK3CA* mutado (38,7%) tratados con Inhibidores de PI3K (p=0, 008). El beneficio clínico no se vio afectado por la presencia de mutaciones de la vía MAPK concomitante (21,1% frente al 26,5% en pacientes con aberraciones de PI3K con o sin mutaciones de KRAS/BRAF respectivamente, p=0,66). .

Se observó un índice de TTF2/1  $\geq$ 1,3 en el 25% de los pacientes que recibieron Inhibidores de PI3K con respecto a la anterior terapia. De los 19 pacientes que obtuvieron beneficio clínico de Inhibidores de PI3K (RP o ES > 16 semanas) y que habían recibido previamente al menos una terapia, 14 (73,7%) mostraron un índice de TTF2/1  $\geq$ 1,3. De los 65 pacientes con un índice de TTF2/1 <1,3, 60 (92,3%) no mostraron beneficio clínico cuando recibieron Inhibidores de PI3K (RP o ES < 16 semanas como mejor respuesta).

Demographics	N	%
Age median (range)	59 (28-79)	
Sex		
Male	44	50.0%
Female	44	50.0%
Tumor type		
Colorectal	42	47.7%
Breast	19	21.6%
Ovary	6	6.8%
Bilio-pancreatic	4	4.5%
Cervix	3	3.4%
Endometrial	3	3.4%
Lung	3	3.4%
Esophageal	2	2.3%
Glioblastoma	2	2.3%
Salivary gland	2	2.3%
Germinal testicular	1	1.1%
Prostate	1	1.1%
Treatment lines - median (range)	3 (0-12)	
0-2	27	30.7%
> 2	61	69.3%
Class of agent		
PI3K/mTOR	40	45.5%
pan-PI3K	33	37.5%
PI3K alpha	15	17.0%
Molecular profiling		
Primary tumor	65	73.9%
Metastatic site	23	26.1%

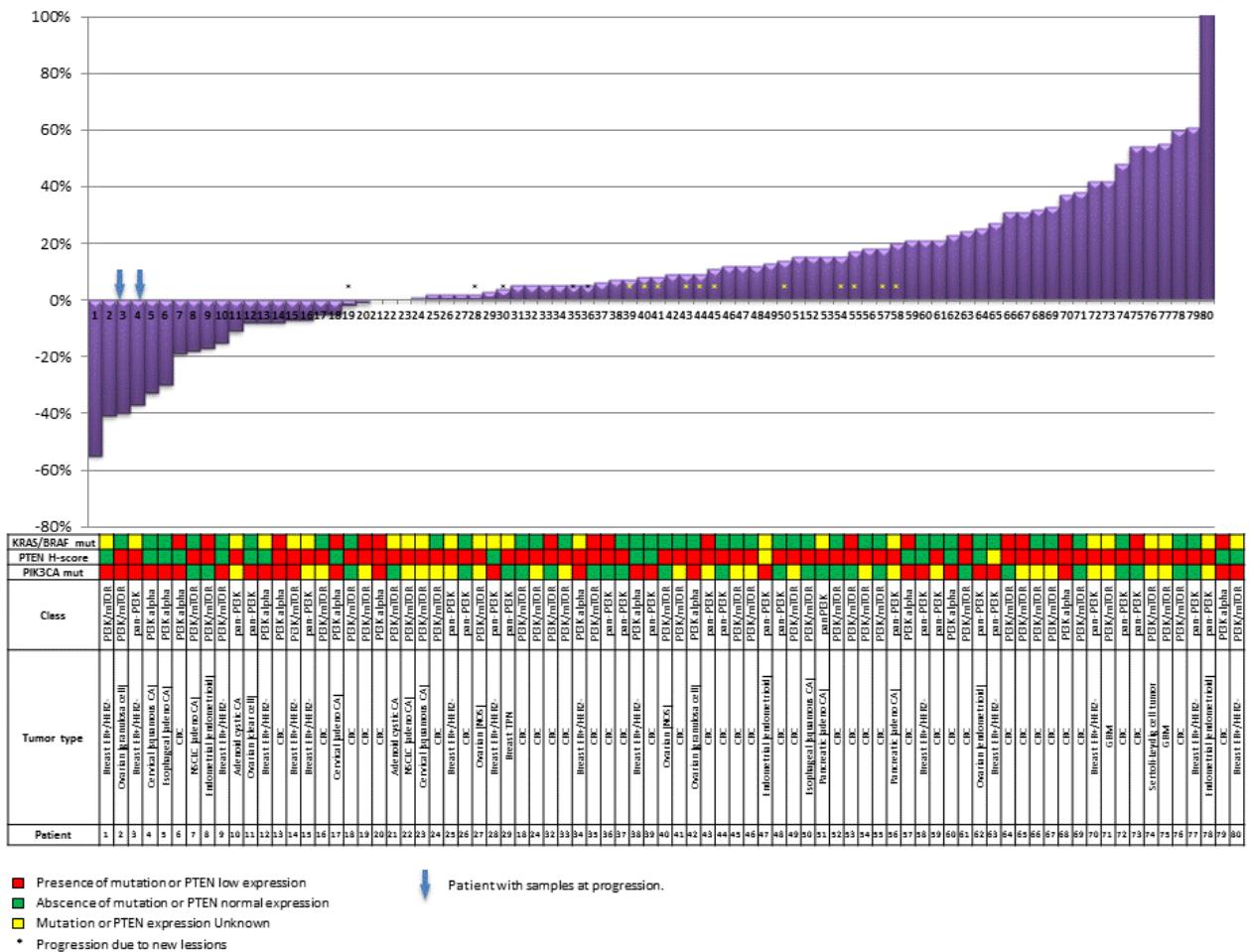
**Tabla 7.** Características demográficas y clínicas de 88 pacientes incluidos en los ensayos con Inhibidores de PI3K en el VHHIO entre enero de 2009 y mayo de 2012.

Molecular profiling	N	% - from assessed
PIK3CA status		
wild-type	30	49.2%
H0147R	14	22.9%
E545K	10	16.4%
E542K	4	6.5%
Q546K	2	3.3%
R88Q	1	1.6%
not assessed	27	
PTEN status		
normal	23	26.1%
low (null)	65 (26)	73.9% (29.5%)
MAPK status		
KRAS/ BRAF wild type	34	64.1%
KRAS mutation	18	34.0%
BRAF mutation	1	1.9%
not assessed	35	
PIK3CA + PTEN status		
PIK3CA mutation + PTEN normal	23	26.1%
PIK3CA wild-type + PTEN low	57	64.9%
PIK3CA mutation + PTEN low	8	9.0%
PI3Kp aberration + RAS/RAF status		
PIK3CA mutation + RAS/RAF wild-type	12	13.6%
PTEN low + RAS/RAF wild-type	22	25.0%
PIK3CA mutation + RAS/RAF mutation	7	8.0%
PTEN low + RAS/RAF mutation	12	13.6%
PI3Kp aberration + RAS/RAF unknown	35	39.8%

**Tabla 8.** Perfil molecular de los pacientes incluidos en los ensayos de fase I con Inhibidores de PI3K combinado en el VHIO.

Response	N	%	Median TTF (range)
All patients	88	100.0%	56 (3-789)
PR	6	6.8%	254 (134-322)
SD	33	37.5%	111 (42-789)
SD > 120 days (10-29% tumor shrinkage)	14 (5)	15.9% (5.7%)	226 (132 - 789)
SD ≤ 120 days	19	21.6%	55 (6-113)
PD	44	50.0%	49 (6-77)
Not assessed	5	5.7%	
<b>Clinical benefit (PR or SD &gt; 120 days)</b>			
Overall	20 (88)	22.7%	
Tumor type			
Colorectal	5 (42)	11.9%	
Breast	5 (19)	26.3%	
Gynecological	6 (12)	50.0%	
Lung	1 (3)	33.3%	
Other	3 (12)	25.0%	
Agent			
PI3K/mTOR (overall)	7 (40)	17.5%	
PI3K/mTOR (in PIK3CA mutant only)	2 (5)	40.0%	
pan-PI3K (overall)	6 (33)	18.2%	
pan-PI3K (in PIK3CA mutant only)	3 (11)	27.3%	
PI3K alpha (in PIK3CA mutant only)	7 (15)	46.7%	
Molecular profiling			
PIK3CA mutation overall	12 (31)	38.7%	
PIK3CA mutation exon 9	8 (16)	50.0%	
PIK3CA mutation exon 20	3 (14)	21.4%	
PTEN low	12 (65)	18.5%	
PTEN null	3 (26)	11.5%	
PIK3CA mutation + PTEN normal	8 (23)	34.8%	
PIK3CA wild-type + PTEN low	8 (57)	14.0%	
PIK3CA mutation + PTEN low	4 (8)	50.0%	
PIK3CA mutation + RAS/RAF wild-type	5 (12)	41.7%	
PIK3CA mutation + RAS/RAF mutation	3 (7)	42.9%	
PTEN low + RAS/RAF wild-type	4 (22)	18.2%	
PTEN low + RAS/RAF mutation	1 (12)	8.3%	
PI3Kp aberration + RAS/RAF unknown	7 (35)	20.0%	
TTF PI3Kp/TTF prior line ≥ 1.3	22 (87)	25.0%	
Colorectal	10 (42)	23.8%	
Breast	4 (19)	21.1%	
Gynecological	6 (12)	50.0%	
Lung	2 (3)	66.7%	
Other	0 (11)	0%	
TTF PI3Kp/TTF prior line < 1.3	65 (87)	73.9%	

**Tabla 9.** Respuesta y beneficio clínico de los pacientes incluidos en los ensayos de fase I con Inhibidores de PI3K combinado en el VHIO.



**Figura 7.** Gráfico en cascada de mejor respuesta en 80 pacientes con lesiones cuantificables a los que se administró Inhibidores de PI3K combinado en ensayos de fase I en el VHIO.

Flecha: pacientes con muestras en progresión.

CRC, cáncer colorrectal; GBM, glioblastoma; NE, No especificado;

### 3.2.3.5 Métodos para el análisis de muestras de pacientes tras mostrar respuesta y desarrollar resistencia secundaria a los inhibidores de PI3K por secuenciación del exón diana

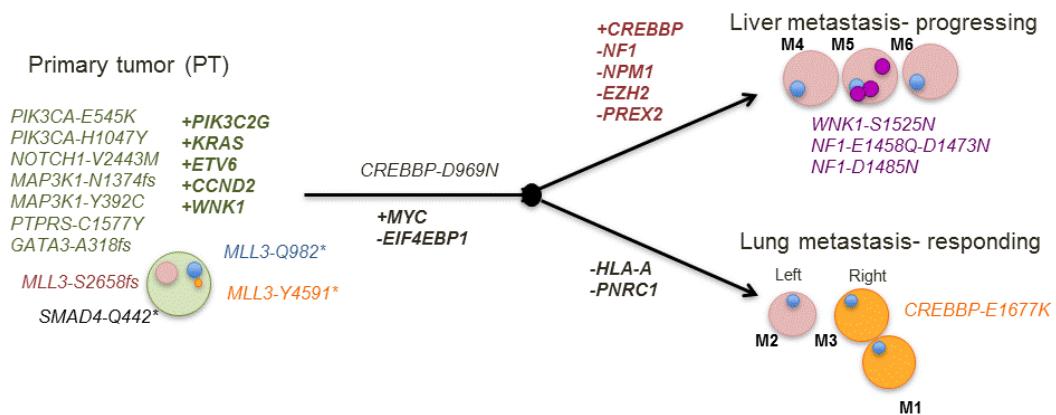
Se tomaron muestras de las lesiones metastásicas al producirse progresión tras tratamiento con Inhibidores de PI3K de dos pacientes con tumores con mutaciones de PIK3CA que inicialmente respondieron a tratamiento. Para evaluar la utilidad de la secuenciación profunda dirigida, analizamos los exones de codificación de unos 200 genes incluidos en el panel

IMPACT de estas muestras. Empleamos ADN de muestras fijadas en formol e incluidas en parafina de esos dos pacientes que respondieron a tratamiento (pacientes A y B), siguiendo el procedimiento descrito por Won HH y col(91). Se analizaron y compararon las frecuencias alélicas de las aberraciones en todas las muestras para verificar la presencia de múltiples subclones. Elaboramos árboles filogenéticos de estos tumores, siguiendo el procedimiento descrito por Gerlinger y col(92). Se realizó inmunofluorescencia de  $\beta$ -catenina siguiendo el procedimiento descrito por Tenbaum SP y col(93).

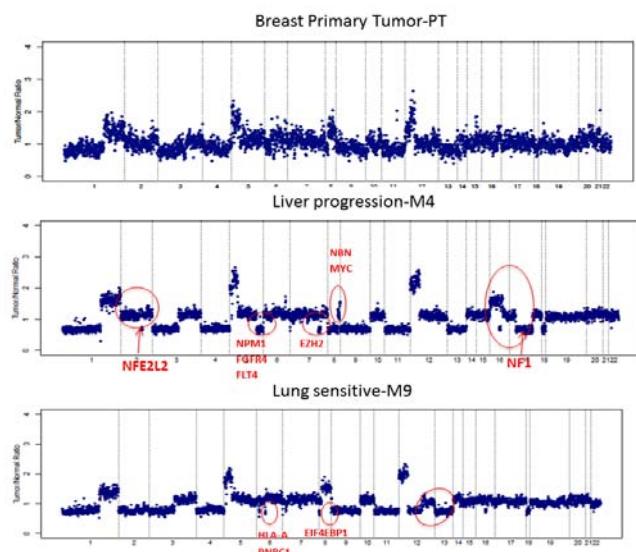
### *3.2.3.6 Resultados de la secuenciación profunda de tumores de dos pacientes que tras responder a tratamiento, experimentaron progresión*

La paciente nº3 (Figura 7) era una mujer con cáncer de mama ER+ PR+ HER2- (cáncer de mama luminal) con metástasis hepática, pulmonar y cerebral (irradiada) tratada anteriormente con cuatro líneas de quimioterapia y tres terapias hormonales. En el análisis de preselección con MassARRAY se observó una mutación de *PIK3CA* (E545K), por lo que se le ofreció participar en el ensayo de fase I con Buparlisib. La enfermedad mostró respuesta parcial (40%) tras cuatro meses de terapia, aunque, desafortunadamente, finalmente la enfermedad hepática progresó con rapidez (aunque la metástasis pulmonar permaneció controlada) y la paciente falleció debido a una disfunción hepática. Se obtuvieron consentimientos escritos para el análisis mediante secuenciación de exones en el tumor primario y en tejido normal y para la realización de una autopsia (metástasis cerebral, pulmonar y hepática). Los resultados desvelaron un tumor complejo multiclonal (Figura 8A). Comparándolo con el tumor primario, los clones del tumor en la metástasis hepática presentaban una importante pérdida en el cromosoma 17, incluyendo el *NF1* completo y la adquisición subclonal de mutaciones de *NF1* (E1458Q-D1473N y D1485N(10, 94)), lo que sugiere un escape de la inhibición de PI3K mediante la activación de la vía Ras/mTORC1(95, 96)(véase Figura 8B).

**Figura 8A**



**Figura 8B**



**Figura 8**

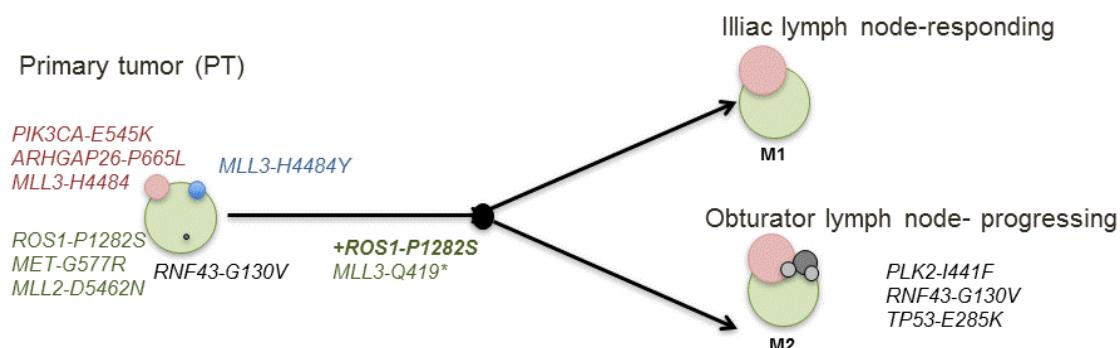
A. Diagrama de clonalidad de muestras de la paciente nº3 (cáncer de mama luminal). En el tumor primario se observaron varias mutaciones fundadoras en los genes *PIK3CA*, *NOTCH1*, *MAP3K1*, *PTPRS* y *GATA3* en el tronco del árbol filogenético, y se detectaron algunas mutaciones menores en *MLL3* y *SMAD4*. Varias regiones cromosómicas estaban amplificadas en el tumor primario y se mantenían en la metástasis, incluida la isoforma gamma de PI3K2 de clase II (*PIK3C2G*) (cromosoma 12p12), entre otras. Se identificó una mutación de *CREBBP-D969N* en las dos

lesiones metastásicas (hígado y pulmón) mientras que *WNK1-S1525N*, *NF1-E1458Q-D1473N* y *NF1-D1485N* solo se encontraron en una de las tres muestras de metástasis hepática en progresión, no estando presente en la metástasis pulmonar, que respondió a tratamiento. Así mismo, en las muestras de metástasis hepática se identificó una pérdida importante en el cromosoma 17, incluido el gen *NF1*.

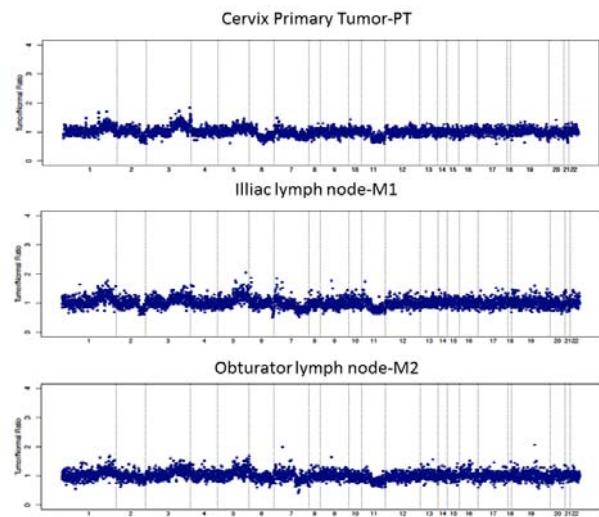
#### B. Alteraciones en el número de copias en la paciente nº4, tumor primario

La paciente nº4 (Figura 7) era una mujer de 65 años con carcinoma cervical de células escamosas con ganglios linfáticos, refractaria tras radioterapia con cisplatino y carboplatino/paclitaxel. El análisis molecular mediante MassARRAY reveló una mutación de *PIK3CA* (E545K), por lo que se le ofreció participar en un ensayo clínico con un inhibidor específico de PI3K $\alpha$  (Alpelisib). La enfermedad mostró respuesta parcial (37%) tras cuatro meses de tratamiento. Tras progresión, la paciente decidió someterse a cirugía pélvica de rescate en la que se pudieron obtener muestras de dos áreas linfáticas, algunas con enfermedad en progresión (zona del obturador) y otras estables (zona ilíaca). Los ganglios linfáticos del obturador (con progresión en tratamiento) presentaban una mutación de *RNF43-G130V*, lo que sugiere un escape a través de la activación de la vía WNT(93) (véase Figura 9A-D).

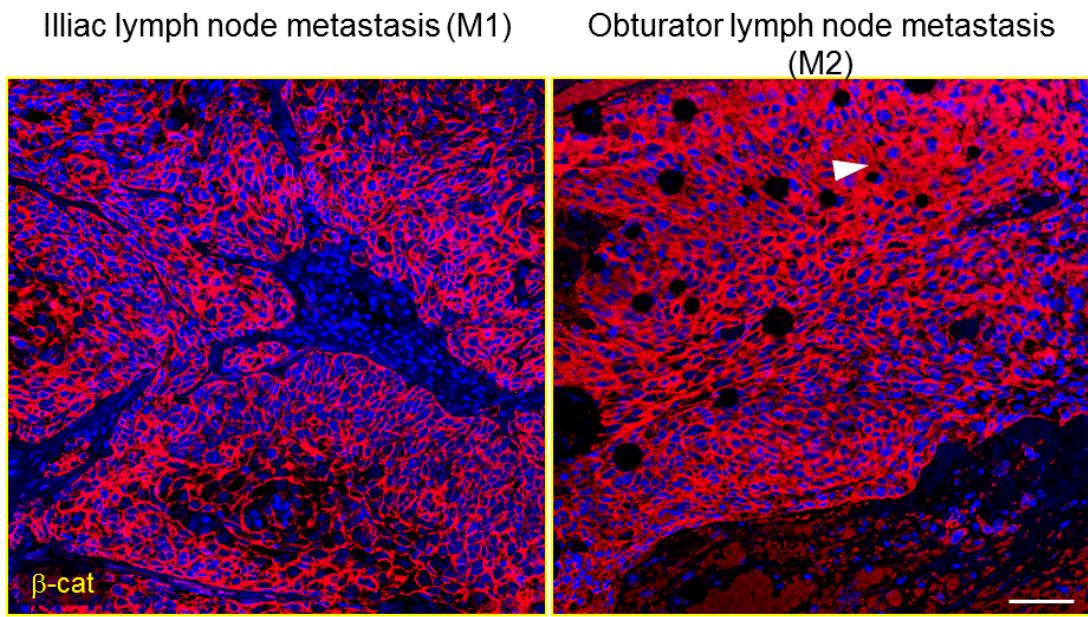
**Figura 9A**



**Figura 9B**

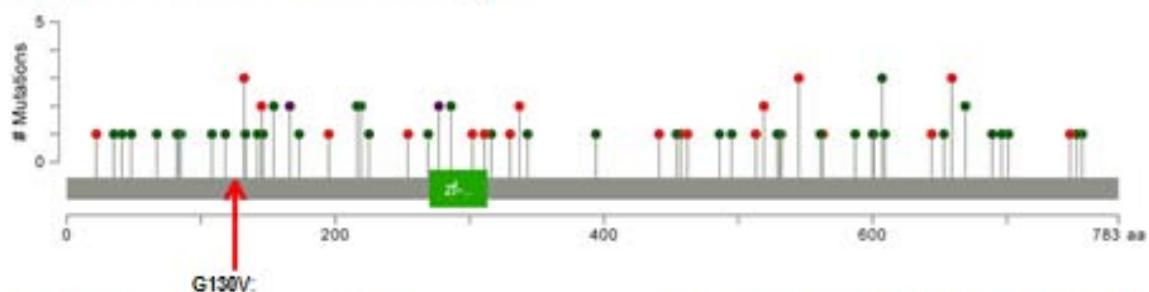


**Figura 9C**

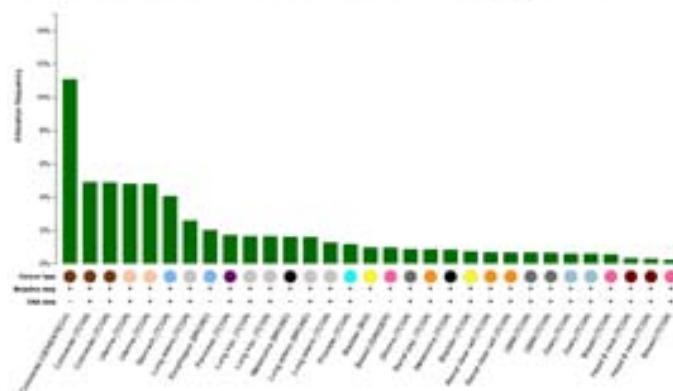


**Figura 9D**

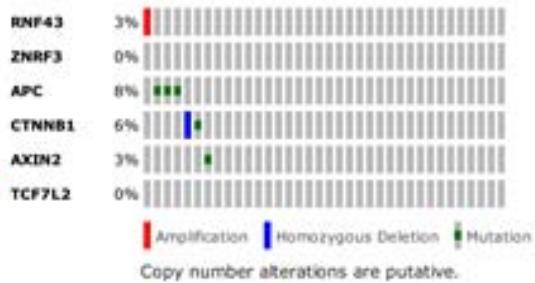
**i. RNF43 mutations in all tumor types**



**ii. RNF43 mutations in all tumor types**



**iii. Alterations in components of the WNT pathway in TCGA-Cervix patients as described in cBioPortal**



**Figura 9**

- A. Diagrama de clonalidad de muestras de la paciente nº4 (cáncer cervical escamoso). Las mutaciones de *ROS1*, *MET* y *MLL2* estaban altamente expresadas en el tumor primario, mientras que *PIK3CA*, *ARHGAP26*, y *MLL3* representaban un clon menor. La mutación de *RNF43* apenas estaba expresada. Parece que las lesiones metastásicas procedían del subclon con el clon mayor *ROS1/MET/MLL2* y haber experimentado una duplicación del alelo mutante de *ROS1*. El clon que expresaba *RNF43-G130V* estaba presente en los ganglios con enfermedad progresiva, en la zona del obturador. Leyenda: PanPI3Ki: pan-inhibidor de PI3K; PI3Ka: inhibidor específico de PI3K alfa.
- B. Alteraciones en el número de copias en la paciente nº5, tumor primario frente a lesiones metastásicas

- C.  $\beta$ -catenina por inmunofluorescencia en metástasis de la cadena ganglionar ilíaca (M1) y enfermedad progresiva en los ganglios linfáticos del obturador (M2 con mutación de *RNF43*), mostrando expresión citoplasmática de  $\beta$ -catenina (flecha)
- D. Alteraciones moleculares en *RNF43* en pacientes del TCGA descritas en cBioPortal for Cancer Genomics. a) Localización de mutaciones en las mutaciones de *RNF43* en todos los tipos de tumor. Nunca antes se habían observado en esos pacientes mutaciones de *RNF G130V*. b) Frecuencia y tipos de tumores donde se han descrito mutaciones de *RNF43*. El cáncer colorrectal y endometrial, donde la vía de señalización WNT suele estar frecuentemente alterada, son los tipos de tumores donde con mayor frecuencia se encuentran mutaciones de *RNF43*. c) Alteraciones en los componentes de la vía WNT en pacientes con cáncer cervical.
- TCGA: The Cancer Genome Atlas

## 3.4 Resultados y discusión

### 3.4.1 Seguridad de los pan-inhibidores de PI3K, Buparlisib y Pilaralisib.

En general, el perfil de los inhibidores de PI3K con respecto a los efectos secundarios resulta aceptable, sin efectos tóxicos inesperados, tal como hemos observado en nuestros ensayos con Buparlisib y Pilaralisib. En términos generales, los efectos tóxicos fueron de leves a moderados y tratables con medicación. Entre los efectos tóxicos limitantes de dosis relacionados con Pilaralisib y Buparlisib se encuentran la hiperglucemia, maculopápulas, intolerancia gastrointestinal (anorexia, náuseas, vómitos, dispepsia, diarrea) y estomatitis.

Aunque algunos de estos efectos tóxicos son inespecíficos (*off-target*) otros podrían estar relacionados con los efectos producidos sobre la diana y estar directamente relacionados con el mecanismo de acción. El tratamiento combinado de Pilaralisib y Buparlisib causó hiperglucemia, que fue tratada con medicación oral antiglucémica y, por lo general, respondió a tratamiento concomitante. En la mayoría de los pacientes se pudo retomar el tratamiento con el inhibidor de PI3K, reduciendo la dosis cuando se producía una hiperglucemia. La hiperglucemia es una toxicidad específica de los inhibidores de PI3K(39). Dado que PI3K es un componente clave en la vía de señalización de la insulina, la inhibición de dicha señalización causa resistencia a la insulina y los niveles de glucosa en sangre aumentan(55, 97, 98). Algunos efectos tóxicos específicos del mecanismo de acción se podrían incluso utilizar como biomarcadores farmacodinámicos o en la toma de decisiones clínicas como, por ejemplo, a la hora de ajustar la dosis: con inhibidores de EGFR se ha usado el rash como marcador, aumentando progresivamente la dosis en aquellos casos en los que no se produzcan efectos secundarios hasta personalizar la dosis(99). De hecho, en el caso de algunos inhibidores de PI3K, la ausencia de hiperglucemia ha sido señalada por algunos como una señal de inhibición insuficiente de la vía, una razón para la interrupción del desarrollo clínico(100). Sin embargo, una limitación a la hora de emplear la hiperglucemia como biomarcador farmacodinámico es

que se produce una liberación compensatoria de insulina (y de C-péptido) y que otros factores, como la dieta, también pueden influir en los niveles de glucosa.

Con frecuencia, se han observado erupciones cutáneas asociadas al uso de inhibidores de Pilaralisib y Buparlisib. La toxicidad cutánea fue frecuente en los pacientes a los que se administraron estos fármacos. La erupción observada en los pacientes tratados con ambos inhibidores era eritematosa, sin ampollas, maculopapilar y no acneiforme, a diferencia de las erupciones causadas por los agentes anti-EGFR(59, 101, 102). Curiosamente, se han observado erupciones similares -y también diarrea- en pacientes tratados con otros inhibidores de la quinasa como sunitinib(103). Normalmente, las erupciones aparecían al principio del tratamiento y estaba relacionada con la dosis. A los pacientes con erupciones se les trató con antihistamínicos o corticosteroides y rara vez hubo que interrumpir o reducir la dosis.

Por otro lado, algunos de los efectos tóxicos esperados que se suelen asociar con los rapálogos (inhibidores de mTOR, inhibiendo por lo tanto la vía de PI3K downstream), como la hiperlipidemia, la neumonitis y la mucositis, no se observaron al administrar Pilaralisib y Buparlisib.

### **3.4.2 Biomarcadores de señalización celular**

Se evaluaron los efectos farmacodinámicos de los inhibidores de PI3K sobre múltiples nodos de la vía PI3K en tejido vicario (*surrogate tissue*) como piel (Buparlisib y Pilaralisib) y cabello (Pilaralisib), así como especímenes de biopsias de tumores (Pilaralisib y Buparlisib). Por el contrario, el cabello humano parece dar mejores resultados que la piel (véase la Figura 1 del segundo manuscrito), pudiendo permitir la toma secuencial de muestras para analizar posteriormente la relación dosis/efecto antitumoral y respuesta/duración del efecto(104, 105). Al analizar los biomarcadores en nuestros dos estudios, observamos un descenso dependiente de la dosis de entre el 30% y el 90% en marcadores como pAKT<sup>S473</sup>, pAKT<sup>T308</sup> pPRAS40<sup>T246</sup>,

p4EBP1<sup>T70</sup> y pS6<sup>S240/S244</sup> al usar los diferentes compuestos a la máxima dosis tolerada (MTD), lo que es indicativo de modulación de la diana molecular.

Para investigar el alcance de la inhibición de PI3K con Pilaralisib se analizaron tejidos vicarios (*surrogate tissue*) derivados de la sangre como lisados o plasma rico en plaquetas. Éstos podrían ser útiles, ya que facilitan la evaluación de las relaciones dosis-tiempo mediante la toma secuencial de muestras (datos no mostrados). No obstante, aún restan dificultades técnicas y científicas que podrían limitar la utilidad de estos métodos; por ejemplo, las concentraciones de fármaco deben ser superiores en sangre que en tejido, por lo que su correlación con los efectos antitumorales no está clara. Por el contrario, el cabello humano parece dar mejores resultados que la piel, pudiendo permitir la toma secuencial de muestras para analizar posteriormente la relación dosis/efecto antitumoral y respuesta/duración del efecto(104, 105).

### **3.4.3 Biomarcadores de efecto metabólico**

Partiendo del papel de la vía de señalización de PI3K en el metabolismo fisiológico de la glucosa, así como de las observaciones de Warburg de que las células tumorales presentan un mayor consumo de glucosa, en estos estudios incluimos biomarcadores del efecto metabólico (98, 106). De este modo, se ha intentado evaluar la relación dosis-respuesta midiendo en ayunas la glucosa, los niveles de insulina y C-péptido en plasma (Pilaralisib y Buparlisib) y la captación de glucosa mediante <sup>18</sup>F-FDG -PET (Buparlisib).

La medición de insulina y C-péptido en plasma sirvieron como marcadores indirectos para demostrar la inhibición de PI3K independiente de la dosis, un efecto que se observó tras solo unos pocos días de tratamiento. Los datos obtenidos en nuestros estudios demuestran que medir el péptido C y la insulina puede ser útil para identificar una dosis biológicamente activa al comparar diferentes niveles de dosis.

La captación de <sup>18</sup>F-FDG disminuyó en la mayoría de los pacientes, y el análisis de regresión lineal indicó que existía una relación entre el nivel de captación de <sup>18</sup>F-FDG y la dosis media de Buparlisib recibida. Aunque estas observaciones demuestran la inhibición específica de la vía PI3K, no pareció existir una relación evidente entre la presencia de una respuesta metabólica parcial (descenso de la captación de <sup>18</sup>F-FDG al cabo de un mes) y la supervivencia sin progresión o mejor respuesta, según los criterios RECIST. El efecto de los inhibidores de PI3K sobre el metabolismo de la glucosa, el pequeño número de pacientes y respuestas observadas, así como la considerable heterogeneidad de pacientes y tipos de tumores tratados en este estudio podrían explicar por qué <sup>18</sup>F-FDG no fue útil como factor de predicción de respuesta a terapia en este estudio, aunque sí demostró su potencial como biomarcador farmacodinámico.

#### ***3.4.4 Señales de actividad y biomarcadores predictivos***

En los dos ensayos de fase I se observaron señales de actividad antitumoral (respuestas parciales y estabilización prolongada de la enfermedad con contracción del tumor o reducción del marcador tumoral). En las pacientes con cáncer de mama, así como en los pacientes con carcinoma ductal de la glándula parótida y hemangioendotelioma epitelioide se observó una respuesta tumoral, y los pacientes con CPNM, cáncer de próstata y cáncer de cabella y cuello se logró una larga estabilización de la enfermedad. Aún así, la eficacia clínica de estos inhibidores de la vía PI3K en monoterapia fue, como mucho, modesta.

Una forma de mejorar los índices de respuesta de la monoterapia es mejorar la selección de pacientes con aberraciones y mutaciones conocidas. Los hallazgos preclínicos según los cuales los tumores con alteraciones de la vía PI3K son más sensibles a los tratamientos con inhibidores de PI3K que los tumores sin dichas alteraciones nos llevaron a implementar un programa de preselección para incluir en estos ensayos pacientes cuyos tumores presentaran mutaciones de *PIK3CA* y PTEN o hubieran perdido la expresión de PTEN(67, 82-84).

En nuestra institución, empleamos la preselección multiplexada de pacientes con enfermedad avanzada. Esta estrategia se empleó para identificar las aberraciones susceptibles de tratamiento que pudieran ser utilizadas como dianas terapéuticas en el futuro. Entre estas aberraciones se encuentran las mutaciones de *PIK3CA* y la pérdida de PTEN para los ensayos con Inhibidores de PI3K (incluyendo estos dos ensayos con Buparlisib y Pilaralisib). Se preseleccionaron 573 pacientes y a aquellos que presentaban alteraciones con probada sensibilidad a los inhibidores de PI3K se les ofreció participar en un ensayo clínico con agentes que incluían un pan-inhibidor de PI3K, PI3K-mTOR o un inhibidor selectivo de PI3K $\alpha$  (n=2). Se incluyeron un total de 88 pacientes con tumores con aberraciones de PI3K en seis ensayos clínicos de fase I diferentes, incluyendo los aquí descritos (NCT00486135, NCT01068483, NCT00485719, NCT00620594, NCT00940498, NCT01219699, NCT01449370). De hecho, la mayoría de los pacientes con alteraciones conocidas de PI3K que fueron incluidos en los ensayos de fase I con Pilaralisib y Buparlisib eran pacientes de nuestro centro. Nuestra estrategia de analizar el perfil molecular mediante el uso de plataformas multiplexadas tuvo un gran impacto en el reclutamiento de pacientes en multitud de ensayos de fase I, lo que permitió probar clínicamente la hipótesis de que los tumores con mutaciones de *PIK3CA* pueden ser más sensibles a los inhibidores de PI3K.

Sin embargo, estos dos ensayos clínicos con pan-inhibidores de PI3K no han demostrado que exista una clara correlación entre las alteraciones moleculares en la vía PI3K y el efecto antitumoral, lo cual contradice, extrañamente, lo sugerido por multitud de ensayos preclínicos(67, 68, 107). En nuestra experiencia, no se observan grandes diferencias en los índices de respuesta en pacientes cuyos tumores presentaban alteraciones en la vía PI3K (definidas en este estudio como una mutación de *PIK3CA* o la pérdida de PTEN) y los que no. Otros estudios, donde se evalúan los inhibidores de PI3K $\alpha$ , Alpelisib y INK1117, y realizados en paralelo a los estudios aquí presentados, se reclutaron únicamente a pacientes con tumores con mutaciones de *PIK3CA*. En este estudio, hemos observado algunas respuestas objetivas,

así como una estabilización prolongada de la enfermedad con contracción del tumor en cáncer de mama (se logró una respuesta parcial en el 33% de las pacientes con cáncer de mama ER+ con mutaciones de *PIK3CA*); cáncer colorrectal (una respuesta parcial) y tumores ginecológicos (una respuesta parcial)(108). No obstante, como se puede comprobar en la Figura 4 del Anexo 2, a pesar de haber seleccionado solo a pacientes cuya enfermedad se suponía muy sensible a los inhibidores de PI3K, solo la mitad pareció haber obtenido algún beneficio clínico. De este modo, el índice de control de la enfermedad, entendido como las respuestas completas y parciales y la estabilización de la enfermedad, fue del 53,2%, con un intervalo de confianza del 95% de 40.1–66.0.

La ausencia de una clara asociación entre las mutaciones de *PIK3CA* y la respuesta a inhibidores de PI3K podría deberse a varios factores, como la pequeña muestra y la heterogénea población de pacientes, que presentaban una gran variedad de tipos de tumores. Con el fin de continuar analizando este hecho con una mayor población de pacientes y evaluar el papel del linaje del tumor a la hora de determinar la sensibilidad de la enfermedad a la terapia, realizamos un análisis combinado de la eficacia clínica de los inhibidores de PI3K estudiados en todos los ensayos de fase I realizados en VH en pacientes cuyos tumores albergaban alteraciones de la vía PI3K. En este conjunto de datos, uno de los mayores en los que se evalúa el agente único Inhibidores de PI3K en pacientes con tumores con alteraciones de PI3K, la actividad antitumoral fue discreta, aunque observamos un mayor beneficio en las pacientes con tumores de mama con mutaciones de *PIK3CA* y con tumores ginecológicos. También realizamos una secuenciación masiva dirigida de las muestras de tumor de dos pacientes cuya enfermedad mostró respuesta para, finalmente, progresar (véase AMPLIACIÓN DE DATOS para ver con más detalle los métodos y resultados obtenidos). La secuenciación paralela masiva en estas muestras metastásicas reveló alteraciones en vías asociadas a la resistencia al inhibidor de PI3K, lo que anima a continuar utilizando en el futuro estas plataformas de preselección de alta capacidad.

El valor predictivo no concluyente de *PIK3CA* para determinar el valor clínico de los inhibidores de PI3K puede deberse a otras razones. En primer lugar, los métodos de detección precoz incluían un número limitado de ensayos (multiplexado limitado), y puede que éstos no tuvieran capacidad para detectar otras alteraciones que podrían determinar la sensibilidad (como las alteraciones en *AKT1/2*, *PIK3R1*, *LKB1*, *NF1*) o resistencia (como las alteraciones en *APC*, *BRAF*, *KRAS*) del tumor. A este respecto, actualmente se pueden emplear los métodos de secuenciación de próxima generación, que han demostrado ser útiles a la hora de determinar los mecanismos primarios y secundarios de resistencia a los inhibidores de PI3K, lo que subraya su utilidad para la determinación del perfil molecular de los tumores de los pacientes (véase la Figura 7 a-e del Anexo 2). En segundo lugar, puede que los tumores con alteraciones de PI3K no respondan debido a la heterogeneidad intratumoral(109). Finalmente, se están obteniendo datos preclínicos (83, 110, 111)que sugieren que el linaje tumoral también parece influir en la sensibilidad del tumor a la terapia.

### 3.5 Conclusiones y seguimiento de líneas de investigación

A partir de los datos aquí presentados, sería razonable afirmar que los pan-inhibidores de PI3K como Buparlisib y Pilaralisib pueden inhibir la vía PI3K/AKT/mTOR con una eficacia razonable y un perfil de seguridad favorable. Los efectos secundarios más habituales asociados a estos fármacos fueron los esperados, teniendo en cuenta los mecanismos de acción de los mismos, es decir, hiperglucemia y erupciones cutáneas.

El análisis de los biomarcadores del metabolismo de la glucosa y las pruebas de <sup>18</sup>F-FDG-PET aquí presentadas respaldan la observación de que estos fármacos inhiben la vía PI3K y perturban el metabolismo de la glucosa.

La reducción de la expresión de pAKT, p4EBP1 y pS6 observada en tejidos vicarios (*surrogate tissue*) y muestras de tumores confirma que Pilaralisib y Buparlisib tienen capacidad para

inhibir la vía de PI3K en tejido tumoral. La actividad antitumoral fue discreta y, aunque estos resultados abren la vía para la realización de otros estudios con pan-inhibidores de PI3K ya sea en monoterapia o combinados con otros agentes para algunas indicaciones (cáncer de mama, ovario y cabeza y cuello), ha quedado demostrado que seleccionar a los pacientes basándose en las alteraciones que presentan los tumores no es suficiente. Los inhibidores de PI3K en tumores con alteraciones de *PIK3CA* no parecen haber mostrado el mismo nivel de dependencia oncogénica y actividad antitumoral que las terapias dirigidas con inhibidores como los de la quinasa dirigidos contra EGFR y BRAF. Las respuestas clínicas a los inhibidores de PI3K en monoterapia no respaldan el uso clínico de las alteraciones en *PIK3CA* como marcadores predictivos en la selección de pacientes. Estas observaciones han tenido un claro impacto a la hora de continuar desarrollando estos fármacos. Para poder desarrollar con éxito estos agentes, anteriormente habría que analizar algunos aspectos farmacológicos, biológicos y traslacionales, con el fin de conocer mejor cómo aplicarlos correctamente.

Para probar la validez de las mutaciones de *PIK3CA* como marcadores predictivos de sensibilidad a los inhibidores de PI3K, así como de otras combinaciones fármaco-biomarcador, y ayudar a tomar decisiones terapéuticas en el contexto del cáncer avanzado, creamos un programa local multiplexado de preselección de pacientes. En primer lugar, empleamos la plataforma de MassArray para comprobar que el programa se adecuaba a su propósito. El programa facilitó el reclutamiento de pacientes para la realización de ensayos clínicos con inhibidores de PI3K. No obstante, cuando aparecieron las plataformas de secuenciación de próxima generación y se mejoraron los tiempos de respuesta, probamos su validez para analizar las muestras de los pacientes. En dos pacientes con mutaciones de *PIK3CA* pudimos detectar los mecanismos de resistencia tras producirse recaída. A continuación, rediseñamos nuestro programa de preselección y adaptamos las plataformas de secuenciación de próxima generación para convertirlas en herramientas de genotipado para facilitar la toma de decisiones terapéuticas en un modelo orientado al paciente.

Tras una primera ronda de ensayos clínicos con inhibidores de vías PI3K, quedaron algunos aspectos sin resolver. Dado el moderado beneficio clínico observado tras la exposición de los pacientes a los pan-inhibidores de PI3K en monoterapia, las muestras clínicas de pacientes tratados con inhibidores de PI3K (con o sin alteraciones en PI3K) se han convertido en valiosas herramientas para identificar los mecanismos de sensibilidad a terapia. De este modo, se utilizaron biopsias de tumores en tratamiento para analizar los mecanismos de acción y definir una dosis biológica óptima, ya que consideramos que estas muestras clínicas podían ayudar a orientar nuestras investigaciones en la dirección correcta (en contraposición con la investigación básica basada en líneas celulares, xenoinjertos o ratones transgénicos que, según se ha demostrado, tienen un valor predictivo limitado). En colaboración con los laboratorios translacionales del VHIR o mediante colaboraciones externas, estas muestras de pacientes sirvieron en muchas ocasiones como punto de partida o, al menos, como referencia orientativa para la investigación preclínica translacional. En la tabla 11 del Anexo 1 se puede ver el resultado de estos esfuerzos, aquí resumidos:

- Tanto Buparlisib como Pilaralisib causaron algunos efectos biológicos inesperados cuya causa se consideró que serían inespecíficos. Por un lado, Buparlisib mostró efectos antitubulares, lo que explica la aparición de algunas toxicidades como trastornos en el estado de ánimo y otros efectos secundarios neuropsiquiátricos. Por otro lado, Pilaralisib mostró tener un efecto inhibidor de la vía MAPK, tal como indicaba la reducción de la señalización por MEK y ERK. Todo esto despertó el interés en inhibidores de PI3K más específicos como INK1117 y Alpelisib, ambos con una alta especificidad hacia la isoforma alfa de PI3K.
- La limitada inhibición de la señalización obtenida a través de tratamiento con Pilaralisib, Buparlisib y Alpelisib (entre el 30 y el 90%, dependiendo del marcador y del fármaco evaluado), podría deberse a la aparición de efectos secundarios que impiden lograr una

inhibición más completa de la vía, o a la presencia de vías de señalización paralelas que podrían burlar la señalización de PI3K. En colaboración con el laboratorio de José Baselga, observamos en las muestras de algunos pacientes una señalización de mTOR permanentemente activa a pesar del tratamiento con inhibidores de PI3K. La investigación preclínica apuntó a la activación de la vía a través de la expresión de IGF1 y/o neuregulina 1(112), lo cual apuntaba al uso de un inhibidor de PI3K en combinación con un inhibidor de mTOR, resultando esta ser sinérgica en modelos preclínicos. Actualmente se está explorando esta hipótesis en dos ensayos clínicos (NCT01899053 y NCT02077933).

- El paciente que más respondió al tratamiento fue un paciente con cáncer de mama triple negativo. Preclínicamente, en esta enfermedad (cáncer de mama estrógeno, progesterona y HER2 negativo), la inhibición de PI3K parecía alterar la expresión de BRCA1/2 y sensibilizar el cáncer de mama triple negativo con BRCA normal a la inhibición del PARP(113). Un ensayo clínico financiado con la beca “Targeting PI3K in Women’s Cancers” del programa Stand-Up-To Cancer de la American Association for Cancer Research trasladó estas observaciones a un ensayo clínico (NCT01623349) en el que se investigó la combinación de olaparib, un inhibidor del PARP, y Buparlisib en el cáncer de mama triple negativo y el cáncer de ovario.
- Dado que la vía PI3K está compuesta por una compleja red de interacciones con cascadas paralelas, su inhibición farmacológica libera una retroalimentación negativa mediante la cual se activan las vías de señalización compensatorias. En contextos preclínicos, se han descrito multitud de mecanismos compensatorios, como la reactivación en retroalimentación dependiente de FOXO de receptores de tirosina quinasa (como HER2, HER3, IGF1R y el receptor de la insulina) y las quinasas descendentes como ERK, amplificación del MYC, y la activación de las vías NOTCH o Wnt-β-catenina(93, 114-120). Estos hallazgos se realizaron en experimentos *in vitro* en el laboratorio, por lo que fue

necesario validarlos en un contexto clínico para poder obtener conclusiones más firmes.

En el cáncer colorrectal, donde la mutación del *PIK3CA* es bastante frecuente, la resistencia a PI3K parece generalizada. Este fenómeno podría estar relacionado con la activación de la vía WNT, tal como indican los estudios preclínicos(93) y las abundantes evidencias clínicas circunstanciales obtenidas (como las frecuentes mutaciones de APC (121)en los tumores resistentes y la aparición de mutaciones activantes en la muestra de una paciente con cáncer cervical en recidiva que inicialmente respondió al inhibidor de PI3K). Estas observaciones alentaron a establecer una colaboración con el laboratorio de Hector Palmer para analizar la activación de la vía WNT (medida mediante β-catenina intranuclear) en muestras de pacientes tratados con inhibidores de PI3K. En este contexto, se observó una correlación entre la activación de la vía WNT y la resistencia a los inhibidores de PI3K (Arqués/Palmer, manuscrito en publicación). Los resultados obtenidos apuntan hacia la combinación de un inhibidor de la vía WNT, por ejemplo, de inhibidores de la tankirasa (aún en las primeras fases de desarrollo), con un inhibidor de la vía PI3K.

Estos resultados y la investigación posterior que suscitaron demuestran que los actuales ensayos de fase I ofrecen el contexto perfecto para la prueba de hipótesis. Junto con la evaluación de seguridad y toxicidad, se analizaron múltiples biomarcadores para determinar si se había dado en la diana y evaluar la respuesta biológica. Estos ensayos han demostrado ser un escenario adecuado para probar el valor predictivo de las mutaciones en el gen *PIK3CA*.



## 4. Segunda parte: El inhibidor de SHH Sonidegib, activación de la vía y desarrollo de una firma de activación de SHH

En esta segunda parte describimos los resultados de un ensayo clínico realizado por primera vez en humanos de Sonidegib, un inhibidor de la vía Hedgehog (Hh), en pacientes con tumores sólidos avanzados, incluido el carcinoma de células basales y el meduloblastoma (sección 4.1). Así mismo, se describen los esfuerzos realizados por determinar la población de pacientes que puede obtener beneficio clínico de este agente (anexo 2).

Cuando iniciamos este trabajo, no estaba claro qué pacientes se podían beneficiar de los inhibidores de Sonic Hedgehog. Las mutaciones de pérdida de función en los reguladores Patched 1 (*PTCH1*) y/o las mutaciones del receptor supresor de Fused (*SUFU*) parecieron responder a la inhibición de la vía Hh, aunque se han descrito otras formas de activar la vía SHH, como las mutaciones de ganancia de función en el regulador positivo Smoothened (*SMO*) y la sobreexpresión del ligando Hh mediante secreción autocrina o paracrina. En el caso del meduloblastoma, por ejemplo, en los estudios para la determinación del perfil de expresión génica se identificaron cuatro subtipos principales, uno de los cuales se caracteriza por la activación de la vía hedgehog (SHH). Aunque solo se hallaron mutaciones en la vía Hh en los casos de la subclase SHH, algunos casos se observaron con activación de SHH en ausencia de mutaciones de la vía Hh detectables. Por lo tanto, parece que la expresión génica tiene mayor valor de predicción de sensibilidad a los inhibidores de *SMO* que el análisis mutacional. Sin embargo, no se dispone de ninguna prueba diagnóstica aplicable para este propósito. De este modo, actualmente no existía ningún método estándar para la identificación de pacientes dentro de los subtipos moleculares de meduloblastoma en el contexto clínico.

Al iniciar el estudio no disponíamos de datos que respaldaran la exclusión de ningún tipo concreto de pacientes, y para identificar a los pacientes con mayor probabilidad de obtener

beneficio clínico, en algunos casos se consideró que era suficiente con aplicar criterios histológicos (considerando activación de SHH en el 90% de los carcinomas de células basales y en el 40% de los meduloblastomas) en lugar del análisis mutacional. De este modo, en este estudio se incluyeron todos los tipos de tumores. Se hizo, eso sí especial hincapie en la inclusión de pacientes con histologías consideradas dependientes de la vía de señalización Hh. Realizamos un análisis retrospectivo de las muestras de los pacientes con el fin de determinar marcadores predictivos (estrategia 1 según descrito en la Introducción) como, por ejemplo, que los tumores con una señalización excesivamente activa de Hh (autocrina/pracrina) o con un perfil de expresión genómica fueran más sensibles al tratamiento con Sonidegib.

#### **4.1 Evaluación clínica de la seguridad, farmacología (farmacocinética y farmacodinamia) y marcadores predictivos de un inhibidor de SMO: Sonidegib o LDE225.**

Sonidegib (LDE225), un nuevo inhibidor selectivo de SMO fue identificado en un cribado celular de alto rendimiento. Las investigaciones preclínicas mostraron una alta penetración en los tejidos (incluyendo la barrera sangre-cerebro) y una buena biodisponibilidad oral, la supresión completa del homólogo 1 del oncogen asociado al glioma (GLI1) y regresión tumoral en los modelos de meduloblastoma en ratones Ptch<sup>+/−</sup> p53<sup>−/−</sup> and Ptch<sup>+/−</sup> Hic1<sup>+/−</sup> (hipermetilado en el cáncer 1)(73).

En este artículo presentamos los resultados del primer estudio de fase 1 con escalada de la dosis realizado en humanos con Sonidegib en pacientes adultos con tumores sólidos avanzados.

*4.1.1 Tercer artículo: Este es el primer estudio multicéntrico, abierto, de fase 1 con aumento progresivo de la dosis realizado en humanos del inhibidor oral de Hedgehog Sonidegib (LDE225) en pacientes dultos con tumores sólidos avanzados.*

# Clinical Cancer Research

## A Phase I, Multicenter, Open-Label, First-in-Human, Dose-Escalation Study of the Oral Smoothened Inhibitor Sonidegib (LDE225) in Patients with Advanced Solid Tumors

Jordi Rodon, Hussein A. Tawbi, Anne L. Thomas, et al.

*Clin Cancer Res* 2014;20:1900-1909. Published OnlineFirst February 12, 2014.

**Updated version** Access the most recent version of this article at:  
doi: [10.1158/1078-0432.CCR-13-1710](https://doi.org/10.1158/1078-0432.CCR-13-1710)

**Supplementary Material** Access the most recent supplemental material at:  
<http://clincancerres.aacrjournals.org/content/suppl/2014/02/12/1078-0432.CCR-13-1710.DC1.html>

**Cited Articles** This article cites by 21 articles, 7 of which you can access for free at:  
<http://clincancerres.aacrjournals.org/content/20/7/1900.full.html#ref-list-1>

**Citing articles** This article has been cited by 3 HighWire-hosted articles. Access the articles at:  
<http://clincancerres.aacrjournals.org/content/20/7/1900.full.html#related-urls>

# A Phase I, Multicenter, Open-Label, First-in-Human, Dose-Escalation Study of the Oral Smoothened Inhibitor Sonidegib (LDE225) in Patients with Advanced Solid Tumors

Jordi Rodon<sup>1,-</sup>, Hussein A. Tawbi<sup>2,-</sup>, Anne L. Thomas<sup>3</sup>, Ronald G. Stoller<sup>2</sup>, Christian P. Turtschi<sup>4</sup>, Jose Baselga<sup>5</sup>, John Sarantopoulos<sup>6</sup>, Devalingam Mahalingam<sup>6</sup>, Yaping Shou<sup>7</sup>, Melissa A. Moles<sup>7</sup>, Lin Yang<sup>7</sup>, Camille Granvil<sup>8</sup>, Eunju Hurh<sup>7</sup>, Kristine L. Rose<sup>8</sup>, Dereck D. Amakye<sup>8</sup>, Reinhard Dummer<sup>4</sup>, and Alain C. Mita<sup>6,-</sup>

## Abstract

**Purpose:** This phase I trial was undertaken to determine the maximum tolerated dose (MTD), dose-limiting toxicities (DLT), safety, tolerability, pharmacokinetics, pharmacodynamics, and preliminary antitumor activity of the novel smoothened inhibitor sonidegib (LDE225), a potent inhibitor of hedgehog signaling, in patients with advanced solid tumors.

**Experimental Design:** Oral sonidegib was administered to 103 patients with advanced solid tumors, including medulloblastoma and basal cell carcinoma (BCC), at doses ranging from 100 to 3,000 mg daily and 250 to 750 mg twice daily, continuously, with a single-dose pharmacokinetics run-in period. Dose escalations were guided by a Bayesian logistic regression model. Safety, tolerability, efficacy, pharmacokinetics, and biomarkers in skin and tumor biopsies were assessed.

**Results:** The MTDs of sonidegib were 800 mg daily and 250 mg twice daily. The main DLT of reversible grade 3/4 elevated serum creatine kinase (18% of patients) was observed at doses  $\geq$  the MTD in an exposure-dependent manner. Common grade 1/2 adverse events included muscle spasm, myalgia, gastrointestinal toxicities, increased liver enzymes, fatigue, dysgeusia, and alopecia. Sonidegib exposure increased dose proportionally up to 400 mg daily, and displayed nonlinear pharmacokinetics at higher doses. Sonidegib exhibited exposure-dependent reduction in GLI1 mRNA expression. Tumor responses observed in patients with medulloblastoma and BCC were associated with evidence of hedgehog pathway activation.

**Conclusions:** Sonidegib has an acceptable safety profile in patients with advanced solid tumors and exhibits antitumor activity in advanced BCC and relapsed medulloblastoma, both of which are strongly associated with activated hedgehog pathway, as determined by gene expression. *Clin Cancer Res*; 20(7); 1900–9. © 2014 AACR.

## Introduction

The hedgehog signaling pathway plays a key role during embryo-fetal development of the brain, bones, and

**Authors' Affiliations:** <sup>1</sup>Vall d'Hebron Institut d'Oncologia and Universitat Autònoma de Barcelona, Barcelona, Spain; <sup>2</sup>University of Pittsburgh Cancer Institute and University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania; <sup>3</sup>University of Leicester, Leicester, United Kingdom; <sup>4</sup>University Hospital of Zurich, Zurich, Switzerland; <sup>5</sup>Memorial Sloan-Kettering Cancer Center, New York, New York; <sup>6</sup>Institute for Drug Development, Cancer Therapy and Research Center, University of Texas Health Science Center, San Antonio, Texas; <sup>7</sup>Novartis Institutes for BioMedical Research, Cambridge, Massachusetts; and <sup>8</sup>Novartis Pharmaceuticals Corporation, East Hanover, New Jersey

**Note:** Supplementary data for this article are available at Clinical Cancer Research Online (<http://clincancerres.aacrjournals.org/>).

Y. Shou and D.D. Amakye are no longer associated with Novartis.

<sup>-</sup>J. Rodon, H.A. Tawbi, and A.C. Mita contributed equally to this article.

**Corresponding Author:** Hussein Tawbi, University of Pittsburgh Cancer Institute, Division of Hematology/Oncology, 5150 Centre Avenue, Pittsburgh, PA 15232. Phone: 412-648-6578; Fax: 412-648-6579; E-mail: tawbih@upmc.edu  
doi: 10.1158/1078-0432.CCR-13-1710

© 2014 American Association for Cancer Research.

muscles (1). During the postnatal period and adulthood, hedgehog pathway activity is involved in the regulation of bone development, tissue maintenance and repair, and maintenance of stem cell populations (hair follicles; refs. 1 and 2). Aberrant hedgehog pathway activation has been linked with the pathogenesis of many human cancers through hedgehog ligand-dependent and ligand-independent mechanisms.

Genetic alterations, including loss-of-function mutations in the negative regulators patched 1 (PTCH1) and/or suppressor of fused (SUFU), or less frequently gain-of-function mutations in the positive regulator Smoothened (SMO), lead to ligand-independent pathway activation and have been linked to basal cell carcinoma (BCC), medulloblastoma, and rhabdomyosarcoma (2). Overexpression of hedgehog ligand has been observed in pancreatic, colorectal, lung, breast, prostate, esophageal, and gastric tumors (2). Therefore, the hedgehog pathway has become an attractive therapeutic target. Inhibitors targeting SMO, including vismodegib, which is approved by the U.S. Food and Drug Administration for the treatment of metastatic or locally advanced BCC, are currently being investigated in clinical trials (3–9).

## Translational Relevance

Aberrant hedgehog pathway activity has been linked to the pathogenesis of many cancers. The results of this phase I trial further advance the emerging clinical experience of hedgehog pathway inhibitors in patients with cancer. Oral sonidegib (LDE225) blocks the hedgehog pathway by selective inhibition of smoothened (SMO). Sonidegib exhibits an acceptable safety profile, exposure-dependent target inhibition, and clinically relevant antitumor effect in patients with locally advanced or metastatic basal cell carcinoma (BCC) and relapsed medulloblastoma. The toxicities identified are manageable and reversible upon discontinuation of treatment. Furthermore, a five-gene hedgehog signature assay demonstrated a strong association between tumor responses and hedgehog pathway activation, thus supporting its use as a patient selection tool in future studies. These data support ongoing clinical investigations of sonidegib as a single agent in BCC and hedgehog pathway-activated medulloblastoma, and as a combination partner with other agents in other malignant disease settings.

Sonidegib(LDE225),N-(6-((2S,6R)-2,6-dimethylmorpholino)pyridin-3-yl)-2-methyl-40-(trifluoromethoxy)biphenyl-3-carboxamide, a novel selective inhibitor of SMO, was identified in a cell-based high-throughput screen (Supplementary Fig. S1; ref. 10). Sonidegib demonstrated high tissue penetration (including blood–brain barrier) and good oral bioavailability in preclinical studies (10). Oral administration of sonidegib in mouse medulloblastoma models *Ptch<sup>b/-</sup> p53<sup>-/-</sup>* and *Ptch<sup>b/-</sup> Hic1<sup>b/-</sup>* (hypermethylated in cancer 1) resulted in complete suppression of glioma-associated oncogene homolog 1 (GLI1) and tumor regression, suggesting targeted inhibition of hedgehog signaling (11).

We report results from a first-in-human, dose-escalation, phase I study with sonidegib in adult patients with advanced solid tumors. The study population was enriched with patients with locally advanced or metastatic BCC and relapsed medulloblastoma. This study established the maximum tolerated dose (MTD) and dose-limiting toxicity (DLT) of continuous daily oral sonidegib administration. In addition, safety, pharmacokinetics, pharmacodynamics, and preliminary antitumor activity were assessed.

## Patients and Methods

### Patient population

Adult patients with histologically or cytologically confirmed advanced solid tumors, including medulloblastoma, whose disease progressed despite standard therapy or for whom no standard therapy was available were eligible. Other key inclusion criteria were measurable or evaluable disease defined by Response Evaluation Criteria In Solid Tumors (RECIST 1.0; ref. 12) or the Neuro-Oncology Criteria for Tumor Response (medulloblastoma only; refs. 13 and 14) and Eastern Cooperative Oncology Group (ECOG)

performance status  $\leq 2$ . In addition, all patients must have had adequate bone marrow (absolute neutrophil count  $\geq 1,500/\mu\text{L}$ , hemoglobin  $\geq 9\text{ g/dL}$ , and platelets  $\geq 100,000/\mu\text{L}$ ), liver (serum total bilirubin  $\leq 1.5 \times$  upper limit of normal [ULN] and aspartate aminotransferase and alanine aminotransferase  $\leq 2.5 \times$  ULN or  $\leq 5.0 \times$  ULN if liver metastases are present), and kidney function (serum creatinine  $\leq 1.5 \times$  ULN or 24-hour creatinine clearance of  $\geq 50\text{ mL/min}$ ). Patients were excluded if they had a history of a brain tumor or brain metastases (except relapsed medulloblastoma), clinically significant cardiac disease, or gastro-intestinal dysfunction that might impair sonidegib absorption. Treatment with strong inhibitors or inducers of cytochrome P450 (CYP) 3A4/5 or drugs metabolized by CYP2B6 or CYP2C9, which have a narrow therapeutic index, was prohibited during the study.

All patients provided written informed consent before enrollment. The study followed the ethical principles of the Declaration of Helsinki, the International Conference on Harmonisation Guideline for Good Clinical Practice, and local regulations (European Directive 2001/20/EC and US Code of Federal Regulations Title 21). The protocol and amendments were approved by the institutional review board, independent ethics committee, or research ethics board at each center.

### Study design

The primary objective of this dose escalation, multicenter, open-label phase I study was to determine the MTD and DLTs of oral sonidegib, administered on a continuous daily schedule. Additional objectives included safety, pharmacokinetics, pharmacodynamics, and antitumor activity. During dose escalation, all patients received a single oral dose of sonidegib in a 7-day pharmacokinetic run-in period to characterize the pharmacokinetic profile of sonidegib. Once the MTD was determined for the once-daily regimen, additional patients were enrolled to ensure a minimum of 22 patients were treated at the MTD to provide a 90% probability of detecting adverse events with an incidence of 10% and permit further assessment of pharmacokinetics, pharmacodynamic effects, and antitumor activity. Twice-daily dosing was also tested to explore the effect of dose fractionation and MTD.

Sequential cohorts of patients were treated with escalating doses of sonidegib once (100, 200, 400, 800, 1,000, 1,500, or 3,000 mg) or twice daily (250, 400, or 750 mg), continuously in a 28-day cycle. Twice-daily dosing was evaluated to address apparent solubility-limited absorption at doses  $>400\text{ mg}$  once daily. A minimum of 3 evaluable patients were required to make dose-escalation decisions after completing cycle 1. Additional patients were enrolled to allow for dropouts and to better define the safety, pharmacokinetics, or pharmacodynamics of sonidegib at a given dose. Enrollment of patients with medulloblastoma and advanced BCC was allowed at previously well-tolerated doses during the dose-escalation phase.

A 2-parameter Bayesian logistic regression model for escalation with overdose control was used to guide dose

escalation decisions (15, 16). A DLT was defined as a significant adverse event or abnormal laboratory parameter adjudged to be Common Terminology Criteria for Adverse Events (CTCAE version 3.0) grade \_3 in severity and considered unrelated to disease progression, intercurrent illness, or concomitant medications. The MTD was defined as the highest dose of sonidegib predicted to have <25% probability of a DLT rate of \_33% during cycle 1 (first 28 days). After tolerating the assigned dose for at least 2 cycles, intrapatient dose escalations were permitted. Dose escalation decisions were impacted by the emergence of late-onset, reversible grade 3/4 elevated serum creatine kinase (CK), occurring primarily during cycle 2.

#### Safety evaluations

Safety was assessed according to CTCAE version 3.0 guidelines (17). Assessments included regular laboratory evaluations, physical examinations, vital signs, weight, and periodic electrocardiogram recordings. All patients were monitored for safety from the first dose until 28 days after the final dose. Additional monitoring, including weekly serum creatine kinase during cycle 2 and on the first day of subsequent cycles, was implemented.

#### Pharmacokinetics assessments and analyses

Blood sample collection and handling. Blood samples for pharmacokinetic analyses were collected throughout the study. For the pharmacokinetic run-in period, serial blood samples were collected starting on day 1 (ending on day 5) at predose and 0.5, 1, 2, 4, 6, 8, 24, 48, 72, and 96 hours postdose. Serial blood samples were also collected on day 15 of cycle 1 at predose and 0.5, 1, 2, 4, 6, and 8 hours postdose. Blood samples were also collected predose on days 1, 8, 16, and 22 of cycle 1; days 1, 2, 8, 15, 16, and 22 of cycle 2; and day 1 of all subsequent cycles. Samples were processed and frozen at \_70°C within 90 minutes of the collection.

Preparation and analysis of plasma samples. Plasma samples were prepared using a protein precipitation extraction procedure, and sonidegib concentrations were determined using a validated liquid chromatography/tandem mass spectrometry (LC/MS-MS) assay using an API 5000 triple quadrupole mass spectrometer from AB Sciex equipped with an electrospray interface. Sample extracts were analyzed using gradient reverse-phase chromatography with a Capcell Pak C18 ACR, 150 \_ 4.6 mm ID, 5-mm particles (Shiseido Co Ltd.). The mobile phase consisting of water/0.1% ammoniac solution followed by acetonitrile/ isopropanol (8:2, v/v) was pumped through the column at a flow rate of 1.0 mL/min. Positive-ion multiple reaction monitoring (MRM) with a labeled internal control and a lower limit of quantitation of 0.0247 ng/mL (using 0.050 mL of plasma) was used for detection. The MRM transition monitored for sonidegib, and the labeled internal standard was m/z 486.07 to 428.08 and 490.07 to 432.08, respectively. The LC/MS-MS chromatograms of all analyzed baseline samples showed no interfering peaks, demonstrating selectivity of the method. Intraday and interday preci-

sion as represented by the coefficient of variation and accuracy as represented by the mean bias were within 20%. The validated method is suitable for the determination of sonidegib in human plasma.

Pharmacokinetics assessments. Pharmacokinetic parameters were calculated using noncompartmental methods with WinNonlin, version 5.2 (Pharsight). Peak plasma concentration ( $C_{max}$ ) and time to reach  $C_{max}$  ( $T_{max}$ ) were obtained from individual sonidegib concentration-time profiles. Area under the plasma concentration-time curve (AUC) values were calculated using the linear trapezoidal rule. Steady state was defined as a stable plasma trough concentration in at least 2 consecutive samples. Accumulation ratios were calculated by dividing the average plasma trough concentration at steady state by the trough concentration after the first dose.

#### Biomarker and antitumor evaluations

Fresh or archival tumor samples were collected when available, and biopsies of normal skin were collected from all patients before sonidegib treatment, at the end of cycles 1 and 2, and within 14 days after the last dose. RNA was extracted from tissue samples and analyzed by reverse transcriptase-PCR (RT-PCR) to measure GLI1 expression and hedgehog pathway activation status, based on the 5-gene hedgehog signature assay (18, 19).

All potential sites of tumor lesions were evaluated by computed tomography, magnetic resonance imaging, or physical examination (locally advanced BCCs) at baseline and every 8 weeks. Antitumor activity was determined according to RECIST 1.0 (12) and the Neuro-Oncology Criteria of Tumor Response (medulloblastoma only; refs. 13 and 14). [<sup>18</sup>F]-fluorodeoxyglucose positron emission tomography (FDG-PET) was performed in a subset of patients at baseline, day 28 of cycles 1 and 2, and posttreatment to supplement RECIST assessments. Percent changes from baseline standardized uptake value (SUV) using the average over lesions per patient were determined for patients with at least one lesion \_2 cm with a tumor-to-background ratio of \_2. Metabolic partial response was defined as a decrease of \_25% in summed maximum SUV in the target lesion as per the recommendations proposed by Weber and colleagues (20).

#### Statistical analyses

Demographics, safety, efficacy, and relevant pharmacokinetics and pharmacodynamic measurements were summarized using descriptive statistics and contingency tables. Study data included all patient data from the dose escalation and enrichment cohorts until all patients had completed at least 3 cycles of treatment or discontinued the study.

## Results

#### Patient demographics and clinical characteristics

A total of 103 patients, comprising 73 and 30 patients on the once-daily and twice-daily schedules, respectively, were enrolled between March 2009 and June 2011. Overall, 16 patients with BCC and 9 patients with medulloblastoma

**Table 1.** Demographic summary and disease characteristics at baseline

Baseline characteristics	All patients (n = 103)
Age, median years (range)	59.0(22–87)
Male sex, %	61.2
Primary site of cancer, n (%)	
Pancreas	19(18.4)
Colorectal	18(17.5)
Other gastrointestinal tumors <sup>a</sup>	8(7.8)
BCC	16(15.5)
Lung	10(9.7)
Medulloblastoma	9(8.7)
Genitourinary tumors <sup>b</sup>	5(4.9)
Breast	3(2.9)
Cutaneous melanoma	3(2.9)
Other <sup>c</sup>	12(11.7)
Prior antineoplastic therapies, n (%)	
Surgery	91(88.3)
Radiotherapy	47(45.6)
Systemic therapy <sup>d</sup>	96(93.2)
Prior systemic therapies <sup>d</sup>	
1	20(19.4)
2	22(21.4)
>2	54(52.4)
ECOG performance status, n (%)	
0	41(39.8)
1	55(53.4)
2	7(6.8)

<sup>a</sup>Other gastrointestinal tumors included cholangiocarcinoma (4), stomach (1), gall bladder (1), esophageal (1), and small intestine (1).

<sup>b</sup>Genitourinary tumors included cervix (1), ovary (1), endometrial (1), prostate (1), and renal (1).

<sup>c</sup>Other included leiomyosarcoma (2), germ cell (2), mesothelioma (2), Merkel cell carcinoma (1), spindle cell carcinoma (1), osteosarcoma (1), adenocarcinoma of unknown primary (1), ciliary body melanoma (1), and ampulloma (1). <sup>d</sup>Included chemotherapy, hormonal therapy, immuno-therapy, and targeted therapy (1 patient with BCC was previously treated with the topical formulation of sonidegib).

were treated. Primary tumor site, previous antineoplastic therapies, and ECOG performance status of patients enrolled are listed in Table 1.

#### Safety findings

Sonidegib was generally well tolerated with typically mild (grade 1/2) adverse events (Table 2). Patients comprising the dose-decision sets are listed in Supplementary Table S1. Most common treatment-related grade 1/2 adverse events experienced by >10% of patients included nausea, dysgeusia, anorexia, vomiting, muscle spasms, myalgia, increased serum creatine kinase, fatigue/asthenia, and alopecia, char-

acterized by gradual thinning of the hair. Notable grade 3/4 adverse events occurring in <5% of all treated patients included weight loss, myalgia, hyperbilirubinemia, dizziness, and asthenia. There were no deaths because of drug-related toxicities. Dose reduction occurred in 17 patients (17%), mostly treated at doses >800 mg. Twenty patients (19%) permanently discontinued treatment because of adverse events, mostly associated with creatine kinase elevation (14 of 20). Reversible grade 3/4 serum creatine kinase elevation was considered to be dose limiting in 19 patients (18%) at doses  $\geq$  800 mg once daily and  $\geq$  250 mg twice daily (Supplementary Table S1). These DLTs tended to occur 3 to 6 weeks after treatment initiation in an exposure-dependent manner (Fig. 1). Because of the delayed onset, reports of DLTs initially seemed to be limited to high-dose cohorts; however, after further evaluation in expanded cohorts, 2 of 26 patients and 2 of 14 patients experienced DLTs at 800 mg once daily and 250 mg twice daily, respectively. Thus, these doses fulfilled the prespecified criteria for MTD. In most cases, elevated creatine kinase was associated with myalgia. However, some patients reported myalgia and muscle spasm without creatine kinase elevation. Treatment-emergent creatine kinase elevation resolved within 4 to 8 weeks of drug discontinuation. No concurrent renal dysfunction was observed in any patient. Of the patients with creatine kinase elevation, 8 resumed treatment on a reduced dose without recurrence. Eight of 19 patients with DLTs also experienced grade 3/4 adverse events, including increased aspartate aminotransferase, alanine aminotransferase, or myoglobin; muscular weakness; and myositis. No clinically significant changes in creatine kinase-myocardial B isoenzyme, suggestive of cardiac muscle injury, were noted. In 3 cases, the DLTs were reported as rhabdomyolysis, primarily based on elevated blood creatine kinase–myoglobin levels without evidence of renal dysfunction. Creatine kinase elevation in these patients resolved following discontinuation of sonidegib. No additional therapy was required in 1 patient—the other 2 patients received sodium chloride (n = 1) or furosemide (n = 1). Treatment with sonidegib was not resumed in these patients.

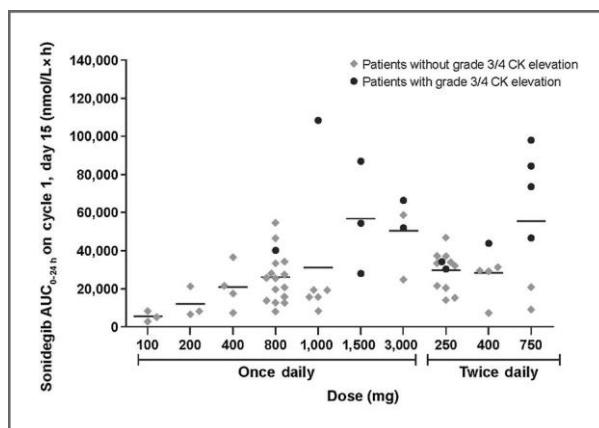
#### Pharmacokinetics

Pharmacokinetic parameters were calculated for 102 patients based on the single-dose pharmacokinetic run-in and for 82 patients on day 15 of daily dosing. Mean sonidegib plasma concentration-time profiles following the pharmacokinetic run-in period before initiating continuous dosing are presented (Supplementary Fig. S2). Relevant pharmacokinetic parameters derived from the plasma concentration-time curves on pharmacokinetic run-in and day 15 of cycle 1 are summarized in Table 3. Sonidegib was absorbed after oral administration, with a median  $T_{max}$  of 2 hours (range 1–48 hours) for all dosing regimens and doses combined. Sonidegib plasma exposure ( $C_{max}$  and AUC) after single-dose administration increased dose proportionally from 100 to 400 mg and less than dose proportionally above 400 mg. After repeated once-daily dosing from 100 to 3,000 mg,  $C_{max}$  and AUC on cycle 1,

Table 2. Most common adverse events (all grades,  $\geq 5\%$  incidence) suspected to be related to sonidegib treatment

Total adverse events (%) Grade 3/4 (%) <sup>a</sup>	Once-daily doses, mg							Twice-daily doses, mg			
	100 (n = 6)	200 (n = 6)	400 (n = 5)	800 (n = 26)	1,000 (n = 11)	1,500 (n = 9)	3,000 (n = 10)	250 (n = 14)	400 (n = 8)	750 (n = 8)	All (n = 103)
<b>Gastrointestinal toxicity</b>											
Nausea	3	1	0	4	3	4	2	3	1	5	26 (25.2)
Dysgeusia	1	1	0	5	3	3	5	6	3	3	30 (29.1)
Anorexia	2	1	1	8	4	0	4	5	3	2	30 (29.1)
				1							1 (1.0)
Vomiting	1	1	0	3	1	1	1	2	1	2	13 (12.6)
Diarrhea	0	0	0	2	0	0	1	1	1	2	7 (6.8)
Constipation	0	1	0	1	1	0	1	0	0	2	6 (5.8)
Muscle spasms	2	2	0	9	3	4	0	5	4	4	33 (32.0)
Myalgia	0	1	0	4	3	2	2	3	0	2	17 (16.5)
				1							1 (1.0)
Blood creatine kinase increased	1	1	0	7	4	3	4	3	4	6	33 (32.0)
Increased transaminases <sup>b</sup>	0	0	0	2	2	3	3	2	2	5	19 (18.4)
Fatigue/asthenia	5	2	0	6	4	3	0	4	3	1	28 (27.2)
				1	1	1					3 (2.9)
Alopecia	1	1	0	4	1	2	1	1	2	0	13 (12.6)
Lethargy	0	0	0	3	1	0	1	1	0	1	7 (6.8)

<sup>a</sup>Italicized numbers indicate grade 3/4 adverse events.<sup>b</sup>Includes increased alanine aminotransferase or aspartate aminotransferase.



**Figure 1.** Relationship between sonidegib exposure and creatine kinase (CK) elevation. Sonidegib area under the plasma concentration–time curve from time 0 to 24 hours ( $AUC_{0-24\text{ h}}$ ) on day 15 of cycle 1 was plotted for each patient by dose cohort. Incidences of creatine kinase elevation were noted for each patient. Incidence of grade 3/4 creatine kinase elevation was associated with increased sonidegib exposure. Gray-filled diamonds indicate patients without grade 3 or 4 creatine kinase elevation; black-filled circles indicate patients who experienced grade 3 or 4 creatine kinase elevation. Black solid line indicates mean  $AUC$ .

day 15 increased approximately dose proportionally up to 400 mg and less than dose proportionally above 400 mg. After twice-daily dosing from 250 to 750 mg,  $C_{max}$  and  $AUC$  on cycle 1, day 15 increased less than dose proportionally. Twice-daily dosing resulted in higher systemic exposures compared with the equivalent once-daily regimen. The 7-day pharmacokinetics run-in phase implemented in this study was not long enough to allow for accurate estimation of the terminal half-life ( $t_{1/2}$ ), oral apparent clearance, or volume of distribution using noncompartmental methods. Based on the trough plasma concentration over time in patients monitored for a sufficiently long period without dose changes, steady state seemed variable and was achieved after 2 to 24 weeks of repeated dosing, with a median accumulation of 16-fold across the dose groups based on  $C_{min}$ . The estimated median effective elimination  $t_{1/2}$  of sonidegib, calculated on the basis of the accumulation ratio, was 11 days. The interpatient coefficients of variation for day 15  $C_{max}$  and  $AUC$  were 39% to 113% and 33% to 122%, respectively, across the dose range of 100 to 3,000 mg/day for all dosing regimens. At the MTD of 800 mg once daily and 250 mg twice daily, the day 15 exposures were similar, with coefficients of variation for

$C_{max}$  of 54% and 44% and  $AUC$  of 50% and 33%, respectively. The median accumulation ratio in 11 patients treated at 800 mg once daily was 16-fold. Increasing sonidegib dose and exposure were associated with increased odds of grade 3/4 creatine kinase elevation (Fig. 1 and Supplementary Fig. S3).

#### Target inhibition

Sonidegib treatment caused a reduction in GLI1 mRNA expression in tumor and skin (Fig. 2). Target inhibition in the tumor, as measured by GLI1 expression, was more

pronounced than in the skin when both tissues were available for analyses (Supplementary Fig. S4). In general, the degree of target inhibition increased in a dose- and expo-sure-dependent manner, consistent with the utility of GLI1 expression as a pharmacodynamic marker for hedgehog pathway activation. However, in the limited number of samples analyzed, the reduction in GLI1 expression did not correlate with tumor response (data not shown).

#### Antitumor activity

Ninety-nine patients (96%) were evaluable for tumor response. Partial tumor responses were observed over the dose range of 100 to 1,500 mg. Six of 16 patients with BCC (37.5%) and 3 of 9 patients with medulloblastoma (33%) achieved objective tumor responses (partial or complete response) according to RECIST and FDG-PET (Supplementary Table S2). In the 3 patients with medulloblastoma with a partial response, who were treated at 200, 800, and 1,500 mg once daily, duration of response ranged from 4 to 8 months. One patient with medulloblastoma, ages 25 years, with largely metastatic bone disease, did not have RECIST-measurable lesions; hence FDG-PET was used to monitor treatment effect. The metabolic partial response in this patient, maintained for 8 months, was associated with symptomatic improvement (reduction in bone pain). A patient with locally infiltrating BCC achieved a histologic complete response confirmed by multiple biopsies of the tumor and surrounding tissue after treatment at 400 mg twice daily (Fig. 3A). Partial responses were also observed in 5 patients with locally advanced or metastatic BCC (spread to the lungs), treated at 100, 800, or 1,000 mg once daily and 250 mg twice daily (Fig. 3B and C). Interestingly, the tumor burden of the patient who achieved a partial response at 250 mg twice daily continued to improve for several months after treatment discontinuation. In patients with BCC and medulloblastoma, there was a strong association between tumor response and hedgehog pathway activation, as determined by a 5-gene hedgehog signature RT-PCR assay (Supplementary Table S2; ref. 18). Best over-all response of stable disease was observed in 24 patients (23%), with duration of stable disease > 6 months in 3 patients with lung adenocarcinoma, spindle cell sarcoma, and BCC.

#### Discussion

Continuous daily oral administration of sonidegib exhibited an acceptable safety profile, exposure-dependent target inhibition, and antitumor activity. The vast majority of adverse events were mild to moderate in severity. Treatment-related adverse events were manageable and reversible after discontinuation of drug. The majority of treatment-related adverse events in this study have been similarly observed with other SMO inhibitors in phase I studies in patients with advanced solid tumors (3, 8). The toxicity profiles of these agents cannot be directly compared in the absence of head-to-head trials; however, the most commonly reported adverse events in >10% across the agents

Table 3. Summary of sonidegib pharmacokinetic parameters after a single dose on day 1 of pharmacokinetic run-in and repeated doses on day 15 of cycle 1

Pharmacokinetic run-in					
Dose, mg	N	C <sub>max</sub> , ng/mL mean (SD; CV%)	AUC <sub>0–168 h</sub> ng · h/mL Mean (SD; CV%)	T <sub>max</sub> , h <sup>a</sup>	Median (min–max)
100 once daily	6	85.8(52.3; 61)	1,880(1,150; 61)	2(1–24)	
200 once daily	6	160(115; 72)	3,670(2,130; 58)	2(2–48)	
400 once daily	5	267(239; 90)	7,450(8,530; 115)	4(4–4)	
800 once daily <sup>b</sup>	25	430(381; 89)	7,870(6,950; 88)	4(1–27)	
1,000 once daily	11	322(258; 80)	7,400(6,340; 86)	2(1–4)	
1,500 once daily	9	376(199; 53)	12,600(7,110; 56)	4(2–24)	
3,000 once daily	10	429(237, 55)	11,800(11,200; 95)	2(1–8)	
250 twice daily <sup>b</sup>	14	150(111; 74)	3,220(2,320; 72)	2(1–4)	
400 twice daily	8	334(300; 90)	7,530(7020; 93)	4(2–4)	
750 twice daily	8	226(180; 80)	6,920(7110; 103)	3(1–23)	
Day 15, cycle 1					
Dose, mg	n <sup>c</sup>	C <sub>max</sub> , ng/mL Mean (SD; CV%)	AUC <sub>0–24 h</sub> , ng · h/mL <sup>c</sup> Mean (SD; CV%)	T <sub>max</sub> , h <sup>a</sup>	Median (min–max)
100 once daily	3	155(63.4; 41)	2,690(1,340; 50)	4(2–6)	
200 once daily	5	269(163; 61)	5,920(3,890; 66)	4(0–6)	
400 once daily	4	558(286; 51)	10,200(5,880; 58)	13(1–24)	
800 once daily <sup>b</sup>	20	840(457; 54)	12,800(6,350; 50)	2(1–6)	
1,000 once daily	8	1,230(1400; 113)	15,200(18,500; 122)	4(2–6)	
1,500 once daily	8	1,320(657; 50)	27,400(14,300; 52)	5(2–24)	
3,000 once daily	6	1,670(1050, 62)	24,600(8770; 36)	3(0–21)	
250 twice daily <sup>b</sup>	13	807(353; 44)	14,500(4780; 33)	2(0–6)	
400 twice daily	7	864(333; 39)	13,800(6390; 46)	2(0–8)	
750 twice daily	8	1570(1020; 65)	26,900(17,300; 64)	4(0–8)	

Abbreviations: AUC<sub>0–168 h</sub>, area under the plasma concentration–time curve from time zero to 168 hours; AUC<sub>0–24 h</sub>, area under the plasma concentration–time curve from time zero to 24 hours; C<sub>max</sub>, maximum plasma drug concentration; T<sub>max</sub>, time to reach C<sub>max</sub>. AUC<sub>0–24 h</sub> for twice-daily doses are calculated as 2·AUC<sub>0–12 h</sub>.

11. Values are median (range) and arithmetic mean (SD; CV%) for all other parameters.

12. Bold values represent maximum tolerated dose for once-daily and twice-daily doses.

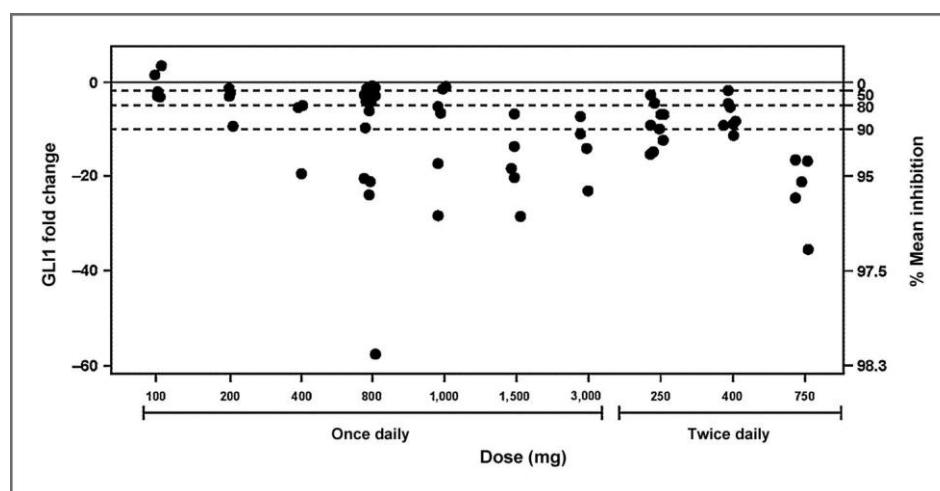
13. AUC analysis on cycle 1, day 15 included 3, 3, 4, 16, 6, 3, 4, 12, 5, and 6 patients from the 100, 200, 400, 800, 1,000, 1,500, and 3,000 mg once-daily and 250, 400, and 750 mg twice-daily dose cohorts, respectively.

include muscle spasms, dysgeusia, fatigue, and alopecia (3, 8).

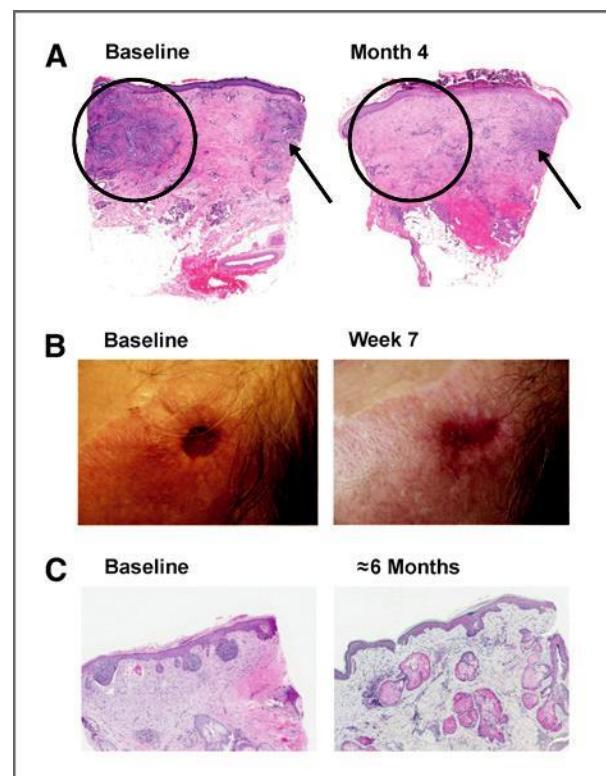
Current understanding of the role for hedgehog signaling suggests that the observed slowly evolving diffuse alopecia, dysgeusia, and muscle-related events are likely mechanistic on-target toxicities (21–23). SMO inhibitors have been shown to induce muscle contraction and muscle fiber twitching in primary human muscle cells, which is thought to be due effects on calcium influx, thus providing a potential mechanism for the muscle spasms observed in patients treated with sonidegib in this study (23). Reversible dose-limiting creatine kinase elevation of skeletal muscle origin (based on total creatine kinase/creatinine kinase–myocardial B ratio) occurred in 18% of patients across all doses (<10% at the MTD, 800 mg once daily), with no evidence of cardiac muscle injury. Overall, hyperCKemia (without evidence of renal impairment) was reported in 46% of patients with

normal creatine kinase at baseline. Six patients with creatine kinase elevation also had grade 3/4 increases in serum transaminases without significant changes in other liver function tests, thus suggesting skeletal muscle origin. High drug exposure was associated with increased odds of grade 3/4 creatine kinase elevations (Fig. 1 and Supplementary Fig. S3). Although resolution of creatine kinase levels was slower than expected for the known half-life of creatine kinase, it was not entirely consistent with the long half-life of sonidegib. Some patients had resolution of creatine kinase despite maintaining high drug concentrations. In addition, recurrence was not observed on retreatment at a reduced dose. For the 3 patients with DLTs documented as rhabdomyolysis, creatine kinase elevation resolved following discontinuation of treatment with supportive care (sodium chloride or furosemide in 2 of the 3 patients). Furosemide was administered as a precaution, apparently to boost

**Figure 2.** Glioma-associated oncogene homolog 1 (GLI1) fold change and percent inhibition in normal skin by dose cohort after sonidegib treatment. GLI1 expression was analyzed in patient skin specimens before and after treatment with sonidegib. Fold change from baseline was determined and plotted by dose cohort. Sonidegib treatment induced a dose-dependent decrease in GLI1 expression. Dotted lines represent 50%, 60%, and 90% mean inhibition.



urinary output in 1 patient, although there was no evidence of impaired renal function. Not surprisingly, there was no clear relationship between the incidence of muscle cramps/ spasms and hyperCKemia, as many patients experience muscle cramps/spasms without creatine kinase elevation



**Figure 3.** Responses in patients with BCC treated with sonidegib. A, IHC of a 76-year-old male patient with BCC treated with 400 mg twice daily. Histologically confirmed complete response was noted after 4 months of treatment. Photographs (B) and IHC (C) of BCCs in a 55-year-old male patient with Gorlin syndrome treated with 800 mg once daily. Partial response was observed after 6 months. Circles in A highlight the presence or absence of tumor tissue; arrows in A highlight fibrosis.

following SMO inhibitor treatments (3, 8). Other drugs with potential to cause toxic myopathy should be used in caution with SMO inhibitors (24).

The underlying reason for the relatively long half-life of sonidegib is unknown, although tight tissue and/or plasma protein binding can be speculated. High-affinity protein binding was also shown to contribute to the long half-life (>7 days) of the SMO inhibitor vismodegib—a methane-sulfonyl benzamide identified in a high-throughput screen (25). Sonidegib does not exhibit a time-dependent pharmacokinetic profile. The drug accumulation pattern over time and extent of accumulation at steady state are consistent with *in vitro* data showing lack of induction or time-dependent inhibition of CYP enzymes (10). Sonidegib displayed nonlinear pharmacokinetics at higher doses, likely because of solubility-limited absorption, and not because of dose-dependent metabolism as shown by parallel decline of the plasma concentration profile across the dose range. Solubility-limited absorption also contributed to the non-linear pharmacokinetics observed for vismodegib, however, slow metabolic elimination was also a factor (25). Although twice-daily dosing provided a higher systemic exposure than equivalent once-daily doses, it did not seem to offer a clinically meaningful advantage over the once-daily regimen in this study. Therefore, the once-daily dosing regimen is currently recommended for further studies with sonidegib. However, the twice-daily dosing regimen may be considered in situations where a faster time to steady-state systemic concentration is desirable.

This proof-of-concept study demonstrated that sonidegib induced target inhibition and antitumor activity at well-tolerated drug exposures in patients with BCC and medulloblastoma, tumor types known to harbor activating mutations (2). Sonidegib exhibited dose- and exposure-dependent inhibition of GLI1 in tumor and normal skin biopsies. GLI1 inhibition at maximum drug exposure at steady state is expected to be higher than that observed at the end of cycle 1. Although GLI1 inhibition in other tumors was comparable to BCC (Supplementary Fig. S4), no objective responses were reported in these tumors. Similarly, GLI1

stromal expression in a patient with rectal cancer treated with sonidegib in a phase I study was reduced (8); however, this patient did not respond to treatment. The lack of response in these patients is most probably because of differences in the tumor dependency on hedgehog signaling (i.e., ligand-dependent vs. ligand-independent). In the case of ligand-dependent tumors, other factors and signaling pathways may be involved in tumorigenesis—therefore, inhibition of hedgehog signaling alone may not be enough to induce a response. In phase I studies, both sonidegib and vismodegib caused a reduction in GLI1 levels in approximately 74% of normal skin biopsies analyzed (3, 8). Taken together, these data suggest that GLI1 is an ideal marker for SMO inhibitor therapy, but not a marker for tumor response. Molecular alterations in other hedgehog pathway components in the patients who responded are unknown as mutational analyses were not conducted in this study; however, hedgehog pathway activity was assessed using the 5-gene hedgehog signature assay, an RT-PCR-based assay, in fresh-frozen paraffin-embedded tumor samples—a strong association between tumor response and activated hedgehog pathway was observed in patients with BCC and medulloblastoma (18, 19).

Similar responses in patients with advanced BCC (29%–58%) have been observed in other phase I and II studies of SMO inhibitors (3, 4). The wide range of responses in these studies may be due in part to differences in patient populations and the methods of tumor evaluation across studies. In particular, assessment of response in BCC is confounded by the presence of residual scarring or fibrosis, making the standard provisions of RECIST suboptimal.

To date, responses in medulloblastoma have been reported only for sonidegib and vismodegib (3, 18, 26–29). Importantly, all responses occurred in patients with hedgehog-activated medulloblastoma (18, 26–29). Complete and partial responses have been observed in patients with medulloblastoma in our study and in a phase I/II study of sonidegib in children with tumors thought to be dependent on hedgehog signaling (phase I) and children and adults with hedgehog-activated medulloblastoma (18). A dramatic but transient regression of systemic metastatic disease (primarily in the bone) was observed in an adult patient treated with vismodegib in the first-in-man phase I study and 3 of 20 adult patients achieved sustained responses in a phase II study in recurrent medulloblastoma (3, 27, 28). Antitumor activity was also observed in 1 pediatric patient with hedgehog-activated medulloblastoma treated with vismodegib in a phase I study (29).

In conclusion, sonidegib treatment at the MTD of 800 mg daily and 250 mg twice daily was well tolerated and demonstrated dose- and exposure-dependent target inhibition. The antitumor activity in BCC and medulloblastoma and mechanism-based toxicities observed demonstrate that

sonidegib effectively inhibits hedgehog signaling. These results support the ongoing development of single-agent sonidegib for treatment of advanced BCC and relapsed medulloblastoma, and further exploration in combination therapies in other cancers (30–33).

### Disclosure of Potential Conflicts of Interest

H.A. Tawbi acted as a consultant/advisor for Novartis. J. Baselga acted as a consultant/advisor for Novartis and received research funding from Novartis. R. Dummer has acted as a consultant/advisor for received honoraria from Novartis. M. Moles, L. Yang, C. Granvil, E. Hurh, and K. Rose are employees of Novartis and have ownership interest. Y. Shou was an employee of Novartis and has ownership interest. D. Amakye was an employee of Novartis. No potential conflicts of interest were disclosed by the other authors.

### Authors' Contributions

Conception and design: J. Rodon, H.A. Tawbi, J. Baselga, Y. Shou, L. Yang, C. Granvil

Development of methodology: H.A. Tawbi, J. Baselga, J. Sarantopoulos, Y. Shou, L. Yang, C. Granvil, D.D. Amakye

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): J. Rodon, H.A. Tawbi, A.L. Thomas, R.G. Stoller, C.P. Turtschi, J. Baselga, J. Sarantopoulos, D. Mahalingam, Y. Shou, M.A. Moles, L. Yang, C. Granvil, R. Dummer, A.C. Mita

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): J. Rodon, H.A. Tawbi, A.L. Thomas, C.P. Turtschi, J. Sarantopoulos, D. Mahalingam, Y. Shou, L. Yang, C. Granvil, E. Hurh, K.L. Rose, D.D. Amakye, R. Dummer, A.C. Mita

Writing, review, and/or revision of the manuscript: J. Rodon, H.A. Tawbi, A.L. Thomas, R.G. Stoller, J. Baselga, J. Sarantopoulos, D. Mahalingam, Y. Shou, M.A. Moles, L. Yang, C. Granvil, E. Hurh, K.L. Rose, D.D. Amakye, R. Dummer, A.C. Mita

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): H.A. Tawbi, D. Mahalingam, A.C. Mita

Study supervision: H.A. Tawbi, J. Sarantopoulos, D. Mahalingam, M.A. Moles, D.D. Amakye, A.C. Mita

### Acknowledgments

The authors thank P. O'Rourke from the Cancer Therapy and Research Center at The University of Texas, L. Felderer from the University Hospital of Zurich, and M. Beltran, R. Dienstmann, I. Brana, G. Argiles, and G. Sala from Vall d'Hebron Institut d'Oncologia in Barcelona, and V. Garcia-Patos from the Department of Dermatology, Hospital Universitari Vall d'Hebron in Barcelona for patient care and data collection. The authors also thank C. Emotte, T. Sharp, and D. Robinson from Novartis Pharmaceuticals Corporation for sample analysis, and J. Brechbill and K. Miller-Moslin for medical editorial assistance.

### Grant Support

Financial support for editorial assistance was provided by Novartis Pharmaceuticals Corporation. The Institute for Drug Development, Cancer Therapy and Research Center, University of Texas Health Science Center, San Antonio, Texas, is also funded by the Cancer Center Support grant P30CA054174. The University of Pittsburgh Cancer Institute shared resources that are supported in part by award P30CA047904 were used for this project. The UPCI-Clinical Translational Research Center supported by the Clinical Translational Science Institute under the award NIH/NCRR/CTSA Grant UL1 RR024153 was used for this project.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received June 21, 2013; revised December 3, 2013; accepted December 19, 2013; published OnlineFirst February 12, 2014.

### References

- Pasca di Magliano M, Hebrok M. Hedgehog signalling in cancer formation and maintenance. *Nat Rev Cancer* 2003;3:903–11.
- Teglund S, Toftgard R. Hedgehog beyond medulloblastoma and basal cell carcinoma. *Biochim Biophys Acta* 2010;1805:181–208.

28. Lorusso PM, Rudin CM, Reddy JC, Tibes R, Weiss GJ, Borad MJ, et al. Phase I trial of hedgehog pathway inhibitor GDC-0449 in patients with refractory, locally-advanced or metastatic solid tumors. *Clin Cancer Res* 2011;17:2502–11.
29. Sekulic A, Migden MR, Oro AE, Dirix L, Lewis KD, Hainsworth JD, et al. Efficacy and safety of vismodegib in advanced basal-cell carcinoma. *N Engl J Med* 2012;366:2171–9.
30. Tang JY, Mackay-Wiggan JM, Aszterbaum M, Yauch RL, Lindgren J, Chang K, et al. Inhibiting the hedgehog pathway in patients with the basal-cell nevus syndrome. *N Engl J Med* 2012;366:2180–8.
31. Erivedge prescribing information. 2012 [cited 2013 May 14] Available from: [http://www.accessdata.fda.gov/drugsatfda\\_docs/label/2012/203388lbl.pdf](http://www.accessdata.fda.gov/drugsatfda_docs/label/2012/203388lbl.pdf).
32. Siu LL, Papadopoulos K, Alberts SR, Kirchoff-Ross R, Vakkalagadda B, Lang L, et al. A first-in-human, phase I study of an oral hedgehog pathway antagonist, BMS-833923 (XL139), in subjects with advanced or metastatic solid tumors. *J Clin Oncol* 2010 ASCO Ann Mtg Proc 2010;28:abstr 2501.
33. Jimeno A, Weiss GJ, Miller WH Jr, Gettinger S, Eigl BJ, Chang AL, et al. Phase I study of the hedgehog pathway inhibitor IPI-926 in adult patients with solid tumors. *Clin Cancer Res* 2013;19:2766–74.
34. Jamieson C, Cortes JE, Oehler V, Baccarani M, Kantarjian HM, Papayanidis C, et al. Phase I dose-escalation study of PF-04449913, an oral hedgehog (Hh) inhibitor, in patients with select hematologic malignancies. *Blood* ASH Ann Mtg Proc 2011;118:abstr 424.
35. Pan S, Wu X, Jiang J, Gao W, Wan Y, Cheng D, et al. Discovery of NVP-LDE225, a potent and selective smoothened antagonist. *Am Cancer Soc Med Chem Lett* 2010;1:130–4.
36. Buonomici S, Williams J, Morrissey M, Wang A, Guo R, Vattay A, et al. Interfering with resistance to smoothened antagonists by inhibition of the PI3K pathway in medulloblastoma. *Sci Transl Med* 2010;2:51ra70.
37. Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, et al. New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. *J Natl Cancer Inst* 2000;92:205–16.
38. Macdonald DR, Cascino TL, Schold SC Jr, Cairncross JG. Response criteria for phase II studies of supratentorial malignant glioma. *J Clin Oncol* 1990;8:1277–80.
39. Wen PY, Macdonald DR, Reardon DA, Cloughesy TF, Sorenson AG, Galanis E, et al. Updated response assessment criteria for high-grade gliomas: response assessment in neuro-oncology working group. *J Clin Oncol* 2010;28:1963–72.
40. Babb J, Rogatko A, Zacks S. Cancer phase I clinical trials: efficient dose escalation with overdose control. *Stat Med* 1998;17:1103–20.
41. Neuenschwander B, Branson M, Gsponer T. Critical aspects of the Bayesian approach to phase I cancer trials. *Stat Med* 2008;27:2420–39.
42. Trott A, Colevas AD, Setser A, Rusch V, Jaques D, Budach V, et al. CTCAE v3.0: development of a comprehensive grading system for the adverse effects of cancer treatment. *Semin Radiat Oncol* 2003;13: 176–81.
43. Shou Y, Robinson D, Amakye D, Rose K, Cho Y, Ligon KL, et al. A five-gene Hedgehog signature developed as a patient preselection tool for Hedgehog inhibitor therapy in medulloblastoma. *Clin Cancer Res*. In press.
19. Amakye D, Robinson D, Rose K, Cho J, Ligon KL, Sharp T, et al. The predictive value of a 5-gene signature as a patient pre-selection tool in medulloblastoma for Hedgehog pathway inhibitor therapy. *Am Assoc Cancer Res Congress* 2012;72:abstr 4818.
20. Weber WA, Petersen V, Schmidt B, Tyndale-Hines L, Link T, Peschel C, et al. Positron emission tomography in non-small-cell lung cancer: prediction of response to chemotherapy by quantitative assessment of glucose use. *J Clin Oncol* 2003;21:2651–7.
21. Rittie L, Stoll SW, Kang S, Voorhees JJ, Fisher GJ. Hedgehog signaling maintains hair follicle stem cell phenotype in young and aged human skin. *Aging Cell* 2009;8:738–51.
22. Liu HX, MacCallum DK, Edwards C, Gaffield W, Mistretta CM. Sonic hedgehog exerts distinct, stage-specific effects on tongue and taste papilla development. *Dev Biol* 2004;276:280–300.
23. Teperino R, Amann S, Bayer M, McGee SL, Loipetzberger A, Connor T, et al. Hedgehog partial agonism drives Warburg-like metabolism in muscle and brown fat. *Cell* 2012;151:414–26.
24. Pasternak RC, Smith SC Jr, Bairey-Merz CN, Grundy SM, Cleeman JL, Lenfant C, et al. ACC/AHA/NHLBI clinical advisory on the use and safety of statins. *J Am Coll Cardiol* 2002;40:567–72.
25. Graham RA, Lum BL, Cheeti S, Jin JY, Jorga K, Von Hoff DD, et al. Pharmacokinetics of hedgehog pathway inhibitor GDC-0449 in patients with locally-advanced or metastatic solid tumors: the role of alpha-1-acid glycoprotein binding. *Clin Cancer Res* 2011;17: 2512–20.
26. Geoerger B, Aerts I, Casanova M, Chisholm J, Hargrave D, Leary SES, et al. Updated results from a phase I study of LDE225, a smoothened antagonist, in pediatric patients with recurrent medulloblastoma or other solid tumors. International Society of Paediatric Oncology meeting abstracts 2012:abstr Q037.
27. Rudin CM, Hann CL, Laterra J, Yauch RL, Callahan CA, Fu L, et al. Treatment of medulloblastoma with hedgehog pathway inhibitor GDC-0449. *N Engl J Med* 2009;361:1173–8.
28. Gajjar AJ, Gururangan S, Qaddumi IA, Packer R, Goldman S, Prados M, et al. A prospective phase II study to determine the efficacy of GDC 0449 (vismodegib) in adults with recurrent medulloblastoma (MB): a pediatric brain tumor consortium study (PBTC 25B). *J Clin Oncol* 2013 ASCO Ann Mtg Proc 2013;31:abstr 2035.
29. Gajjar A, Stewart CF, Ellison DW, Kaste S, Kun LE, Packer RJ, et al. Phase I study of vismodegib in children with recurrent or refractory medulloblastoma: a pediatric brain tumor consortium study. *Clin Cancer Res* 2013;19:6305–12.
30. ClinicalTrials.gov. A phase II study of efficacy and safety in patients with locally advanced or metastatic basal cell carcinoma (BOLT). 2011 [cited 2013 May 14]. Available from: <http://ClinicalTrials.gov/show/NCT01327053>.
31. ClinicalTrials.gov. Efficacy, safety and pharmacokinetics of oral LDE225 in treatment of patients with nevoid basal cell carcinoma syndrome (NBCCS BCC). 2011 [cited 2013 May 14]. Available from: <http://clinicaltrials.gov/ct2/show/NCT01350115>.
32. ClinicalTrials.gov. A dose finding and safety study of oral LDE225 in children. 2010 [cited 2013 May 14]. Available from: <http://ClinicalTrials.gov/show/NCT01125800>.
33. ClinicalTrials.gov. A phase III study of oral LDE225 versus (vs) temozolamide (TMZ) in patients with hedgehog (Hh)-pathway activated relapsed medulloblastoma (MB). 2012 [cited 2013 May 14]. Available from: <http://www.clinicaltrials.gov/ct2/show/NCT01708174>.

En los estudios de determinación de perfil de expresión génica se han identificado cuatro subgrupos moleculares de meduloblastoma entre los que se incluye uno caracterizado por la activación de la señalización de Hedgehog (Hh). Aunque en este estudio hemos demostrado la actividad de los inhibidores de la vía Hh como sonidegib (LDE225) en pacientes con meduloblastoma, actualmente no existe ningún método estándar para la identificación de los subtipos moleculares de en esta enfermedad, y los métodos actuales de clasificación de pacientes no son válidos en el contexto de los estudios clínicos: los métodos de determinación del perfil génico precisan muestras tumorales de gran tamaño y no son eficaces con tejido fijado en formol e incluido en parafina. Del mismo modo, para la preselección de pacientes mediante secuenciación génica directa es necesario realizar un cribado mutacional de genes de la vía Hh, como *PTCH1* y *SMO* que resultan ser de gran tamaño y complejidad, ya que no disponen de puntos calientes donde se acumulen las mutaciones. Además, la correlación entre el estado mutacional y la activación de la vía no está clara, dada la observación de casos de meduloblastomas con SHH activado en ausencia de mutaciones de la vía Hh.

En el artículo descrito en el anexo 2, describimos el desarrollo de una prueba de firma génica en la que se analiza la expresión de cinco genes mediante RT-PCT para identificar pacientes con meduloblastoma con la vía Hh activada. Observamos que esta firma estaba asociada a la respuesta a tratamiento con sonidegib, lo que llevó a su aplicación como herramienta de preselección de pacientes en un ensayo clínico fase II posterior.

## 4.2 Resultados y discusión

### 4.2.1 Seguridad de Sonidegib y marcadores farmacodinámicos de la inhibición de la vía

La administración oral continuada de sonidegib mostró poseer un perfil de seguridad aceptable. Los efectos secundarios derivados del tratamiento se pudieron tratar y revertir tras la interrupción del mismo. Los efectos secundarios más habituales (en más del 10% de los pacientes) fueron los espasmos musculares, disgeusia, fatiga y alopecia.

Se observó una elevación de creatina quinasa (CK) de origen musculo-esquelético (basada en el índice total CK/CK-MB) en el 18% de los pacientes con todas las dosis (pero en < 10% de los pacientes en la dosis máxima tolerada, 80 mg una vez al día). La elevación de CK sin evidencia de insuficiencia renal ("hyperCKemia") se produjo en ≈ 46% de los pacientes y la exposición a altas dosis del fármaco se asoció a una mayor probabilidad de experimentar una elevación de CK de grado 3/4. Aunque también se observaron espasmos musculares, no se identificó una correlación clara entre éstos y la *hyperCKemia*.

Algunos de estos efectos secundarios, como la alopecia, la disgeusia y los espasmos musculares probablemente son toxicidades específicas de los inhibidores de SHH(122, 123), ya que la mayoría de ellos también se han dado en estudios de fase 1 con otros inhibidores de SMO en pacientes con tumores sólidos avanzados(123, 124).

El periodo de semidesintegración de sonidegib demostró ser prolongado, probablemente debido a la fuerte unión de las proteínas en los tejidos y/o el plasma, y a la ausencia de inducción o inhibición dependiente del tiempo de las enzimas CYP.

Demostramos que sonidegib inducía la inhibición de la diana molecular mediante la inhibición dosis-dependiente de GLI1 en biopsias tumorales y cutáneas. A pesar de la inhibición de GLI1 observada en los tumores, no esperábamos que ésta estuviera directamente correlacionada con la respuesta

tumoral, debido a las diferentes dependencias que los tumores muestran con respecto a la señalización de Hh (esto es, ligando-dependientes frente a ligando-independientes). En el caso de los tumores ligando-dependientes, puede que otros factores y vías de señalización participen en la tumorigénesis, y la inhibición de la señalización Hh por sí misma puede no ser suficiente para inducir una respuesta. En resumen, GLI1 parece ser un marcador ideal de la inhibición de SHH en las terapias de inhibición de SMO, aunque no es útil como marcador predictivo de respuesta tumoral.

#### ***4.2.2 Estudio de fase I de la eficacia y predictores de eficacia y firma de cinco genes.***

En el estudio fase I (sección 4.1) se observó un prometedor efecto antitumoral en pacientes con carcinoma de células basales (una respuesta completa y cinco respuestas parciales) y meduloblastoma (dos respuestas parciales y una respuesta parcial metabólica), unos tipos de cáncer que presentan activación de la vía Hh. Se desconocen las alteraciones moleculares que pudieran presentar los componentes de la vía Hh en los pacientes incluidos en este estudio de fase I, ya que no se realizaron análisis mutacionales completos. La razón es que se consideró que la vía Hh podría activarse a través de diferentes mecanismos y que el análisis mutacional completo en muestras en parafina no era factible debido a su complejidad, esto es, por la multitud de genes de gran tamaño implicados, como *PTCH*, *SMO* y *SUFU*, sin puntos calientes claros (base de datos COSMIC(125)).

En cuanto al desarrollo clínico posterior de Sonidegib y la selección de pacientes en subsiguientes estudios, el caso del meduloblastoma parecía más complicado que el del carcinoma basocelular. En este último la selección en base a la histología bastaría, ya que se ha sabe que el 90% de los carcinomas basocelulares presentan mutaciones en la vía SHH que inducen la activación ligando-independiente de la vía. Sin embargo, en el caso del meduloblastoma, estas mutaciones solo están presentes en una fracción de pacientes (alrededor del 25%) y aunque el perfil de expresión génica revela que hasta el 30% de los meduloblastomas tienen la vía Hh activada(126-128), no existe una

correlación clara entre la firma genética de SHH y las mutaciones en los genes *PTCH1*, *SMO* y *SUFU*(129).

Otros estudios, como un estudio con sonidegib en adultos asiáticos (NCT01208831), un estudio de sonidegib en niños (NCT01125800) y varios estudios de vismodegib en pacientes adultos y pediátricos, también hallaron actividad preliminar de los inhibidores de SHH en algunos pacientes con meduloblastoma(130). Esta actividad antitumoral, añadida a las limitaciones implícitas en el uso de análisis mutacionales o análisis de expresión génica mediante Affymetrix para la preselección de pacientes, nos llevó a colaborar con otros centros para desarrollar un ensayo en el que se empleara RT-PCR (reacción en cadena de la polimerasa de transcripción reversa) y que fuese válido para muestras tumorales conservadas en parafina.

Como se indica en el artículo "**Desarrollo de una firma de cinco genes de la vía Hedgehog como herramienta para la preselección de pacientes para terapia con inhibidores de Hedgehog en el meduloblastoma**", descrito en el anexo 2, aplicamos el enfoque por etapas descrito a continuación:

1. Desarrollo de la prueba de firma de cinco genes de la vía Hh
  - a. Selección de genes candidatos: análisis combinado de tres conjuntos de datos públicos de meduloblastoma.
  - b. Optimización de la prueba: selección de genes diana con expresiones diferenciales en pruebas RT-PCR, optimizadas para muestras fijadas en formalina y conservadas en parafina.
  - c. Construcción del modelo: construcción de un modelo multigenético predictivo mediante el método *elastic net* empleando 40 muestras tumorales fijadas en formalina y conservadas en parafina.
2. Validación independiente de la prueba de firma de cinco genes de la vía Hh

- a. Concordancia entre el estado de la vía Hh determinado mediante el perfil de expresión génica (usando la plataforma de Affymetrix) y prueba de firma de cinco genes de la vía Hh, en muestras pareadas frescas/fijadas en formalina y conservadas en parafina.
3. Validación clínica de la prueba de firma de cinco genes en la vía Hh
- a. Los datos clínicos y las biopsias de los tumores de pacientes con meduloblastoma recurrente de tres estudios de fase I fueron usadas para la validación clínica: el estudio X2101, realizado en adultos con tumores sólidos avanzados aquí descrito (NCT00880308), el estudio X1101, en adultos asiáticos con tumores sólidos avanzados (NCT01208831), y el estudio X2104, en niños con tumores sólidos avanzados (NCT01125800).

La prueba de firma de cinco genes (que finalmente incluyó a *GLI1*, *SPHK1*, *SHROOM2*, *PDLIM3* y *OTX2*) resultó extremadamente efectiva, con un 100% de concordancia con el perfil obtenido por Affymetrix, e identificando que la vía de señalización Hh en los tumores de los seis pacientes que respondieron a sonidegib se encontraba activada, e identificando como no activada la vía Hh en los tumores de los pacientes que no respondieron a tratamiento. Con la excepción de *GLI1*, el resto de genes identificados no habían sido identificados antes con la señalización canónica de SHH. Sin embargo *OTX2* sí es un conocido gen con expresión alterada en meduloblastomas. Partiendo de estos resultados, actualmente se está realizando un estudio clínico en el que se están testando tanto el test como el fármaco (NCT01708174), siguiendo las directrices de la FDA para el desarrollo simultáneo de fármacos y biomarcadores(131). En este estudio, pacientes con meduloblastoma SHH+ (en base al test de 5 genes), se randomizan a temozolamida o sonidegib.

No obstante y ante los espectaculares avances tecnológicos que actualmente se están produciendo(132), hoy en día el panorama parece haber cambiado. Cabe cuestionarnos si hoy

empleo de plataformas de secuenciación de próxima generación, capaces de secuenciar de 50 a 200 genes en muestras fijadas en formalina y conservadas en parafina, podrían capturar la mayoría de los tumores con la vía de señalización Hh activada(133). Sería interesante estudiar si una prueba de expresión genética como la aquí descrita es más efectiva a la hora de predecir la sensibilidad del tumor a los inhibidores de SHH que un análisis mutacional realizado con plataformas de secuenciación de segunda generación como las disponibles hoy en día.

#### **4.3 Conclusiones y líneas de investigación de seguimiento**

Los resultados de este estudio de fase 1 y el desarrollo de una prueba para la selección de pacientes con meduloblastoma suponen una aportación a las recientes experiencias clínicas con inhibidores de la vía Hh en pacientes oncológicos.

En nuestro estudio, demostramos que sonidegib (LDE225) bloquea la vía Hh mediante la selección selectiva de los receptores Smoothened, mostrando un perfil de seguridad aceptable y una inhibición de la diana dependiente de la exposición. Esto se traduce en un efecto antitumoral clínicamente relevante en pacientes con carcinoma de células basales avanzado o metastásico y meduloblastoma en recidiva. El test capaz de determinar una firma de cinco genes en la vía Hh demostró una fuerte asociación entre la respuesta tumoral y la activación de la vía Hh en el meduloblastoma, lo cual avala su uso en la selección de pacientes en futuros estudios. Partiendo de estos datos, actualmente se están realizando ensayos clínicos con sonidegib como agente único en pacientes con carcinoma de células basales y con meduloblastoma con la vía Hh activada, y empleado en combinación con otros agentes en pacientes oncológicos (ver anexo 3).

En el caso del meduloblastoma, a diferencia del carcinoma basocelular, la selección de pacientes mediante criterios histológicos no se consideró eficiente. Y, en contraste con otros casos donde el análisis mutacional fue considerado factible (Tabla 1), en este estudio desarrollamos una firma molecular que se pudiera trasladar al contexto clínico. El empleo de muestras de pacientes tratados

previamente en estudios de fase I como el descrito aquí resultó ser clave para la validación clínica de la prueba. La innegable utilidad clínica de esta prueba a la hora de analizar las muestras de estos pacientes desembocó en el traslado de la prueba y el fármaco directamente a un estudio de fase III (esto es, de un estudio de fase I a un estudio de fase III), acelerando así el desarrollo del fármaco.

Nuestra experiencia en el desarrollo clínico precoz de fármacos, en la realización de estudios de fase I y en la aplicación de estrategias de combinación así como en la experimentación con inhibidores de PI3K y SHH en concreto, unido a algunos datos preclínicos obtenidos nos alentó a proponer un estudio de fase I en el que se combinara Buparlisib con Sonidegib. Existían varias razones por las que combinar un inhibidor de PI3K (como Buparlisib) con un inhibidor de SHH (como Sonidegib):

- Atacar varias vías para obtener un efecto sumatorio, y quizás un efecto sinérgico, al inhibir simultáneamente las vías de PI3K y SHH en tipos de tumores en cuya carcinogénesis estén implicadas ambas vías (por ejemplo, el glioblastoma y el meduloblastoma(70, 134, 135)), así como ampliar la cantidad de tipos de tumores tratables, ya que con este método se pueden atacar dos poblaciones celulares distintas.
- Vencer a los mecanismos conocidos de resistencia. En el meduloblastoma, donde las alteraciones de SHH son causa del tumor, se ha observado que tras la inhibición de SMO, las células tumorales hiperactivan la vía PI3K como mecanismo de resistencia(136).
- Las células madre cancerosas son una subpoblación de células que muestran un fenotipo relacionado con las células madre embrionarias. En la última década se han descrito los mecanismos moleculares subyacentes en la carcinogénesis en el cáncer y la autorrenovación en las células madre y las principales vías de señalización implicadas tanto en las células madre cancerosas como en las embrionarias parecen ser las vías SHH, PI3K/AKT, Notch, Wnt y TGF-β, entre otras. Inhibiendo dos de las principales vías de señalización de células madre

cancerosas, se espera obtener un efecto sinérgico en esta extremadamente importante población de células tumorales.

Partiendo de esta sólida justificación para el uso combinado de Buparlisib y Sonidegib, propusimos la realización de un ensayo clínico de fase Ib iniciado por un investigador para determinar la(s) dosis máxima(s) tolerada(s) de Buparlisib y Sonidegib en combinación, cuando son administrados oralmente a pacientes adultos con tumores sólidos avanzados seleccionados (con especial hincapie en el glioblastoma y el meduloblastoma), y evaluar la actividad antitumoral preliminar de dicha combinación. Ante el interés de la propuesta, Novartis Pharmaceuticals aceptó financiar el ensayo, así como implementar esta idea en un estudio internacional multicéntrico de fase Ib (NCT01576666). Los resultados de este estudio, pendientes de publicación, se presentaron en el congreso ESMO 2014.



## 5. Conclusiones

### 5.1 Los estudios de fase I como entorno para probar hipótesis y realizar ensayos en poblaciones de pacientes seleccionados molecularmente.

Las recientes alteraciones genómicas descubiertas, subyacentes en los fenotipos malignos, unidas a un mayor repertorio de agentes dirigidos han abierto un abanico de oportunidades para el desarrollo de fármacos basado en criterios genómicos. La capacidad para obtener el perfil molecular de cada paciente para identificar aberraciones tratables de forma rentable y precisa mediante el uso de tecnologías de alto rendimiento planea un sinfín de posibilidades a la hora de intentar seleccionar la mejor terapia en base al estatus molecular (emparejar alteración molecular y terapia) y usar terapéuticas en una población de pacientes seleccionados(137). Aprovechar los avances en el diseño y ejecución de las investigaciones clínicas, pasando de las terapias basadas en la histología de los tumores a la oncología clínica molecular es una forma de hacer frente a la elevada tasa de renuencia en los ensayos orientados al desarrollo de nuevos agentes antitumorales(138, 139).

Desde esta perspectiva, la prueba precoz de hipótesis, en estudios de fase I, con el uso de biomarcadores de selección (aberraciones moleculares concretas que pueden predecir la respuesta tumoral) pueden demostrar la prueba de concepto y avalar posteriores investigaciones con un fármaco o diana molecular concreta en poblaciones de pacientes que más se puedan beneficiar.

Con el fin de optimizar el potencial beneficio clínico para cada paciente, en nuestra institución hemos desarrollado un programa interno de preselección molecular, abogando por un programa de análisis molecular local, multiplexado, optimizado para su propósito, con el fin de facilitar la toma de decisiones terapéuticas en pacientes que se estén planteando participar en un estudio, y reclutar a aquellos pacientes con mayor probabilidad de obtener beneficio clínico de cada terapia en estudio ("emparejar pacientes con ensayos"). Mediante esta estrategia, los estudios de fase I con agentes

dirigidos molecularmente son utilizados para la prueba temprana de hipótesis empleando biomarcadores, lo cual acelerará el desarrollo de nuevos fármacos.

Tal como se indica en este estudio, los datos obtenidos en estos estudios de fase I resultaron clave para el desarrollo de Pilaralisib, Buparlisib y Sonidegib:

- De este modo, pudimos demostrar que el tratamiento ya sea con Pilaralisib o con Buparlisib es seguro y tolerable a dosis suficientes para inhibir la vía de señalización PI3K. Así lo demostraron marcadores farmacodinámicos como los marcadores metabólicos (insulina, péptido C, imágenes de PET) y los biomarcadores de señalización celular analizados en tejidos vicarios (*surrogate tissue*) como piel (Buparlisib y Pilaralisib) y cabello (Pilaralisib), así como especímenes de biopsias de tumores (Pilaralisib y Buparlisib).
- En nuestros estudios hemos demostrado que tanto Pilaralisib como Buparlisib (así como otros inhibidores de PI3K incluidos en nuestro programa de desarrollo clínico precoz de fármacos) tiene una considerable actividad antitumoral.
  - Estas observaciones permitieron seguir investigando con inhibidores de PI3K en estudio de fase Ib, II y III con múltiples histologías (Anexo 3).
- Una estrategia de análisis molecular extenso orientada al paciente es viable y avala el desarrollo de un programa de Medicina Genómica (anexo 4).
- La actividad antitumoral de los inhibidores de PI3K no parece estar correlacionada con las mutaciones de *PIK3CA*, lo que contradice multitud de datos preclínicos publicados, al menos en el caso de los inhibidores de PI3K/mTOR y inhibidores pan-PI3K.
  - Esta observación desencadenó un importante cambio en la estrategia aplicada en el desarrollo de fármacos inhibidores de PI3K. Por un lado, nuestras observaciones sugieren que el análisis solo de mutaciones de *PIK3CA* como único elemento

activador de la vía es simplista y reduccionista, por lo que se generando multiples estudios más exhaustivos de las alteraciones de la vía. Por otro lado, múltiples ensayos clínicos posteriores cambiaron de estrategia y se centraron en poblaciones de pacientes no seleccionados con la esperanza de estudiar de forma retrospectiva las alteraciones de la vía (lo que implica el pasar de la estrategia 2 a la 1 según descrito en la Introducción).

- Sonidegib bloquea la vía Hh mediante la selección selectiva de los receptores Smoothened, mostrando una inhibición de la diana dependiente de la dosis. Esto quedó demostrado al producirse una reducción de la expresión de mRNA en el gen GLI1 en piel y tejido tumoral.
- Los pacientes presentaron buena tolerancia al tratamiento con sonidegib, que mostró actividad preliminar en pacientes con cáncer avanzado, especialmente en el caso del carcinoma basocelular y el meduloblastoma.
- Pudimos desarrollar un test que permita evaluar, mediante la determinación de una firma de cinco genes, la activación de la vía Hh. La utilidad clínica de esta firma para detectar pacientes que se puedan beneficiar de sonidegib quedó demostrada con los datos obtenidos en el estudio de fase I.
  - Esta observación permitió pasar rápidamente de un estudio de fase I a un estudio de fase III donde actualmente se están evaluando tanto la prueba como la actividad del fármaco en el meduloblastoma (NCT01708174).
- La experiencia del programa de preselección molecular mediante MassArray, así como con el desarrollo de ambos tipos de inhibidores (de PI3K y SHH) resultaron clave a la hora de adaptar las nuevas plataformas de secuenciacion de próxima generación en nuestro programa de prescreening molecular. Así, hoy en dia podemos determinar con mayor

eficacia el perfil de alteraciones moleculares complejas que pueden servir como marcadores predictivos de sensibilidad a agentes dirigidos.

## 5.2 Evolución del programa de prescreening molecular fundamentada en las experiencias aquí descritas

Ideamos un programa de análisis molecular para identificar las posibles dianas terapéuticas asociadas a la vulnerabilidad del tumor como proceso dinámico y poliédrico. El proceso comenzaría identificando los estudios clínicos y las dianas terapéuticas (por ejemplo, inhibidores de PI3K) disponibles en nuestro Programa de Desarrollo Clínico Precoz de fármacos para los pacientes, así como las alteraciones que influyen en la sensibilidad tumoral y que pueden ser analizadas (mutaciones de *PIK3CA*, pérdida de función del gen *PTEN*...). Conforme la tecnología y el conocimiento vayan avanzando, se irían identificando nuevos mecanismos de vulnerabilidad o resistencia tumoral a un fármaco dirigido concreto (como las mutaciones *KRAS* concomitantes) y se introducirían nuevas plataformas diagnósticas, junto con nuevas dianas y ensayos clínicos [24]; de este modo, nuestro programa iría incorporándolos, rediseñándose en ciclos repetitivos.

A medida que el número de variantes genéticas clínicamente relevantes ha aumentado, y el progreso en las plataformas genómicas, hemos pasado de evaluar mutación a mutación, a llevar a cabo evaluaciones multiplexadas en regiones genéticas calientes (hot spots) en múltiples genes del cáncer (usando, por ejemplo MassArray). En nuestro caso, sosteníamos la idea de que las instituciones académicas deberían invertir en el desarrollo, evaluación e implementación de la biotecnología, para que el actual paradigma de diagnóstico molecular (una prueba, un fármaco) pudiera ser sustituido por el uso de tecnologías multiplexadas de genotipado que ayuden a optar entre múltiples alternativas terapéuticas.

Inicialmente, comenzamos empleando la plataforma MassARRAY para la labor de preselección, ya que ésta requiere muy poco tejido, se puede realizar con rapidez y resulta menos costosa. Conforme fuimos avanzando, nos dimos cuenta de que con MassARRAY no se pueden evaluar lesiones

importantes relativas a los genes supresores tumorales, ya que al poderse dar en cualquier parte del gen, no únicamente en los puntos calientes, estas plataformas son insuficientes. Además, estas plataformas no capturan las delecciones, amplificaciones y traslocaciones génicas, que podrían ser importantes factores predictivos de sensibilidad también. Los dos casos de resistencia secundaria a los inhibidores de PI3K aquí descritos, donde se encontraron alteraciones en genes que no serían analizables con MassArray ejemplifican esta situación. De este modo, y a diferencia de las plataformas como la de MassArray, identificamos que las estrategias de secuenciación masiva en paralelo proporcionarían un análisis genómico más completo del tumor, e incluirían el número de copias del genoma y reordenamientos estructurales. Estas plataformas, al aumentar la profundidad de cobertura mejorarían la precisión en la detección de mutaciones, especialmente en muestras con poca celularidad tumoral o con múltiples subclones tumorales(92, 140). Junto con el cada vez mayor número de genes y tipos de alteraciones genómicas susceptibles de ser utilizadas como dianas de terapias experimentales, la disminución del coste de las tecnologías de secuenciación de próxima generación hacen planteable su uso para la caracterización más completa de numerosas aberraciones genómicas tumorales(132).

En base a esta experiencia, actualmente hemos pasado del análisis basado en la espectrometría de masas (MassArray) a una estrategia de secuenciación personalizada por Amplicon, permitiendo un diagnóstico molecular más preciso y adaptado a la era contemporánea de las terapias dirigidas. La experiencia con los dos proyectos aquí presentados ha resultado útil para desarrollar un programa de preselección de segunda generación basado en la secuenciación de próxima generación. Actualmente, al emplear estrategias basadas en la identificación de aberraciones, podemos secuenciar un panel personalizado de 50 genes que permita la detección de todos los genes que podrían ser indicadores de sensibilidad y resistencia tumoral a PI3K (incluyendo *PTEN*, *PIK3CA*, *AKT*, *TSC1*, *TSC2*...), e inhibidores de SHH (*PTCH*, *SMO*, *SUFU*...), entre otros. A este panel de análisis de mutaciones, hemos añadido un panel para determinar la presencia de alteraciones en el numero de

copias genéticas y otro para determinar translocaciones genómicas, ambos usando la tecnología de nCounter (Nanostring).

Un beneficio añadido del esfuerzo de analizar las muestras tumorales y realizar el reclutamiento de pacientes seleccionados conforme a los marcadores predictivos candidatos es que la obtención precoz de datos clínicos pueda impulsar enormemente la investigación traslacional. Reconociendo las limitaciones de los modelos tumorales preclínicos y su baja predictibilidad, los datos clínicos en el mejor escenario posible (como el tratar pacientes que presenten un tumor con el *PIK3CA* mutado con inhibidores de PI3K) permitirá redirigir investigaciones preclínicas y traslacionales en la dirección correcta. Conocer dichas limitaciones proporcionará un mayor conocimiento de la vía, así como de los mecanismos de resistencia clínicamente relevantes (al analizar el estado mutacional de los pacientes que no respondan a tratamiento) y de las terapias combinadas pertinentes. A este respecto, los datos y las muestras clínicas de los pacientes que participaron en el programa de prescreening molecular paralelo al desarrollo de los estudios con inhibidores de PI3K permitieron la realización de las colaboraciones científicas, como las anteriormente descritas en cáncer de mama y colon (véase tabla 11 en Anexo 1).

### **5.3 Estudios clínicos en el área de la Medicina Genómica**

La nueva era de la Medicina Oncológica de Precisión, auspiciada por el empleo de una serie de biomarcadores para guiar el tratamiento con agentes dirigidos (tal vez en un pequeño subconjunto de pacientes), requiere de la adaptación de diseños de ensayos clínicos, así como de la colaboración internacional. Estos diseños de ensayos clínicos para el diagnóstico y tratamiento del cáncer deben tener en cuenta una serie de limitaciones (como la frecuencia del biomarcador, el coste y tiempo de respuesta del análisis, entre otros), con el fin de incorporar los datos genómicos de manera eficaz y dinámica y evaluar la utilidad de asociar a los pacientes cuyo perfil molecular haya sido determinado con intervenciones o terapias dirigidas concretas.

Dado que las investigaciones de nuestra institución están enfocadas al desarrollo clínico precoz de fármacos para terapias dirigidas, y fundamentándonos en la experiencia adquirida con programa de preselección molecular para los inhibidores de PI3K, hemos desarrollado una cartera de ensayos de Medicina Genómica (Anexo 4) que son el resultado de la fructífera colaboración entre las instituciones académicas y la industria. Según su diseño, estos ensayos de Medicina Genómica se pueden clasificar en diferentes categorías:

- **Bases de datos clínicos y genómicos y estudios longitudinales de cohortes con estudios de casos y controles anidados** (Anexo 4A): Actualmente, en nuestra institución determinamos prospectivamente el perfil de una gran número de pacientes, con el fin de crear una cohorte longitudinal caracterizada molecularmente y con anotaciones clínicas. Se trata de una estrategia inclusiva, en la que se criban las dianas susceptibles de ser tratadas en todos los pacientes, independientemente del tipo de cáncer que padeczan. De este modo, los pacientes se convierten en "donantes de información genómica" y estas bases de datos (donde, en contraposición con las bases de datos COSMIC o el Cancer Genome Atlas, la mayoría de los pacientes presentan enfermedad refractaria(14, 141)), aportan valiosos datos relativos a resultados clínicos. Así, estas bases de datos proporcionan información real sobre las dianas más relevantes en nuestra área, sobre la que sustentar los planes de investigación clínica. A los pacientes con aberraciones moleculares concretas también se les ofrece participar en ensayos clínicos donde recibir tratamiento con agentes dirigidos asociados a alteraciones concretas o con fármacos ya aprobados.
- **Estudios independientes de histología (histology-agnostic), específicos de alteración** (Anexo 4B): En este marco, los pacientes con diferentes histologías tumorales pero con la misma aberración molecular son incluidos en un ensayo clínico donde reciben una terapia dirigida contra dicha aberración y cuyo principal criterio de valoración es la eficacia. Estos ensayos son flexibles, de modo que el reclutamiento de pacientes se puede extender a todos

los pacientes con tipos de tumor que hayan demostrado señales iniciales de actividad antitumoral, o bien se pueden excluir aquellos tipos de tumor donde no se haya observado respuesta preliminar con los primeros pacientes. En caso de que se realicen nuevos descubrimientos, se pueden añadir más cohortes con otros tipos de tumor.

- **Estudios de resistencia:** Diseño de ensayo secuencial con inclusión no aleatorizada de pacientes y realización de repetidas biopsias Los pacientes son seleccionados conforme a un biomarcador concreto y tratados según dicho marcador. En caso de recidiva, en la segunda parte del estudio se utilizan los biomarcadores putativos identificados en las biopsias tumorales repetidas con el fin de dirigir la terapia, que podrán ser un fármaco de segunda generación o una combinación de agentes dirigidos (Anexo 4C).
- **Diseño de ensayo clínico “N-de-1”** (Anexo 4D): El diseño “N-de-1” es una prometedora estrategia para investigar el valor de una terapia oncológica individualizada, en pacientes con aberraciones moleculares raras, independientemente del tipo de tumor que padezcan. Cada paciente es utilizado como su propio control y se compara el efecto de la actual combinación terapéutica fármaco-aberración con el efecto de la anterior terapia recibida. El estudio Winther es un ejemplo de diseño “N-de-1” modificado; en él se están empleando una serie de avanzadas tecnologías de determinación de perfil molecular para caracterizar de forma exhaustiva los eventos oncogénicos en pacientes con diferentes tipos de cáncer (Anexo 4E). En el ensayo se compara la supervivencia sin progresión de los pacientes tratados según el perfil molecular de su tumor con los resultados obtenidos en el tratamiento inmediatamente anterior a la inclusión en el estudio.

## 5.4 Controversias y dificultades del desarrollo clínico precoz de fármacos basado en el empleo de biomarcadores

Como se ha podido comprobar en el caso de las mutaciones de *PIK3CA* con los inhibidores de PI3K y las mutaciones de *PTCH* en el meduloblastoma y los inhibidores de SHH, la cuestión fundamental con respecto a cualquier alteración individual (esto es, si se trata de un evento indicador importante), normalmente aún no se ha resuelto cuando se inicia el desarrollo clínico de un fármaco. Una importante limitación es la carencia de tests plenamente validados y reproducibles que puedan ser realizadas en laboratorios debidamente certificados que funcionen conforme a las Clinical Laboratory Improvement Amendments (CLIA) o las Buenas Prácticas de Laboratorio Clínico (GLCP) de la OMS. Conseguir validar los tests antes de iniciar el desarrollo de un fármaco podría retrasar todo el proceso. Alternativamente, si se optara por no seleccionar a la población correcta de pacientes y tratar un grupo de pacientes al azar, se podría perder el beneficio del fármaco a causa del efecto de "dilución". Además, si se seleccionara el biomarcador erróneo, los posibles efectos beneficiosos del agente dirigido en una población de pacientes menos específica se perdería.

Con el fin de evitar esto, se ha propuesto un proceso paralelo y simultáneo para el desarrollo clínico de fármacos oncológicos y de biomarcadores predictivos: en los ensayos clínicos de fase I, donde la selección de pacientes se suele fundamentar en la mejor estimación ("the best guess"), los biomarcadores farmacodinámicos deben seguir unos criterios muy rigurosos a la hora de definir la "prueba de mecanismo", mientras que se pudieran usar estándares menos estrictos para los biomarcadores predictivos usados para la selección de pacientes (46, 142) en estas fases. En estudios clínicos más avanzados (estudios fase II y III, sobretodo los de registro), se podrían usar estándares más estrictos.

En este trabajo hemos podido ver cómo la vía PI3K quedaba claramente inhibida al usar las dosis biológicamente óptimas, que se definieron empleando varios marcadores farmacodinámicos. Del mismo modo, la terapia con Sonidegib, así como el empleo de la expresión del gen GLI1 en la piel resultaron útiles a la hora de definir una dosis biológicamente activa para la inhibición de la vía de SHH. Por otro lado, las mutaciones de *PIK3CA* fueron analizadas en nuestros laboratorios para

comprobar su valor predictivo en los tratamientos con inhibidores de PI3K, revelando que el mero análisis de este biomarcador no era suficiente. Igualmente, la selección según el tipo de tumor, como el meduloblastoma, no fue suficiente a la hora de seleccionar a aquellos pacientes que más se podrían beneficiar de los inhibidores de SHH, aunque los datos aportados por los estudios de fase I permitieron el desarrollo de una prueba de firma de cinco genes que sí podía ayudar a seleccionar eficazmente a los pacientes que se podían beneficiar del tratamiento. Estos dos esfuerzos demuestran que los estudios de fase I y el empleo de biomarcadores de selección aceleran el desarrollo de fármacos al ayudar a tomar las decisiones sobre si administrar o no un tratamiento.

Lo que sabemos hasta ahora sobre el cáncer nos ha enseñado que los tumores son entidades complejas y a la Medicina Genómica le restan muchos obstáculos por superar. El descubrimiento de la heterogeneidad de los tumores, así como la rápida adquisición de resistencia a los agentes únicos observada en múltiples casos son sol una muestra de algunos de estos obstáculos. El análisis detallado del genoma del cáncer en toda su complejidad, así como la heterogeneidad intertumoral e intratumoral han revelado diferencias topográficas, temporales y funcionales en las mutaciones, las variaciones en el número de copias de los cromosomas y las firmas de expresión génica(143). En algunos casos, la presencia de un biomarcador puede no ser representativa de la enfermedad en su conjunto, mientras que en otros observamos aberraciones ubícuas inductoras de alteraciones que se mantienen durante el avance del cáncer. En el cáncer colorrectal y el NSCLC, por ejemplo, existe una elevada concordancia entre las mutaciones de *KRAS* y *EGFR* entre el tumor primario y la metástasis en estadíos avanzados(144, 145). Junto con la heterogeneidad en las alteraciones, también se empieza a observar en muchos tipos tumorales una “evolución convergente”, es decir la activación de una serie de vías moleculares, siguiendo unos patrones, mas usando mutaciones diversas. Es decir, diferentes alteraciones moleculares en diferentes genes darían lugar a similares efectos biológicos, activando las mismas vías moleculares.

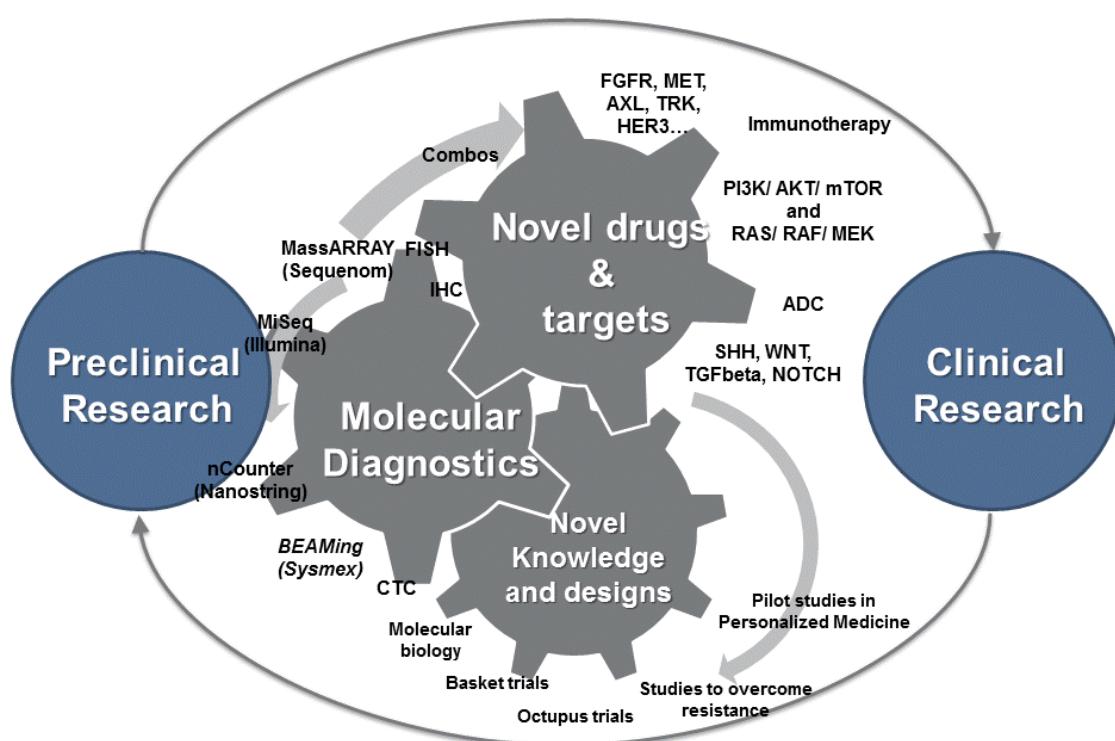
La resistencia a la terapia podría deberse a la presencia de una señalización paralela a través de bien retroalimentaciones negativas adaptables o de receptores tirosina quinasas con aberraciones existentes (resistencia primaria(146)) o ser adquirida a causa de la presión terapéutica (resistencia secundaria(147)), mediante una respuesta adaptativa (pérdida de la diana, selección de subclones resistentes, activación paralela de otra vía de señalización). Una forma de abordar la heterogeneidad de los tumores y la aparición de resistencia es realizando una biopsia de la recidiva con el fin de detectar tanto las mutaciones adquiridas como aquellas alteraciones infrarepresentadas en el tumor original y que aumentaron posteriormente durante la progresión o con la selección del tratamiento. Dado que la obtención de múltiples biopsias es costoso y conlleva riesgos, también se están intentando identificar otras alternativas como el análisis de células o DNA tumoral circulante(148). Si se demuestra que estos marcadores no invasivos reflejan con exactitud el espectro y frecuencia de las aberraciones presentes en las múltiples lesiones micrometastásicas ocultas y que son predictivos de respuesta a terapia con agentes dirigidos concretos, estas estrategias no invasivas se podrán utilizar para el tratamiento y la selección de pacientes en ensayos clínicos(149).

## 5.5 Observaciones finales

En conclusión, para optimizar el beneficio clínico de cada paciente, evitar gastos innecesarios y la administración de tratamientos fútiles a otros pacientes, así como acelerar el desarrollo de nuevos fármacos contra el cáncer, hemos reflejado aquí que la prueba de hipótesis y el análisis de biomarcadores en los estudios clínicos de fase I es una estrategia razonable y viable. La identificación de aberraciones moleculares inductoras de alteraciones y una sólida base preclínica y biológica para el agente dirigido son esenciales en este proceso. En el caso de las mutaciones de *PIK3CA*, no logramos confirmar su valor para predecir la sensibilidad tumoral a los inhibidores de PI3K, lo que indica que habrá que realizar más investigaciones antes de emplear marcadores predictivos en los

estudios de fase III con estos fármacos. Por otro lado, una firma genética de la activación de Hh sí que mostró estar correlacionada con la actividad antitumoral de sonidegib, lo que avala su uso en un estudio pivotal del meduloblastoma.

El coste cada vez menor de las tecnologías moleculares de alto rendimiento facilitará la aplicación de una estrategia oncológica personalizada. Por lo tanto, el cribado de los tumores de pacientes elegibles para los estudios clínicos es un paso importante en el desarrollo de nuevos fármacos. En las actuales Unidades de Ensayos Clínicos, se han incorporado plataformas de genotipado para poder detectar alteraciones susceptibles de tratamiento que puedan orientar las decisiones terapéuticas. Nosotros abogamos por un programa de preselección adaptable, flexible, local y multiplexado que incorpore nuevos fármacos y dianas, nuevo conocimiento, métodos de genotipado y diseños novedosos de estudios, donde el conocimiento, la tecnología y la farmacología se encuentren en continua revisión para poder proporcionar la mejor atención a los pacientes.



**Figura 11.** Un modelo de investigación translacional en el desarrollo clínico precoz de fármacos.

## 6. Anexos

### Anexo 1. Estudios y publicaciones del grupo en el área de trabajo descrita

**Tabla 10.** Estudios con inhibidores de PI3K/AKT/mTOR desarrollados en la Unitat de Investigació en

Terapia Molecular de l'Hospital Universitari de la Vall d'Hebron.

<b>Tipo de tumor</b>	<b>Código ensayo</b>	<b>Título</b>	<b>Fase</b>
Tumores sólidos avanzados	14H-MC-JWAA	A Phase I Trial of Single-Agent LY2780301 in Patients With Advanced or metastatic cancer	I
Tumores sólidos avanzados	CBY719X2105J	A phase Ib/II open-label, multi-center study of the combination of BYL719 plus AMG 479 (ganitumab) in adult patients with selected advanced solid tumors	Ib/II
Tumores sólidos avanzados	CC-115-ST-001	A phase 1a/1b, multicenter, open label, dose-finding study to assess the safety, tolerability, pharmacokinetics and preliminary efficacy of the dual DNA-PK and TOR kinase inhibitor, CC-115, administered orally to subjects with advanced solid tumors, Non-Hodgkin's lymphoma (NHL) or Multiple myeloma (MM)	Ia/Ib
Tumores sólidos avanzados	B1271002	A Multi-arm Phase 1 Dose Escalation Study Of The Safety, Pharmacokinetics, And Pharmacodynamics Of The Dual PI3K/MtorInhibitors Pf-04691502 And Pf-05212384 In Combination With Experimental Or Approved AntiCancer Agents In Patients With advanced solid tumors	I
Tumores sólidos avanzados	B2151001	A Phase 1 Study Of PF-05212384 (Also Known as PKI-587) Administered As An Intravenous Infusion To Subjects With advanced solid tumors	I
Tumores sólidos avanzados	CBEZ235A2101	A Phase I/II, Multi-center, Open-label Study of BEZ235, Administered Orally on a Continuous Daily Dosing Schedule in Adult Patients With Avanzado Solid Malignancies Including Patients With advanced solid breast tumors	I
Tumores sólidos avanzados	CBEZ235A2118	A Phase Ib Multi-center, Open-label, 4-arm Dose-escalation Study of Oral BEZ235 and BKM120 in Combination With Weekly Paclitaxel in Patients With Advanced Solid Tumorsand Weekly Paclitaxel/Trastuzumab in Patients With HER2+ advanced solid breast tumors	Ib
Tumores sólidos avanzados	GE280079	A Study Evaluating the Safety, Tolerability, and Pharmacokinetics of GDC-0973 in Combination With GDC-0068 When Administered in Patients With advanced solid tumors	Ib
Tumores sólidos avanzados	CBKM120B2101	A Phase Ib, Open-label, Multi-center, Dose-escalation Study of Oral BKM120 in Combination With Oral GSK1120212 in Adult Patients With Selected advanced solid tumors.	I
Tumores sólidos avanzados	CBKM120X2101	A Phase IA, Multicenter, Open-label Dose Escalation Study of BKM120, Administered Orally in Adult Patients With advanced solid tumors	Ia
Tumores sólidos avanzados	CBY719X2101	A Phase IA, Multicenter, Open-label Dose Escalation Study of Oral BYL719, in Adult Patients With advanced solid tumors, Whose Tumors Have an Alteration of the PIK3CA Gene	I
Tumores sólidos avanzados	CLDE225X2114	A Phase Ib, Multi-center, Open Label, Dose Escalation Study of Oral LDE225 in Combination With BKM 120 in Patients With Advanced Solid Tumors	I
Tumores sólidos avanzados	CMEK162X2101	A Phase Ib, Open-label, Multi-center, Dose-escalation and Expansion Study of an Orally Administered Combination of BKM120 Plus MEK162 in Adult Patients With Selected advanced solid tumors	I
Tumores sólidos avanzados	CMEK162X2103	A Phase Ib, Open-label, Multi-center, Dose-escalation and Expansion Study of an Orally Administered Combination of BEZ235 Plus MEK162 in Adult Patients With Selected advanced solid tumors	Ib
Tumores sólidos avanzados	CMEK162X2109	A Phase Ib Open-label, Multi-center, Dose Escalation and Expansion Study of Orally Administered MEK162 Plus BYL719 in Adult Patients With Selected advanced solid tumors	I
Tumores sólidos avanzados	CMEK162X2111	A Phase Ib/II Open-label, Multi-center Study of the Combination of MEK162 Plus AMG 479 (Ganitumab) in Adult Patients With Selected advanced solid tumors	I/II
Tumores sólidos	CRAD001X2109	An Open-label, Multi-center Phase I Dose-finding Study of	I

avanzados		RAD001 (Everolimus, Afinitor®) in Combination With BEZ235 in Patients With advanced solid tumors	
Tumores sólidos avanzados	14-MC-JWAA	A Phase I Trial of Single-Agent LY2780301 in Patients With advanced solid tumors	I
Tumores sólidos avanzados	PAM4743G	An Open-Label, Phase I, Dose-Escalation Study Evaluating the Safety and Tolerability of GDC-0068 in Patients With Refractory and advanced solid tumors	I
Tumores sólidos avanzados	INK1117-001	A Phase I, Dose Escalation Study of MLN1117 in Subjects With Advanced Solid Malignancies Followed by Expansion in Subjects With Measurable Disease	I
Tumores sólidos avanzados	INK128-001	A Phase I, Open Label, Dose Escalation Study of Oral Administration of Single Agent INK128 in Subjects With Malignancies Followed by an Expansion in Subjects With Measurable Disease	I
Tumores sólidos avanzados	MK8669-049	Phase I Parallel Protocol of MK-8669 (Ridaforolimus) + MK-2206 and MK-8669 (Ridaforolimus) + MK-0752 Doublets (MK-MK) in Patients With advanced solid tumors	I
Tumores sólidos avanzados	PAM4983G	A Phase Ib, Open-label, Dose-escalation Study of the Safety and Pharmacology Of GDC-0068 in Combination With Docetaxel, Fluoropyrimidine Plus Oxaliplatin, Paclitaxel, or Enzalutamide in Patients With advanced solid tumors	I
Tumores sólidos avanzados	PCD4989g	A Phase I, Open Label, Dose Escalation Study of the Safety and Pharmacokinetics of MPDL3280A Administered Intravenously As a Single Agent to Patients With Locally Advanced or Metastatic Solid Tumors or Hematologic Malignancies	I
Tumores sólidos avanzados	PIM4945g	A Phase Ib, Open Label, Dose Escalation Study of the Safety and Pharmacology of GDC-0980 in Combination With a Fluoropyrimidine, Oxaliplatin, and Bevacizumab in Patients With advanced solid tumors	Ib
Tumores sólidos avanzados	PMT 4979g	An Open-Label, Phase I/II, Dose Escalation Study Evaluating the Safety and Tolerability of GDC-0032 in Patients With Locally Advanced or Metastatic Solid Tumors and in Combination With Endocrine Therapy in Patients With Locally advanced solid Hormone Receptor-Positive breast cancer	I
Tumores sólidos avanzados	XL147-001	A Phase 1 Dose-Escalation Study of the Safety and Pharmacokinetics of XL147 Administered Orally Daily to Subjects with advanced solid tumors	I
Tumores sólidos avanzados	C32001	A Multicenter, Open-label, Phase 1b Study of MLN0128 (an Oral mTORC1/2 Inhibitor) in Combination With MLN1117 (an Oral PI3K $\alpha$ Inhibitor) in Adult Patients With advanced solid tumors	Ib
Tumores sólidos avanzados	CBGJ398X2102	A phase Ib, open-label study of oral BGJ398 in combination with oral BYL719 in adult patients with select advanced solid tumors.	Ib
Tumores sólidos avanzados	CBYL719Z2101	A phase Ib, open label, dose finding study of BYL719 in combination with paclitaxel in Advanced Solid Tumorsfollowed by two expansion phases in locally advanced or metastatic chemotherapy naive HER2-breast cancer patients and recurrent or metastatic head-and-neck squamous cell carcinoma patients pre-treated with platinum based therapy	Ib
Tumores sólidos avanzados	MK-3475-028	Fase IB Study of MK-3475 in Subjects with Select advanced solid tumors	Ib
Tumores sólidos avanzados	XL765-001	A Phase 1 Dose-Escalation Study of the Safety and Pharmacokinetics of XL765 Administered Orally Daily to Subjects with advanced solid tumors	I
Tumores sólidos avanzados	MK-8669/004	A Phase I Study of Ridaforolimus (MK8669) and MK0646 in Patients With advanced solid tumors	I
Tumores sólidos avanzados	XL765-003	A Phase 1 Dose-Escalation Study of XL765 in Combination with Erlotinib in Subjects with advanced solid tumors	I
Tumores sólidos avanzados	PM1183-A-001-08	Phase I, Multicenter, Open-label, Dose-escalating, Clinical and Pharmacokinetic Study of PM01183 in Patients With advanced solid tumors	I
Tumores sólidos avanzados	3265K1-1002-WW/B2151001	A Phase 1 Study Of PF-05212384 (Also Known as PKI-587) Administered As An Intravenous Infusion To Subjects With advanced solid tumors	I
Tumores sólidos avanzados	CCLR457X2101	A Phase I/II Multicenter, Open-label Study of CLR457, Administered Orally in Adult Patients With advanced solid tumors	Ia
Tumores sólidos avanzados	PQR309-001/SAKK 67/13	Phase I Study of Oral PQR309 in Patients With advanced solid tumors	Ia
Tumores sólidos avanzados	C32001	A Multicenter, Open-label, Phase 1b Study of MLN0128 (an Oral mTORC1/2 Inhibitor) in Combination With MLN1117 (an Oral PI3K $\alpha$ Inhibitor) in Adult Patients With advanced solid tumors	Ib

Glioblastoma	CBKM120E2101	A Phase I, two-stage, multi-center, open-label dose escalation study of BKM120 in combination with adjuvant temozolamide and with concomitant radiation therapy and temozolamide in patients with newly diagnosed glioblastoma	I
Glioblastoma	GO028070	An open-label, phase I, dose-escalation study evaluating the safety and tolerability of GDC-0084 administered to patients with progressive or recurrent high-grade gliomas	I
Glioblastoma	CINC280X2204	A Phase Ib/II, Multi-center, Open-label Study of Single-agent INC280 in Combination With Buparlisib in Patients With Recurrent Glioblastoma	Ib/II
Cáncer de mama	CLEE011X2106	A phase Ib/II trial of LEE011 in combination with everolimus (RAD001) and exemestane in the treatment of postmenopausal women with estrogen receptor breast cancer.	Ib/II
Cáncer de mama	CBKM120x2107	A Phase Ib/II, Open Label, Multi-center Study Evaluating the Safety and Efficacy of BKM120 in Combination With Trastuzumab in Patients With Relapsing HER2 Overexpressing breast cancer Who Have Previously Failed Trastuzumab	Ib/II
Cáncer de mama	CBKM120ZES02T/SOLTI-1103	A Phase II Trial of BKM120 (a PI3K Inhibitor) in Patients With Triple Negative metastatic breast cancer	II
Cáncer de mama	GDC4950g	A Phase II, Double-Blind, Placebo Controlled, Randomized Study of GDC-0941 or GDC-0980 With Fulvestrant Versus Fulvestrant in advanced solid breast cancer in Patients Resistant to Aromatase Inhibitor Therapy	II
Cáncer de mama	MK-8669-041	A Study of Ridaforolimus (MK-8669) in Combination With Dalotuzumab (MK-0646) Compared to Standard of Care Treatment in Estrogen Receptor Positive breast cancer Patients (MK-8669-041 AM3)	II
Cáncer de mama	MK-8669-64	A Randomized Trial of the Combination of Ridaforolimus and Exemestane, compared to Ridaforolimus, Exemestane and Dalotuzumab in High Proliferation Estrogen Receptor Positive Breast cancer Patients	II
Cáncer de mama	D3610C0002	A Phase I/II Study of AZD5363 Combined With Paclitaxel in Patients With advanced solid tumors breast cancer, comprising a Safety Run-In and a Placebo-controlled Randomised Expansion in ER+ve Patients Stratified by PIK3CA Mutation Status.	I
Cáncer de mama	XL147-203	Study of XL147 (SAR245408) in Combination With Trastuzumab or Paclitaxel and Trastuzumab in Subjects With advanced solid breast cancer Who Have Progressed on a Previous Trastuzumab-based Regimen	Ib/II
Cáncer de mama	B1271003	An Open-Label, Randomised Phase 1b/2 Study Of PF-04691502 In Combination With Letrozole Compared With Letrozole Alone In Patients With Estrogen Receptor Positive, Her-2 Negative Early Breast Cancer	I
Cáncer de mama	MK8669-050	A Clinical Trial to Qualify the Growth Factor Signature (GFS) as an Intermediate Biomarker of Response for Development of PI3K-Pathway Inhibitors in Patients With Breast Cancer	0/Ib
Cáncer de mama	MK0646-013	Study to Establish Proof-of-Biology for MK-0646 in Breast Cancer	I
Cáncer de mama	MK-8669-050	A Clinical Trial to Qualify the Growth Factor Signature (GFS) as an Intermediate Biomarker of Response for Development of PI3K-Pathway Inhibitors in Patients With Breast Cancer	I
Cáncer de mama	XL147-202	A Phase 1/2 Dose-Escalation Study of XL147 or XL765 in Combination with Letrozole in Subjects with Hormone Receptor-Positive and HER2 Negative Breast Cancer Refractory to a Nonsteroidal Aromatase Inhibitor	I/II
Cáncer de mama	XL147-203	Study of XL147 (SAR245408) in Combination With Trastuzumab or Paclitaxel and Trastuzumab in Subjects With Metastatic Breast Cancer Who Have Progressed on a Previous Trastuzumab-based Regimen	I
Cáncer de mama	1280.4	A Phase Ib/II Randomized Study of BI 836845 in Combination With Exemestane and Everolimus Versus Exemestane and Everolimus Alone in Women With Advanced Breast Cancer	Ib/II
Cáncer de mama	CLEE011X2106	A Phase Ib/II Trial of LEE011 in Combination With Everolimus (RAD001) and Exemestane in the Treatment of Postmenopausal Women With Estrogen Receptor Positive, Her2- Advanced Breast Cancer	Ib/II
Cáncer de mama	PMT 4979g	An Open-Label, Phase I/II, Dose Escalation Study Evaluating the Safety and Tolerability of GDC-0032 in Patients With Locally Advanced or Metastatic Solid Tumors and in Combination With	Ib/II

		Endocrine Therapy in Patients With Advanced Breast Cancer Hormone Receptor-Positive	
Cáncer de mama	GO27802	A Phase Ib Open-Label, Dose-Escalation Study of the Safety and Pharmacology of GDC-0032 in Combination With Either Docetaxel or Paclitaxel in Patients With HER2-Negative, Advanced Breast Cancer	Ib/II
Cáncer Colorectal	TTD08-06	Open, Multicenter Phase II Study to Evaluate the Efficacy and Safety of the Combination of Panitumumab With Irinotecan in Patients With Wild-Type KRAS Metastatic Colorectal Cancer Refractory to Irinotecan Based Chemotherapy	II
Cáncer Colorectal	CLGX818X2103	A Phase Ib/II Multi-center, Open-label, Dose Escalation Study of LGX818 and Cetuximab or LGX818, BYL719, and Cetuximab in Patients With BRAF Mutant Colorectal cancer	Ib/II
Cáncer de Esofago	CLJM716X2103	A Phase Ib/II, Open-label Study of LJM716 in Combination With BYL719 Compared to Taxane or Irinotecan in Patients With Previously Treated Esophageal Squamous Cell Carcinoma (ESCC)	Ib/II
Cáncer de Esofago	CLJM716X2103	A phase Ib/II, open-label study of LJM716 in combination with BYL719 compared to taxane or irinotecan in patients with previously treated esophageal squamous cell carcinoma	Ib/II
Cáncer Colorectal	MK-0646-025	A Phase IIA Open Label, Adaptive, Randomized Clinical Trial of Dalotuzumab (MK-0646) Treatment in Combination With Irinotecan Versus Cetuximab and Irinotecan for Patients With Metastatic Rectal Cancers (mRC) Expressing High IGF-1/Low IGF-2 Levels	II
Cáncer de Endometrio	B1271004	A Randomized Phase 2 Non-comparative Study Of The Efficacy Of Pf-04691502 And Pf-05212384 In Patients With Recurrent Endometrial Cancer	II
Cáncer Endometrio de	LLO-TEM-2011-01/TEM IIG-4 (POEM)	A Phase IIa Pharmacokinetic-pharmacodynamic Study to Confirm the Inhibitory Effect of Temsirolimus, Targeting the mTOR Pathway in Endometrial Carcinoma	IIa
Tumores neuroendocrinos	CBEZ235Z2401	Randomized Phase II Study of BEZ235 or Everolimus in Advanced Pancreatic Neuroendocrine Tumors	II
Tumores neuroendocrinos	GETNE 1003	A Phase II Study on Everolimus, an mTOR Inhibitor (Oral Formulation), With Octreotide LAR® in Adult Patients With Advanced, Non-functioning, Well-differentiated Gastrointestinal Neuroendocrine Tumours (GI NET)	II
Tumores neuroendocrinos	SOM230I2201	A randomized, open-label phase II multicenter study evaluating the efficacy of oral Everolimus alone or in combination with Pasireotide LAR i.m. in Advanced progressive pancreatic neuroendocrine tumors (PNET) - The COOPERATE-2 study	II
NSCLC	CBKM120D2201	An Open Label Two-stage Study of Orally Administered BKM120 in Patients With Metastatic non small-cell lung cancer With Activated PI3K Pathway	II
NSCLC	CC-223-NSCL-001	A Phase 1b, Multi-Center, Open-Label Study of the mTOR Kinase Inhibitor CC-223 in Combination With Erlotinib or Oral Azacitidine in non small-cell lung cancer	Ib
NSCLC	20060534	Amgen Protocol 20060534 - A Phase 1b/2 Trial of AMG 479 or AMG 102 in Combination With Platinum-based Chemotherapy as First-Line Treatment for Extensive Stage Small Cell Lung Cancer	Ib/II
NSCLC	B7461001	Phase 1/2 Study Of Pf 06463922 (an Alk/ros1 Tyrosine Kinase Inhibitor) In Patients With non small-cell lung cancer Harboring Specific Molecular Alterations	I/II
NSCLC	MK3475-001	Phase I Study of Single Agent MK-3475 in Patients With Progressive non small-cell lung cancer, Melanoma, and Non-Small Cell Lung Carcinoma	I
Renal	PIM4973g	A Phase II, Open Label, Randomized Study of GDC-0980 Versus Everolimus in Patients With Renal Cell Carcinoma Who Have Progressed on or Following VEGF-Targeted Therapy	II
Sarcoma-GIST	CSTI571X2103	A Dose-finding Phase Ib Multicenter Study of Imatinib in Combination With the Oral Phosphatidyl-inositol 3-kinase (PI3K) Inhibitor BYL719 in Patients With Gastrointestinal Stromal Tumor (GIST) Who Failed Prior Therapy With Imatinib and Sunitinib	Ib
Sarcoma-GIST	CSTI571X2101	A multi-arm dose-finding phase Ib multicenter study of imatinib in combination with the oral phosphatidyl-inositol 3-kinase (PI3-K) inhibitor BKM120 in patients with Gastrointestinal Stromal Tumor (GIST) who failed prior therapy with imatinib and sunitinib	I

**Tabla 11** Trabajos conjuntos en los que se estudian los mecanismos de eficacia y resistencia a los inhibidores de PI3K donde se aplicaron los descubrimientos y/o muestras extraídos en los ensayos.

Publicados
Prat A, Adamo B, Fan C, Peg V, Vidal M, Galván P, Vivancos A, Nuciforo P, Palmer HG, Dawood S, Rodón J, Ramony Cajal S, Del Campo JM, Felip E, Tabernero J, Cortés J. Genomic analyses across six cancer types identify basal-like breast cancer as a unique molecular entity. <i>Sci Rep.</i> 2013 Dec 18;3:3544.
Elkabets M, Vora S, Juric D, Morse N, Mino-Kenudson M, Muranen T, Tao J, Campos AB, Rodon J, Ibrahim YH, Serra V, Rodrik-Outmezguine V, Hazra S, Singh S, Kim P, Quadt C, Liu M, Huang A, Rosen N, Engelman JA, Scaltriti M, Baselga J. mTORC1 inhibition is required for sensitivity to PI3K p110α inhibitors in PIK3CA-mutant Breast cancer. <i>Sci Transl Med.</i> 2013 Jul 31;5(196):196ra99.
Ibrahim YH, García-García C, Serra V, He L, Torres-Lockhart K, Prat A, Anton P, Cozar P, Guzmán M, Grueso J, Rodríguez O, Calvo MT, Aura C, Díez O, Rubio IT, Pérez J, Rodón J, Cortés J, Ellisen LW, Scaltriti M, Baselga J. PI3K inhibition impairs BRCA1/2 expression and sensitizes BRCA-proficient triple-negative Breast cancer to PARP inhibition. <i>Cancer Discov.</i> 2012 Nov;2(11):1036-47.
Dienstmann R, Serpico D, Rodon J, Saura C, Macarulla T, Elez E, Alsina M, Capdevila J, Perez-Garcia J, Sánchez-Ollé G, Aura C, Prudkin L, Landolfi S, Hernández-Losa J, Vivancos A, Tabernero J. Molecular profiling of patients with colorectal Cancer and matched targeted therapy in phase I clinical trials. <i>Mol Cancer Ther.</i> 2012 Sep;11(9):2062-71. doi: 10.1158/1535-7163.
Huang HJ, Angelo LS, Rodon J, Sun M, Kuenkele KP, Parsons HA, Trent JC, Kurzrock R. R1507, an anti-insulin-like growth factor-1 receptor (IGF-1R) antibody, and EWS/FLI-1 siRNA in Ewing's sarcoma: convergence at the IGF/IGFR/Akt axis. <i>PLoS One.</i> 2011;6(10):e26060.
Yan Y, Serra V, Prudkin L, Scaltriti M, Murli S, Rodríguez O, Guzman M, Sampath D, Nannini M, Xiao Y, Wagle MC, Wu JQ, Wongchenko M, Hampton G, Ramakrishnan V, Lackner MR, Saura C, Roda D, Cervantes A, Tabernero J, Patel P, Baselga J. Evaluation and clinical analyses of downstream targets of the Akt inhibitor GDC-0068. <i>Clin Cancer Res.</i> 2013 Dec 15;19(24):6976-86.
Juric D, Castel P, Griffith M, Griffith OL, Won HH, Ellis H, Ebbesen SH, Ainscough BJ, Ramu A, Iyer G, Shah RH, Huynh T, Mino-Kenudson M, Sgroi D, Isakoff S, Thabet A, Elamine L, Solit DB, Lowe SW, Quadt C, Peters M, Derti A, Schegel R, Huang A, Mardis ER, Berger MF, Baselga J, Scaltriti M. Convergent loss of PTEN leads to clinical resistance to a PI(3)Kα inhibitor. <i>Nature.</i> 2014 Nov 17.
Enviados
Therapeutic Wnt/β-catenin signaling inhibition for the treatment of colorectal Cancer patients: Molecular determinants of Fármaco-response and combinationwith PI3K and AKT inhibitors. O Arqués, I Chicote, I Puig, SP. Tenbaum, G Argilés, R Dienstmann, N Fernández, G Caratù, J Matito, D Silberschmidt, J Rodon, S Landolfi, A Prat, E Espín, R Charco, P Nuciforo, A Vivancos, W Shao, J Tabernero, HG Palmer. Submitted.
PI3K inhibition results in enhanced estrogen receptor function and dependence in hormone receptor-positive Cancer de mama. A Bosch, Z Li, A Bergamaschi, H Ellis, E Tosca, A Prat, JJ Tao, DE Spratt, NT Viola-Villegas, P Castel, G Minuesa, N Morse, J Rodón, Y Ibrahim, V Serra, J Cortes, J Perez-Garcia, P Galvan, J Grueso, M Guzman, J Katzenellenbogen, M Kharas, JS Lewis, M Dickler, N Rosen, S Chandarlapaty, M Scaltriti, J Baselga.
AXL mediates resistance to PI3Kα inhibition by activating the EGFR/PKC/mTOR axis in head and neck and esophageal squamous cell carcinomas. M Elkabets, E Pazarentzos, D Juric, Q Sheng, RA Pelossof, S Brook, A Oaknin , J Rodon, N Morse, JJ Yan, M Liu, R Das, Y Chen, A Tam, H Wang, J Liang, JM.

Gurski, DA Kerr, R Rosell, C Teixidó, A Huang, RA Ghossein, N Rosen, TG Bivona, M Scaltriti, J Baselga.

**Tabla 12** Artículos en los que se describe nuestra experiencia en la aplicación clínica de las metodologías de cribado.

Vidwans SJ, Turski ML, Janku F, Garrido-Laguna I, Munoz J, Schwab R, Subbiah V, Rodon J, Kurzrock R. A framework for genomic biomarker actionability and its use in clinical decision making. <i>Oncoscience</i> . 2014 Oct 22;1(10):614-23.
de Gramont A, Watson S, Ellis LM, Rodón J, Tabernero J, de Gramont A, Hamilton SR. Pragmatic issues in biomarker evaluation for targeted therapies in Cancer. <i>Nat Rev Clin Oncol</i> . 2014 Nov 25
Dienstmann R, Rodon J, Tabernero J. Optimal design of trials to demonstrate the utility of genomically-guided therapy: Putting Precision Cancer Medicine to the test. <i>Mol Oncol</i> . 2014 Jul 15
Rodon J. An (only) partially established paradigm of Fármaco development of targeted therapies. <i>Eur J Cancer</i> . 2014 Aug;50(12):2037-9.
Prat A, Adamo B, Fan C, Peg V, Vidal M, Galván P, Vivancos A, Nuciforo P, Palmer HG, Dawood S, Rodón J, Ramony Cajal S, Del Campo JM, Felip E, Tabernero J, Cortés J. Genomic analyses across six Cancer types identify basal-like Breast cancer as a unique molecular entity. <i>Sci Rep</i> . 2013 Dec 18;3:3544.
Dienstmann R, Rodon J, Prat A, Perez-Garcia J, Adamo B, Felip E, Cortes J, Iafrate AJ, Nuciforo P, Tabernero J. Genomic aberrations in the FGFR pathway: opportunities for targeted therapies in Tumores sólidos. <i>Ann Oncol</i> . 2014 Mar;25(3):552-63.
De Mattos-Arruda L, Rodon J. Pilot studies for personalized Cancer medicine: focusing on the patient for treatment selection. <i>Oncologist</i> . 2013;18(11):1180-8.
Dienstmann R, Rodon J, Barretina J, Tabernero J. Genomic medicine frontier in human Tumores sólidos: prospects and challenges. <i>J Clin Oncol</i> . 2013 May 20;31(15):1874-84.
Dienstmann R, Rodon J, Tabernero J. Biomarker-driven patient selection for early clinical trials. <i>Curr Opin Oncol</i> . 2013 May;25(3):305-12.
Rodon J, Dienstmann R, Serra V, Tabernero J. Development of PI3K inhibitors: lessons learned from early clinical trials. <i>Nat Rev Clin Oncol</i> . 2013 Mar;10(3):143-53.
Argilés G, Rodon J, Tabernero J. Depicting the evolving scenario of translational-guided Fármaco development. <i>Clin Transl Oncol</i> . 2012 Dec;14(12):881-2.
Dienstmann R, Serpico D, Rodon J, Saura C, Macarulla T, Elez E, Alsina M, Capdevila J, Perez-Garcia J, Sánchez-Ollé G, Aura C, Prudkin L, Landolfi S, Hernández-Losa J, Vivancos A, Tabernero J. Molecular profiling of patients with colorectal Cancer and matched targeted therapy in phase I clinical trials. <i>Mol Cancer Ther</i> . 2012 Sep;11(9):2062-71.
Rodón J, Saura C, Dienstmann R, Vivancos A, Ramón y Cajal S, Baselga J, Tabernero J. Molecular prescreening to select patient population in early clinical trials. <i>Nat Rev Clin Oncol</i> . 2012 Apr 3;9(6):359-66.
Dienstmann R, Rodon J, Tabernero J. Fármaco development in the era of personalized oncology: from population-based trials to enrichment and prescreening strategies. <i>Am Soc Clin Oncol Educ Book</i> . 2012:168-72.

**Tabla 13.** Publicaciones sobre la vía IGFR1-PI3K-AKT-mTOR de nuestro grupo.

Publicados
Shapiro GI; Rodón J; Bedell C; Kwak EL; Baselga J; Braña I; Pandya SS; Scheffold C; Laird AD; Nguyen LT; Xu Y; Egile C; Edelman G. Phase I safety, pharmacokinetic and pharmacodynamic study of SAR245408 (XL147), a novel oral pan-Class I PI3K inhibitor, in patients with advanced Solid tumors.2013. Clin Cancer Res. 20: 233-245.
Atzori F, Tabernero J, Cervantes A, Prudkin L, Andreu J, Rodríguez-Braun E, Domingo A, Guijarro J, Gamez C, Rodon J, Di Cosimo S, Brown H, Clark J, Hardwick JS, Beckman RA, Hanley WD, Hsu K, Calvo E, Roselló S, Langdon RB, Baselga J. A phase I pharmacokinetic and pharmacodynamic study of dalotuzumab (MK-0646), an anti-insulin-like growth factor-1 receptor monoclonal antibody, in patients with advanced solid tumors. Clin Cancer Res. 2011 Oct 1;17(19):6304-12. Epub 2011 Aug 2.
Tolcher AW, Sarantopoulos J, Patnaik A, Papadopoulos K, Lin CC, Rodon J, Murphy B, Roth B, McCaffery I, Gorski KS, Kaiser B, Zhu M, Deng H, Friberg G, Puzanov I. Phase I, pharmacokinetic, and pharmacodynamic study of AMG 479, a fully human monoclonal antibody to insulin-like growth factor receptor 1. J Clin Oncol. 2009 Dec 1;27(34):5800-7. Epub 2009 Sep 28.
Tabernero J, Rojo F, Calvo E, Burris H, Judson I, Hazell K, Martinelli E, Ramon y Cajal S, Jones S, Vidal L, Shand N, Macarulla T, Ramos FJ, Dimitrijevic S, Zoellner U, Tang P, Stumm M, Lane HA, Lebwohl D, Baselga J. Dose- and schedule-dependent inhibition of the mammalian target of rapamycin pathway with everolimus: a phase I tumor pharmacodynamic study in patients with advanced solid tumors . J Clin Oncol. 2008 Apr 1;26(10):1603-10. Epub 2008 Mar 10. Erratum in: J Clin Oncol. 2010 Dec 20;28(36):5350.
Rodon J; Dienstmann R; Serra V; Tabernero J. Development of PI3K inhibitors: lessons learned from early clinical trials. 2013. Nat. Rev. Clin. Oncol.. 10: 143-153.
Markman B, Tabernero J, Krop I, Shapiro GI, Siu L, Chen LC, Mita M, Melendez Cuero M, Stutvoet S, Birle D, Anak O, Hackl W, Baselga J. Phase I safety, pharmacokinetic, and pharmacodynamic study of the oral phosphatidylinositol-3-kinase and mTOR inhibitor BGT226 in patients with advanced solid tumors . Ann Oncol. 2012 Feb 22.
Dienstmann R, Rodon J, Serra V, Tabernero J. Picking the point of inhibition: a comparative review of PI3K/AKT/mTOR pathway inhibitors. Mol Cancer Ther. 2014. May;13(5):1021-31
Rodon J, Dienstmann R, Serra V, Tabernero J. Development of PI3K inhibitors: lessons learned from early clinical trials. Nat Rev Clin Oncol. 2013 Mar;10(3):143-53.
Elkabets M, Vora S, Juric D, Morse N, Mino-Kenudson M, Muranen T, Tao J, Campos AB, Rodon J, Ibrahim YH, Serra V, Rodrik-Outmezguine V, Hazra S, Singh S, Kim P, Quadt C, Liu M, Huang A, Rosen N, Engelman JA, Scaltriti M, Baselga J. mTORC1

inhibition is required for sensitivity to PI3K p110 $\alpha$  inhibitors in PIK3CA-mutant Breast cancer. *Sci Transl Med.* 2013 Jul 31;5(196):196ra99.

Ibrahim YH, García-García C, Serra V, He L, Torres-Lockhart K, Prat A, Anton P, Cozar P, Guzmán M, Grueso J, Rodríguez O, Calvo MT, Aura C, Díez O, Rubio IT, Pérez J, Rodón J, Cortés J, Ellisen LW, Scaltriti M, Baselga J. PI3K inhibition impairs BRCA1/2 expression and sensitizes BRCA-proficient triple-negative Breast cancer to PARP inhibition. *Cancer Discov.* 2012 Nov;2(11):1036-47.

Huang HJ, Angelo LS, Rodon J, Sun M, Kuenkele KP, Parsons HA, Trent JC, Kurzrock R. R1507, an anti-insulin-like growth factor-1 receptor (IGF-1R) antibody, and EWS/FLI-1 siRNA in Ewing's sarcoma: convergence at the IGF/IGFR/Akt axis. *PLoS One.* 2011;6(10):e26060.

Yan Y, Serra V, Prudkin L, Scaltriti M, Murli S, Rodríguez O, Guzman M, Sampath D, Nannini M, Xiao Y, Wagle MC, Wu JQ, Wongchenko M, Hampton G, Ramakrishnan V, Lackner MR, Saura C, Roda D, Cervantes A, Tabernero J, Patel P, Baselga J. Evaluation and clinical analyses of downstream targets of the Akt inhibitor GDC-0068. *Clin Cancer Res.* 2013 Dec 15;19(24):6976-86.

Juric D, Castel P, Griffith M, Griffith OL, Won HH, Ellis H, Ebbesen SH, Ainscough BJ, Ramu A, Iyer G, Shah RH, Huynh T, Mino-Kenudson M, Sgroi D, Isakoff S, Thabet A, Elamine L, Solit DB, Lowe SW, Quadt C, Peters M, Derti A, Schegel R, Huang A, Mardis ER, Berger MF, Baselga J, Scaltriti M. Convergent loss of PTEN leads to clinical resistance to a PI(3)K $\alpha$  inhibitor. *Nature.* 2014 Nov 17.

Rodon J, Braña I, Siu LL, De Jonge MJ, Homji N, Mills D, Di Tomaso E, Sarr C, Trandafir L, Massacesi C, Eskens F, Bendell JC. Phase I dose-escalation and -expansion study of buparlisib (BKM120), an oral pan-Class I PI3K inhibitor, in patients with advanced solid tumors. *Invest New Fármacos.* 2014 Aug;32(4):670-81.

#### Enviados

Therapeutic Wnt/ $\beta$ -catenin signaling inhibition for the treatment of colorectal Cancer patients: Molecular determinants of Fármaco-response and combinationwith PI3K and AKT inhibitors. O Arqués, I Chicote, I Puig, SP. Tenbaum, G Argilés, R Dienstmann, N Fernández, G Caratù, J Matito, D Silberschmidt, J Rodon, S Landolfi, A Prat, E Espín, R Charco, P Nuciforo, A Vivancos, W Shao, J Tabernero, HG Palmer. Enviado.

A First in Human Phase I Trial of LY2780301, a dual p70 S6 kinase and Akt Inhibitor, in Patients with Advanced or Metastatic Cancer. A.Azaro, J.Rodon, A Calles, I. Braña, M. Hidalgo, P. Lopez-Casas, M. Munoz, P Westwood, J Miller, BA. Moser, U. Ohnmacht, W. Bumgardner, KA. Benhadji, E. Calvo. Enviado

Phase I trial of the pan-PI3K inhibitor SAR245408 (XL147) in patients with chronic lymphocytic leukemia (CLL) or relapsed/refractory lymphoma. JR Brown, MS Davids, J. Rodon, P Abrisqueta, J Lager, J Jiang, W Huang, C Egile, F Awan. Submitted

Implementation of a molecular prescreening program to identify PI3K alterations that enable enrichment of early clinical trials with PI3K-altered tumors and analysis of clinical benefit. J Rodon, R Dienstmann, A Vivancos, A Navarro, P Nuciforo, J Cortes, J Carles, JM del Campo, E Felip, C Aura, J Hernandez, H Palmer, J Perez, M Scaltriti, M Berger, V Serra, J Tabernero. Enviado

PI3K inhibition results in enhanced estrogen receptor function and dependence in

hormone receptor-positive Breast cancer. A Bosch, Z Li, A Bergamaschi, H Ellis, E Tosca, A Prat, JJ Tao, DE Spratt, NT Viola-Villegas, P Castel, G Minuesa, N Morse, J Rodón, Y Ibrahim, V Serra, J Cortes, J Perez-Garcia, P Galvan, J Grueso, M Guzman, J Katzenellenbogen, M Kharas, JS Lewis, M Dickler, N Rosen, S Chandarlapaty, M Scaltriti, J Baselga.

AXL mediates resistance to PI3K $\alpha$  inhibition by activating the EGFR/PKC/mTOR axis in head and neck and esophageal squamous cell carcinomas. M Elkabets, E Pazarentzos, D Juric, Q Sheng, RA Pelossof, S Brook, A Oaknin , J Rodon, N Morse, JJ Yan, M Liu, R Das, Y Chen, A Tam, H Wang, J Liang, JM. Gurski, DA Kerr, R Rosell, C Teixidó, A Huang, RA Ghossein, N Rosen, TG Bivona, M Scaltriti, J Baselga.

**Anexo 2. Manuscrito “Desarrollo de una firma de cinco genes de Hedgehog como herramienta para la preselección de pacientes para terapia con inhibidores de Hedgehog en el meduloblastoma”**

# Clinical Cancer Research

## A Five-Gene Hedgehog Signature Developed as a Patient Preselection Tool for Hedgehog Inhibitor Therapy in Medulloblastoma

Yaping Shou, Douglas M. Robinson, Dereck D. Amakye, et al.

*Clin Cancer Res* 2015;21:585-593. Published OnlineFirst December 3, 2014.

**Updated version** Access the most recent version of this article at:  
doi: [10.1158/1078-0432.CCR-13-1711](https://doi.org/10.1158/1078-0432.CCR-13-1711)

**Supplementary Material** Access the most recent supplemental material at:  
<http://clincancerres.aacrjournals.org/content/suppl/2014/12/06/1078-0432.CCR-13-1711.DC1.html>

**Cited Articles** This article cites by 31 articles, 15 of which you can access for free at:  
<http://clincancerres.aacrjournals.org/content/21/3/585.full.html#ref-list-1>

# A Five-Gene Hedgehog Signature Developed as a Patient Preselection Tool for Hedgehog Inhibitor Therapy in Medulloblastoma

Yaping Shou<sup>1</sup>, Douglas M. Robinson<sup>1</sup>, Dereck D. Amakye<sup>2</sup>, Kristine L. Rose<sup>2</sup>, Yoon-Jae Cho<sup>3</sup>, Keith L. Ligon<sup>4,5,6</sup>, Thad Sharp<sup>2</sup>, Asifa S. Haider<sup>2</sup>, Raj Bandaru<sup>1</sup>, Yuichi Ando<sup>7</sup>, Birgit Geoerger<sup>8</sup>, Francois Doz<sup>9</sup>, David M. Ashley<sup>10</sup>, Darren R. Hargrave<sup>11</sup>, Michela Casanova<sup>12</sup>, Hussein A. Tawbi<sup>13</sup>, Jordi Rodon<sup>14</sup>, Anne L. Thomas<sup>15</sup>, Alain C. Mita<sup>16</sup>, Tobey J. MacDonald<sup>17</sup>, and Mark W. Kieran<sup>4</sup>

## Abstract

**Purpose:** Distinct molecular subgroups of medulloblastoma, including hedgehog (Hh) pathway–activated disease, have been reported. We identified and clinically validated a five-gene Hh signature assay that can be used to preselect patients with Hh pathway–activated medulloblastoma.

**Experimental Design:** Gene characteristics of the Hh medulloblastoma subgroup were identified through published bioinformatic analyses. Thirty-two genes shown to be differentially expressed in fresh-frozen and formalin-fixed paraffin-embedded tumor samples and reproducibly analyzed by RT-PCR were measured in matched samples. These data formed the basis for building a multi-gene logistic regression model derived through elastic net methods from which the five-gene Hh signature emerged after multiple iterations. On the basis of signature gene expression levels, the model computed a propensity score to determine Hh activation using a threshold set *a priori*. The asso-

ciation between Hh activation status and tumor response to the Hh pathway inhibitor sonidegib (LDE225) was analyzed.

**Results:** Five differentially expressed genes in medulloblastoma (GLI1, SPHK1, SHROOM2, PDLIM3, and OTX2) were found to associate with Hh pathway activation status. In an independent validation study, Hh activation status of 25 medulloblastoma samples showed 100% concordance between the five-gene signature and Affymetrix profiling. Further, in medulloblastoma samples from 50 patients treated with sonidegib, all 6 patients who responded were found to have Hh-activated tumors. Three patients with Hh-activated tumors had stable or progressive disease. No patients with Hh-nonactivated tumors responded.

**Conclusions:** This five-gene Hh signature can robustly identify Hh-activated medulloblastoma and may be used to preselect patients who might benefit from sonidegib treatment. Clin Cancer Res; 21(3); 585–93. © 2014 AACR.

## Introduction

Medulloblastoma, a malignant primitive neuroectodermal tumor arising in the cerebellum, is the most common brain tumor in children ages <4 years (1). The current standard of care involves surgery followed by craniospinal radiation and chemotherapy, which can be given concurrently with or following radiation (2). Not infrequently, young children treated with chemotherapy alone will respond to second-line salvage therapy, including

radiation; however, there is no standard, effective salvage treatment for recurrence following craniospinal radiation and the prognosis following recurrence is dismal (3). Moreover, especially for young children, radiation can lead to long-term toxicities, including neurocognitive damage (2). Therefore, targeted therapies, with improved efficacy and reduced toxicity, are greatly needed for young children and for patients with relapsed disease.

Currently, there is no gold-standard method for the identification of molecular subtypes of medulloblastoma. Recently

<sup>1</sup>Novartis Institutes for BioMedical Research, Inc., Cambridge, Massachusetts. <sup>2</sup>Novartis Pharmaceuticals Corporation, East Hanover, New Jersey. <sup>3</sup>Departments of Neurology and Neurosurgery, Stanford University School of Medicine, Stanford, California. <sup>4</sup>Pediatric Neuro-Oncology, Dana-Farber Cancer Institute and Boston Children's Hospital and Harvard Medical School, Boston, Massachusetts. <sup>5</sup>Department of Pathology, Children's Hospital Boston, Brigham and Women's Hospital, and Harvard Medical School, Boston, Massachusetts. <sup>6</sup>Department of Medical Oncology and Center for Molecular Oncologic Pathology, Dana-Farber Cancer Institute, Boston, Massachusetts. <sup>7</sup>Nagoya University Hospital, Nagoya, Japan. <sup>8</sup>Institut Gustave Roussy, University Paris-Sud, Villejuif, France. <sup>9</sup>Institut Curie and University Paris Descartes, Sorbonne Paris Cite, France. <sup>10</sup>Deakin University/Barwon Health, Melbourne, Australia. <sup>11</sup>Great Ormond Street Hospital for Children, London. <sup>12</sup>Fondazione IRCCS Istituto Nazionale dei Tumori, Milano, Italy. <sup>13</sup>University of Pittsburgh Cancer Institute and University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania. <sup>14</sup>Vall d'Hebron Institut d'Oncologia, and Universitat Autònoma de Barcelona, Barcelona, Spain. <sup>15</sup>University of Leicester, Leicester, United Kingdom. <sup>16</sup>Cancer Therapy and Research Center, University of Texas

<sup>17</sup>Children's Healthcare of Atlanta, Aflac Cancer and Blood Disorders Center, Emory University School of Medicine, Atlanta, Georgia.

Note: Supplementary data for this article are available at Clinical Cancer Research Online (<http://clincancerres.aacrjournals.org/>).

Current address for Y. Shou: Takeda Pharmaceuticals International Co., Cambridge, Massachusetts; current address for D.D. Amakye: Bayer HealthCare, Whippany, New Jersey; and current address for R. Bandaru: AstraZeneca Pharmaceuticals, Waltham, Massachusetts.

Corresponding Author: Mark W. Kieran, Dana-Farber Cancer Institute, 450 Brookline Avenue, D-3154, Boston, MA 02215. Phone: 617-632-4907; Fax: 617-632-4897; E-mail: mark\_kieran@dfci.harvard.edu  
doi: 10.1158/1078-0432.CCR-13-1711

© 2014 American Association for Cancer Research.

## Translational Relevance

A significant proportion of patients with medulloblastoma experience relapse after primary treatment. Because of the lack of standard salvage regimens and long-term toxicities associated with available therapies, especially for younger patients, novel targeted therapies are greatly needed for patients with relapsed disease. Gene expression profiling studies have identified 4 molecular subgroups of medulloblastoma, including one characterized by activated hedgehog (Hh) signaling. Inhibitors targeting the Hh pathway, including sonidegib (LDE225), have demonstrated activity in patients with medulloblastoma. An RT-PCR-based five-gene signature assay has been developed to identify patients with Hh pathway-activated medulloblastoma and is associated with response to sonidegib treatment. Given the potential for drug-induced premature growth plate closure in children who have not achieved skeletal maturity, the ability to preselect patients who are most likely to obtain clinical benefit is a valuable advance in the development of Hh inhibitor therapy.

however, several studies have identified distinct molecular subgroups of medulloblastoma through gene expression profiling (4–7). Four subtypes, wingless (WNT), sonic hedgehog (SHH), group 3, and group 4, with distinct gene expression profiles and molecular abnormalities, have been described (5–8). Mutations that activate the hedgehog (Hh) pathway have been identified and found exclusively in the SHH subclass, which constitutes approximately one third of medulloblastomas (4, 6, 7, 9, 10); however, medulloblastomas can be classified within the SHH subclass in the absence of Hh pathway mutations (4–7, 11).

Several small-molecule inhibitors of smoothened (SMO), the G protein-coupled receptor-like transducer of Hh signaling, are being explored as novel, targeted therapies designed to treat cancers associated with aberrant Hh signaling (12). Sonidegib (LDE225) is a potent and selective SMO inhibitor that has demonstrated dose-dependent tumor regression in patched<sup>b/-</sup> (Ptch<sup>b/-</sup>) p53<sup>-/-</sup> and Ptch<sup>b/-</sup> hypermethylated in cancer 1 (Hic1<sup>b/-</sup>) mouse medulloblastoma models (13, 14). In recent phase I studies testing single-agent sonidegib in adult and pediatric patients with advanced solid tumors, antitumor activity was demonstrated in several patients with medulloblastoma (15, 16).

Genetic analyses of medulloblastomas have identified mutations in several Hh pathway genes; however, due to the low incidence of medulloblastoma, reports of recurrent mutations are limited (4–7). In addition, because few of these are hot-spot mutations, targeted genotyping cannot be used and mutational screening of large Hh pathway genes such as PTCH1 and SMO would be required (17). Furthermore, several studies have demonstrated that in medulloblastoma, the incidence of Hh pathway activation is more prevalent than Hh pathway mutations (4–7, 11).

For these reasons, preselection of patients by direct gene sequence analysis is not suitable for use in clinical studies, especially considering the importance of a short turnaround time to determine patient eligibility. Recent genomic-based molecular subclassification of medulloblastoma suggests that identification

of an Hh gene signature through gene expression profiling could be used for treatment decisions (18). However, the current expression profiling methods require a large quantity of tumor and are time intensive, both of which are not amenable for translation to the clinic (18). In addition, these methodologies do not work efficiently with the formalin-fixed and paraffin-embedded (FFPE) tumor specimens that are widely available in clinical practice. In this study, we developed a five-gene Hh signature assay as a clinically applicable tool for preselecting patients with Hh-activated medulloblastoma who are most likely to derive benefit from Hh inhibitor therapy.

## Patients and Methods

### Patient samples

Medulloblastoma tumor specimens were obtained from the Dana-Farber Cancer Institute/Boston Children's Hospital (Boston, MA; see Supplementary Methods for additional information; n = 40) and Children's Healthcare of Atlanta (CHOA; Atlanta, GA; n = 25).

Medulloblastoma samples (n = 50) were also obtained from patients enrolled in three separate phase I studies of oral sonidegib in patients with advanced solid tumors (Supplementary Table S1; refs. 19–21). The protocols and amendments of each study were approved by the institutional review board, independent ethics committee, or research ethics board at each center. Medulloblastoma and normal cerebral tissue samples used for assay validation were obtained from commercial vendors, Asterand (n = 1, medulloblastoma), and Biochain (n = 1, medulloblastoma; n = 3, normal cerebral tissue).

### Candidate gene selection

For selection of the initial list of candidate genes for further development of the signature, gene expression data from three independent external studies were reanalyzed. The first dataset from Thompson and colleagues (4) included data obtained by the Affymetrix HG-U133Av2 microarray chip from 46 patients (ages < 21 years) with medulloblastoma tumors. Nine of the samples were defined as group D with target genes of the SHH pathway significantly overrepresented among upregulated genes in their profiles. The second dataset from Kool and colleagues (5) included 62 medulloblastoma tumor samples profiled on the Affymetrix U133 Plus 2.0 chip. The 62 samples were classified into five subgroups, with subgroup B comprising 15 cases defined mostly by SHH-activated pathway genes. The third dataset, reported by Cho and colleagues (7), included 194 medulloblastoma tumor samples profiled on the Affymetrix HT-U133A. Six stable molecular subgroups of medulloblastoma were classified (c1 to c6), including the c3 subgroup that showed enrichment of gene sets associated with SHH signaling. Data from five, 20, and 11 normal cerebellum samples (profiled on Affymetrix U133Av2, Affymetrix U133 Plus 2.0, and HT-133A, respectively) obtained from the Gene Expression Omnibus (<http://www.ncbi.nlm.nih.gov/geo/>) database were combined with the Thompson, Kool, and Cho datasets, respectively, to enhance verification of the clustering below (4, 5, 7).

Data normalization and unbiased filtering. Normalization was performed independently for the three separate datasets using the robust multiarray averaging normalization algorithm in R Bioconductor package (<http://www.r-project.org/>).

Without regard to Hh activation status, the datasets were reduced by filtering out probe sets that showed variability and  $\log_2$  expression intensities below a defined threshold in all of the samples for that specific dataset [Thompson dataset coefficient of variation (CV) < 5% and  $\log_2$  expression <4; Kool dataset CV < 5% and  $\log_2$  expression intensity <5; Cho dataset CV < 5% and  $\log_2$  expression <5; refs. 4, 5, 7]. Distinct thresholds were used for each of the three datasets to account for the inherently different background noise caused by the use of different versions of the Affymetrix platform in different laboratories.

**Candidate gene selection.** Two parallel methods to determine candidate genes were initiated and later compared for consistency. One approach assessed each dataset independently and for each, collapsed the clusters defined previously into Hh active or Hh inactive. A univariate logistic regression analysis approach was employed on each dataset to identify genes that were potentially predictive of Hh activation status. The probe sets were then ranked by their significance and area under the receiver operating characteristic curve, and compared for consistency across datasets.

The second approach combined all 3 datasets, necessitating the use of distinct hierarchical clustering (performed using the Pearson dissimilarity distance metric and complete linkage; ref. 4). Distinct clusters were selected to define samples as Hh active ( $n = 63$ ) or Hh inactive ( $n = 236$ ). Samples that clustered less distinctly with either subgroup ( $n = 3$ ) were excluded from further analysis. The resulting dataset, with gene expression intensity for 10,995 probe sets, was used to run both a para-metric t test and Wilcoxon rank-sum test to compare the two classes.

**Identification of candidate genes and control genes.** Candidate genes that showed at least a 2-fold difference in mean expression levels between the Hh-active and Hh-inactive groups were identified. Initially, a false discovery rate P value of 0.01 was employed. This rate, however, resulted in a large pool of candidate genes, leading to a more complex selection strategy, in which statistical significance ( $P < 0.00001$  and average area under the curve  $\geq 95\%$ ) in at least two of the three independent logistic regression fits and significance ( $P < 0.001$ ) by Wilcoxon rank-sum or parametric t test were required for selection. In some rare instances, a candidate gene was selected if it showed high predictivity in a single experiment because different versions of the Affymetrix chip that were used contained their own unique gene candidates. Considering the potential for bias, the use of P values should be interpreted as a statistical filtering mechanism for identifying a reasonable number of potential candidate genes for the next stage of the analysis. Thirty-two genes were identified, including 21 genes upregulated and 11 genes downregulated in Hh pathway-activated samples.

Candidate control genes that were expressed nondifferentially across all subclasses of medulloblastoma tumors were also selected from the normalized dataset of 302 fresh-frozen medulloblastoma samples from three published sources (4, 5, 7). Criteria for selection included low variability in expression across all three medulloblastoma datasets (CV  $\leq 4\%$ ) and a target gene-specific Affymetrix probe set with robust expression in medulloblastoma tumors ( $\log_2 > 8$ ). Twenty-two potential control genes were selected that fit these criteria. Zona pellucida glycoprotein 2, identified as distinctly upregulated in the set of 25

normal cerebellum samples, was used to analyze the nontumor contamination present in the FFPE samples and was therefore included on the TaqMan low-density array (TLDA) array (data not shown).

#### Assay design and optimization for FFPE

TaqMan-based RT-PCR assays (Applied Biosystems Inc) designed with small amplicon size, and targeted at different regions of the gene, were screened for each of the 54 candidate genes (32 differentially expressed genes and 22 nondifferentially expressed or control genes) to assess their feasibility, utility, and robustness in FFPE samples. On average, three assays per gene were evaluated using a titration of complementary DNA (cDNA) from three FFPE tissue specimens (one  $Hh^+$  medulloblastoma, one  $Hh^-$  medulloblastoma, and one normal cerebellum). Eighteen of 32 differentially expressed candidate genes and four of 22 candidate control genes that had at least one assay that showed robust expression (raw cycle threshold value  $\leq 30$  using 50 ng of cDNA input) in at least one of the three FFPE samples and high assay efficiency across all three samples (90% to 110%) were selected. A custom TLDA (Applied Biosystems) was built using these 22 selected genes to further the signature identification process.

#### RNA extraction, RT-PCR, and gene expression profiling

RNA was extracted from FFPE sections using the QiagenRNeasy FFPE extraction Kit (Qiagen) and reverse transcribed to cDNA using random hexamers and a high-capacity cDNA archive kit (Applied Biosystems). Real-time PCR on the TLDA array was performed using the PRISM 7900HT sequence detection system (Applied Biosystems) with a universal human reference RNA (740000; Agilent Technologies, Inc) included as a technical control in every array. The delta cycle threshold (Ct) method was used to compute the expression levels of individual genes after data normalization as described below.

RNA was extracted from 25 frozen medulloblastoma tumor samples using the Trizol protocol (15596-018; Invitrogen) and was profiled by AROS Biosciences on the Affymetrix human genome U133 Plus 2.0 array with the 3' IVT express labeling Kit (Affymetrix).

**Data normalization.** The 18 differentially expressed genes and high assay efficiency in FFPE samples were normalized using the average expression of four control genes (HECT, UBA, and WWE domain containing 1; YME1-like 1; superoxide dismutase 1; and La ribonucleoprotein domain family member 1). The common control gene glyceraldehyde-3-phosphate dehydrogenase showed high variability across the sample sets and therefore was not used in the analysis (data not shown). With the delta Ct method, the raw expression level of each candidate gene was subtracted from the average of the four control genes. Following normalization, the 18 differentially expressed genes formed the basis of the model-building exercise described below.

#### Computational methods/model building

**Model building.** The elastic net is a regularized regression method that uses a weighted sum of the L1 and L2 penalty terms used in the lasso (22) and ridge (23) regression models, respectively. This method was selected due to the potential for correlation among the genes. In practice, the ridge portion of the penalty tended to group correlated genes together, whereas

Table 1. Five-gene Hh signature and control genes

Gene	Uni-gene accession number	Expression level <sup>a</sup>
GLI1	2139596	High
SHROOM2	1782725	High
SPHK1	139253	High
PDLIM3	5795643	High
OTX2	178376	Low
HUWE1	150675	Control
YME1L1	714304	Control
SOD1	238951	Control
LARP1	179374	Control

Abbreviations: SOD1, superoxide dismutase 1, soluble; LARP1, La ribonucleoprotein domain family member 1; YME1L1, YME1-like 1.

<sup>a</sup>Relative to Hh-negative medulloblastoma samples.

the lasso portion of the penalty then included or excluded these groups as a set in the final model. This algorithm can be used to calculate optimal values for  $\alpha$  and  $\lambda$ , which control the weight applied to each of the penalty terms and the amount of shrinkage exerted on the data, respectively. For optimization of the model, a series of 100  $\alpha$ s and 91  $\lambda$ s were generated. For each  $\alpha$  and  $\lambda$  pair, a model was fit in a 5-fold cross-validation framework, resulting in an estimate of deviance or model error, the lowest of which was deemed optimal. In addition to model error, the optimal model was also defined by the exact number and identity of genes, as well as model coefficients.

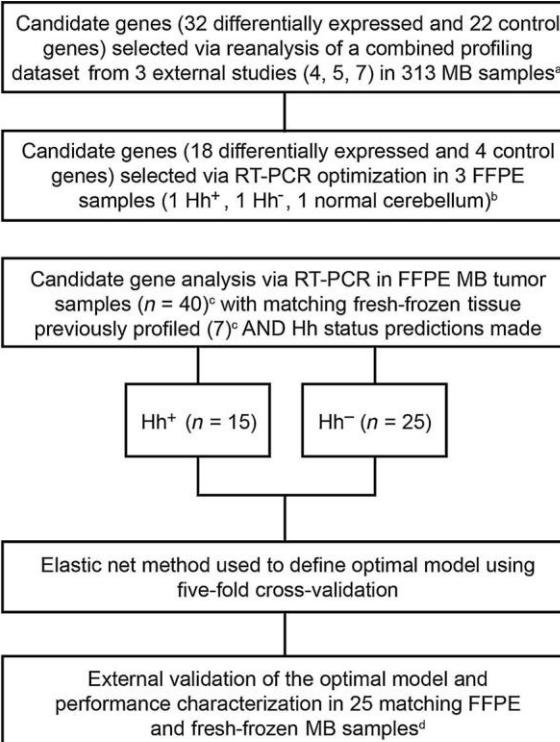
**Optimal model selection.** To account for potential cross-validation error, the cross-validation procedure was iterated 10,000 times. Eight- and five-gene models, defined by selection of  $\alpha$  and  $\lambda$ , were selected in approximately 80% of the iterations. In each case, the same eight genes [glioma-associated oncogene homolog 1 (GLI1), orthodenticle homeobox 2 (OTX2), shroom family member 2 (SHROOM2), PDZ and LIM domain 3 (PDLIM3), sphingosine kinase 1 (SPHK1), secreted frizzled-related protein 1, amyloid b A4 precursor protein-binding family A member 2, and spermatogenesis-associated 20; Table 1], or a subset of five genes (GLI1, OTX2, SHROOM2, PDLIM3, and SPHK1) were selected, with slight variations in the precise model coefficients. For each model size, the most frequently selected  $\alpha$  and  $\lambda$  pair was selected as the optimal model and used for further evaluation in the independent validation dataset. Ultimately, the five-gene model was selected for further development.

**Model thresholds.** The elastic net method generates a multigene logistic regression model, which produces a propensity score in the range of 0 to 1 that estimates the probability of being Hh activated for a given sample. A threshold was then selected to separate Hh activated and non-Hh-activated classes. During the model generation/discovery phase of this exercise, the separation between tumors categorized as Hh activated versus those categorized as Hh nonactivated was relatively large; thus, any threshold selected within that range was indistinguishable in terms of predictive performance. A threshold of 0.500 was selected as the score threshold because it partitions the range of scores into equal parts and may also be interpreted as the least biased choice. With the threshold set at 0.500, a value of  $>0.500$  was classified as Hh activated, whereas a value of  $<0.500$  was classified as non-Hh activated.

## Results

### Development of the five-gene Hh signature model

Briefly, a panel of 32 candidate genes differentially expressed in Hh<sup>b</sup> versus Hh<sup>-</sup> tumors and another 22 potential normalization genes were selected from data derived from 313 medulloblastomas in three independently published profiling studies (4, 5, 7). RT-PCR assays for these candidate genes were developed and optimized for use in FFPE specimens. Assays with robust



<sup>a</sup>Data from normal cerebellum samples obtained from the Gene Expression Omnibus (<http://www.ncbi.nlm.nih.gov/geo/>) database was also included in the analysis.

<sup>b</sup>FFPE samples were obtained from BioChain Institute, Inc.

<sup>c</sup>FFPE MB tumor samples were obtained from the Dana-Farber Cancer Institute/Boston Children's Hospital. The 40 matching fresh-frozen samples were a subset of the 194 MB samples previously profiled (7).

<sup>d</sup>MB tumor samples were obtained from the Children's Healthcare of Atlanta.

Figure 1.

Development of the five-gene Hh signature. A panel of 32 candidate genes differentially expressed in Hh<sup>b</sup> vs. Hh<sup>-</sup> tumors and 22 potential normalization genes were selected from a combined dataset of 313 medulloblastoma (MB) samples in three independently published profiling studies (4, 5, 7). Data from normal cerebellum samples obtained from the Gene Expression Omnibus (<http://www.ncbi.nlm.nih.gov/geo/>) database were also included in the analysis. RT-PCR assays for these candidate genes were developed and optimized for use in 3 FFPE specimens (1 Hh<sup>b</sup>, 1 Hh<sup>-</sup>, 1 normal cerebellum) obtained from BioChain. Assays with robust performance for 18 differentially expressed genes and four control genes were further selected. This panel of genes was assayed in the 40 FFPE medulloblastoma specimens with established Hh activation status (7) and subjected to model building by the elastic net method for signature identification. FFPE medulloblastoma tumor samples were obtained from the Dana-Farber Cancer Institute/Boston Children's Hospital. The 40 matching fresh-frozen samples were a subset of the 194 medulloblastoma samples previously profiled (7). The five-gene Hh signature was externally validated in a set of 25 matching FFPE and fresh-frozen medulloblastoma samples obtained from Children's Healthcare of Atlanta.

performance in FFPE for 18 differentially expressed genes (ten upregulated and eight downregulated in  $Hh^b$  vs.  $Hh^-$  tumors) plus four control genes were further selected and assembled onto a TLDA card. The expression of this panel of genes was assayed in the 40 FFPE medulloblastoma specimens and formed the basis of the multigene model-building exercise (Fig. 1). The matching fresh-frozen specimens of these 40 cases were previously profiled as part of a larger group of 194 medulloblastoma fresh-frozen samples as described by Cho and colleagues (7), and each case was classified as  $Hh^b$  or  $Hh^-$  based on its Affymetrix gene expression profile.

The 18 differentially regulated candidate genes normalized by the average of the control genes were subjected to a model-building exercise using the elastic net algorithm (24) in a 5-fold cross-validation framework. The elastic net method selects the optimal model that uses the least number of genes to identify  $Hh$  activation status with minimal error (Fig. 2; ref. 25). To understand the impact of variation in the cross-validation procedure, the entire model-building exercise was iterated 10,000 times, resulting in a distribution of optimal models. Models with five genes were strongly represented in this distribution. The most frequently selected five-gene model was deemed optimal (Fig. 2B). The five-gene signature includes four upregulated genes, *GLI1*, *SPHK1*, *SHROOM2*, and *PDLIM3*, and one downregulated gene, *OTX2*.

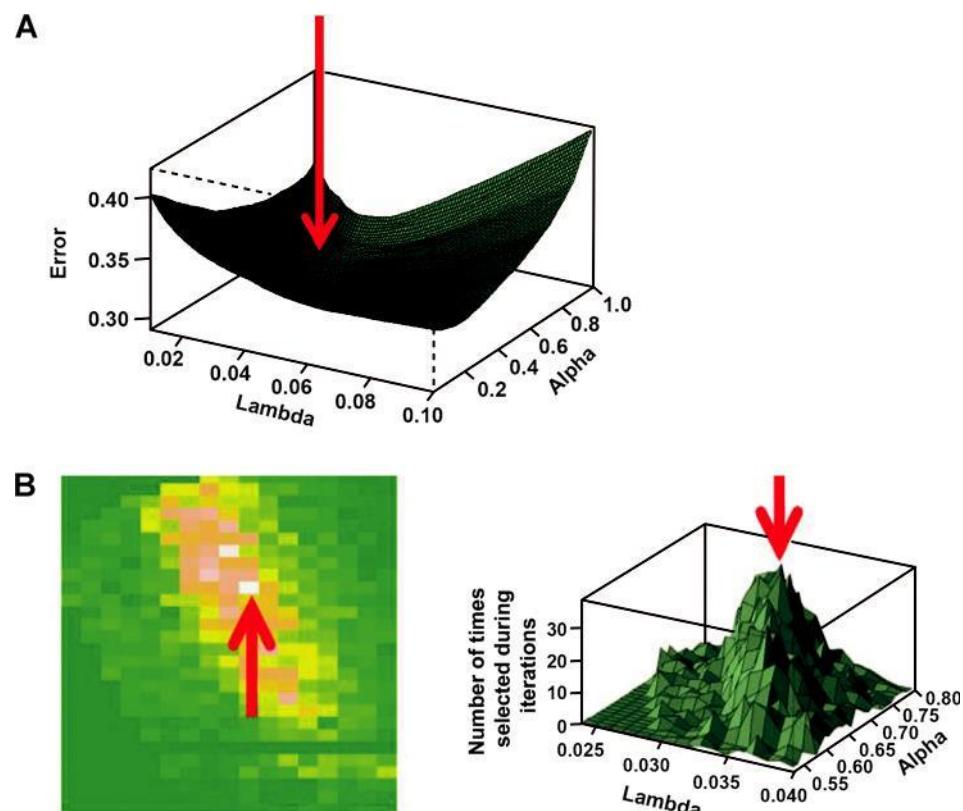
As described above, the model computes a propensity score of being  $Hh^b$  (0 to 1) for a given sample based on the expression levels of the five genes. A cutoff of 0.5 was set *a priori* and used to determine the  $Hh$  activation status ( $Hh^b$  vs.  $Hh^-$ ; Fig. 3).

Detailed methods are provided (see Patients and Methods section).

#### Validation of the five-gene model

The five-gene model was fixed and an analysis plan was written and approved in our validated clinical repository. The model was then validated with a sample set of 25 matched FFPE and fresh-frozen medulloblastoma tumors (CHOA) that were not used during the model-building exercise (Fig. 1). Tissue specimens from 25 patients with a median age of 3 years (range, 0.5–16 years) were collected at diagnosis ( $n = 24$ ) or following chemo-therapy ( $n = 1$ ). The fresh-frozen samples were analyzed by Affymetrix gene expression profiling, and  $Hh$  activation status was determined as previously described (7). The matching FFPE medulloblastoma samples were processed and subjected to gene expression analysis using the custom TLDA. The five-gene model was used to calculate the propensity score and predict  $Hh$  activation status for each tumor in this validation set.

Eight patients were classified as  $Hh^b$  and 17 as  $Hh^-$  based on Affymetrix profiling. The identification of  $Hh$  activation status from the five-gene signature showed 100% agreement with the determination made by Affymetrix profiling. With the five-gene model, the eight tumors identified to be  $Hh^b$  had a median propensity score of 0.879 (range, 0.691–0.976), and the remaining 17 tumor samples were considered to be  $Hh^-$ , with a median propensity score of 0.007 (range, 0.001–0.03; Fig. 3). The considerable difference in propensity scores between the positive and negative cases reflects a robustness of  $Hh$  status determination by the five-gene model.



**Figure 2.**

Selection of the five-gene model. An optimal model with minimal error (red arrow) was determined using the elastic net algorithm (24) in a five-fold cross-validation framework. A, cross-validation was run for each model parameter pair, shown by the x and y axes, in which each model provided an estimate of model error (z axis). The optimal model was the one that produced minimal model error in cross-validation (red arrow). B, to better understand the potential cross-validation error, the procedure was iterated 10,000 times. The frequency distribution of the models with five genes was plotted in a heat-map format (B, left) and in three dimensions (B, right) for each model parameter pair, in which the most frequently selected model was chosen as optimal (red arrow).

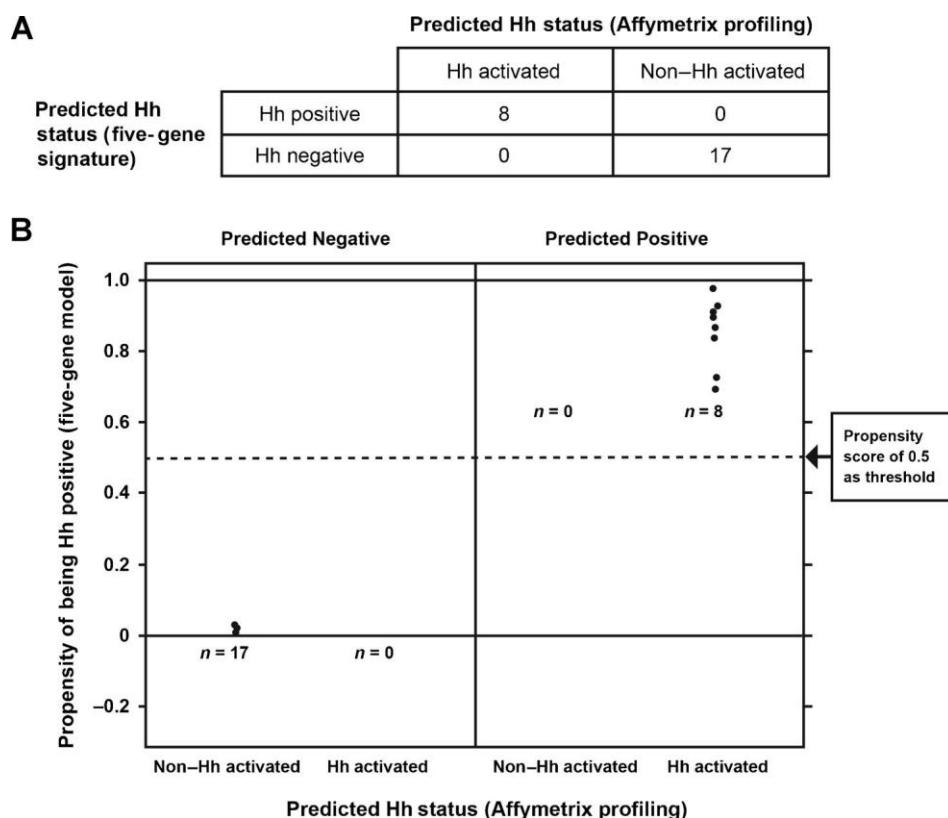


Figure 3.

External validation data for the five-gene Hh signature. The five-gene Hh signature was validated in an independent set of 25 medulloblastoma specimens. A, the prediction of Hh activation status from the five-gene signature showed 100% agreement with the determination made by Affymetrix profiling for all tumor specimens. B, the eight tumors predicted to be Hh activated had a median propensity score of 0.879 (range, 0.691–0.976), and the remaining 17 tumor samples were predicted to be non-Hh activated, with a median propensity score of 0.007 (range, 0.001–0.03). Model-predicted propensity score threshold for Hh activation call was 0.5 (dotted line).

#### Predictive value of five-gene Hh signature for tumor response

Classification of tumors by the five-gene signature and Affymetrix profiling both rely on measures of gene expression; therefore, a strong agreement between the two methods may be somewhat expected. A more rigorous assessment of the five-gene signature may be determined using patient samples with accompanying data showing clinical response to treatment with the potent Hh pathway inhibitor sonidegib. If the signature is accurate, only those patients who are classified as Hh activated should respond to sonidegib therapy, whereas those who are Hh non-activated should not receive benefit.

To test this hypothesis, the same locked model used in the validation above was then applied to different samples from patients enrolled in clinical trials; these data were independent from those used during the model-building exercise. Pretreatment archival FFPE tumor specimens from 50 patients with relapsed medulloblastoma who were enrolled in three phase I clinical studies of sonidegib were profiled for the five-gene Hh signature by RT-PCR (19–21). Six patients who achieved partial or complete response were determined to have Hh<sup>b</sup> tumors. One patient with an Hh<sup>b</sup> tumor had stable disease for 112 days and 2 patients progressed after 36 and 65 days. All 41 remaining patients were predicted to have Hh<sup>-</sup> tumors and had disease progression (n = 30), stable disease (n = 5), or were not evaluable for tumor response (n = 6). Duration on treatment for patients with Hh<sup>b</sup> tumors ranged from 36 to 288 days, whereas duration on treatment for patients with Hh<sup>-</sup> tumors was lower, ranging from 10 to 169 days. In retrospect, Hh<sup>b</sup> tumors appeared to be particularly sensitive to sonidegib, with tumor responses observed after just 2 months of treatment. For the three adult patients who obtained partial responses on sonidegib (at doses of 200, 800, and 1,500

mg once daily), the duration of tumor responses ranged from four to eight months before documented disease progression (15). Two pediatric and one adult patient (dosed at 372 mg/m<sup>2</sup>, 425 mg/m<sup>2</sup>, and 800 mg sonidegib once daily, respectively) also responded and were in complete remission for 22, >18, and >2 months, respectively (16).

The association between Hh activation status and tumor response to sonidegib treatment is shown in Tables 2 and 3. The positive and negative predictive values of the signature for tumor response are estimated to be 0.67 [95% confidence interval (CI), 0.30–0.93] and 1.00 (95% CI, 0.91–1.00), respectively. All model scores were derived using patient samples with blinded response data to remove any potential bias. Thirty-one patients with medulloblastoma from the three studies did not have tumors evaluated with the five-gene signature assay.

Of particular interest, one patient with confirmed partial response underwent five independent surgeries over a 10-year period, from initial diagnosis to the latest relapse just before treatment with sonidegib. Tissue from all five specimens was determined to be Hh activated by the five-gene signature, indicating that Hh pathway activation status can be stably maintained over an extended period of time and following recurrence. Several other patients had multiple tumor samples tested—four patients (1 stable disease, 3 progressive disease) were determined to have Hh<sup>-</sup> tumors over the course of their disease, one patient (complete response) had a Hh<sup>b</sup> tumor throughout the disease course, and another patient (complete response) had two tumor samples analyzed with different results (1 Hh<sup>b</sup>, 1 Hh<sup>-</sup>; Table 2). The reason for this discrepancy is being investigated; however, it could be due in part to a large difference in tumor content between the two samples.

Table 2. Observed responses to sonidegib treatment correlated with Hh activation status as determined by the five-gene signature in individual patients with medulloblastoma

Study	Dose (once daily)	Best overall response <sup>a</sup>	Five-gene signature–predicted Hh status	Propensity score
NCT00880308	200 mg	PR	Activated	0.983
	800 mg	PR <sup>b</sup>	Activated	0.929, 0.607 <sup>c</sup> , 0.931, 0.880, 0.873
	1,500 mg	PR	Activated	0.967
	250 mg <sup>d</sup>	PD	Activated	0.806
	800 mg	PD	Nonactivated	0.384
	1,500 mg	PD	Nonactivated	0.092
NCT01208831	400 mg	PD	Nonactivated	0.006
	600 mg	PD	Nonactivated	0.003
NCT01125800	372 mg/m <sup>e</sup>	CR <sup>f</sup>	Activated	0.327, 0.874
	425 mg/m <sup>e</sup>	CR	Activated	0.949
	800 mg	CR <sup>g</sup>	Activated	0.906, 0.729
	800 mg	SD	Activated	0.866
	680 mg/m <sup>2</sup>	PD	Activated	0.603
	800 mg	PD	Nonactivated	0.246
	800 mg	PD	Nonactivated	0.017
	800 mg	SD	Nonactivated	0.306
	233 mg/m <sup>2</sup>	SD <sup>h</sup>	Nonactivated	0.001, 0.002
	233 mg/m <sup>2</sup>	PD	Nonactivated	range, 0.001–0.007 <sup>i</sup>
	233 mg/m <sup>2</sup>	Unknown	Nonactivated	0.024
	372 mg/m <sup>2</sup>	PD <sup>j</sup>	Nonactivated	range, 0.001–0.012 <sup>i</sup>
	372 mg/m <sup>2</sup>	Unknown	Nonactivated	0.007
	425 mg/m <sup>2</sup>	SD	Nonactivated	0.038
	425 mg/m <sup>2</sup>	PD	Nonactivated	range, 0.001–0.014 <sup>j</sup>
	425 mg/m <sup>2</sup>	Unknown	Nonactivated	0.003, 0.011 <sup>k</sup>
	680 mg/m <sup>2</sup>	SD	Nonactivated	0.004, 0.304 <sup>j</sup>
	680 mg/m <sup>2</sup>	PD	Nonactivated	range, 0.011–0.403 <sup>m</sup>
	680 mg/m <sup>2</sup>	Unknown	Nonactivated	0.022, 0.445 <sup>n</sup>

Abbreviations: CR, complete response; PD, progressive disease; PR, partial response; SD, stable disease.

<sup>a</sup>Tumor response was determined by RECIST 1.0 (ref. 33; NCT01208831) and the Neuro-Oncology Criteria of Tumor Response (ref. 34; NCT00880308, NCT01125800). <sup>b</sup>Patient provided five blocks of tumor tissues collected throughout the disease course; all samples were Hh activated per the five-gene signature assay.

<sup>c</sup>Low sample quantity.

<sup>d</sup>This patient was treated on a twice-daily schedule.

<sup>e</sup>Patient provided two blocks of tumor tissues collected throughout the disease course; first sample was Hh nonactivated and the second sample was Hh activated per the five-gene signature assay.

<sup>f</sup>Patient provided two blocks of tumor tissues collected throughout the disease course; both samples were Hh activated per the five-gene signature assay.

<sup>g</sup>Patient provided two blocks of tumor tissues collected throughout the disease course; both samples were Hh nonactivated per the five-gene signature assay. <sup>h</sup>Data from 6 patients treated at 233 mg/m<sup>2</sup>, each with progressive disease.

<sup>i</sup>Data from 8 patients treated at 372 mg/m<sup>2</sup>, each with progressive disease. Three patients provided two (n ¼ 2) or three (n ¼ 1) blocks of tumor tissues throughout the disease course; all samples were Hh nonactivated per the five-gene signature assay.

<sup>j</sup>Data from 4 patients treated at 425 mg/m<sup>2</sup>, each with progressive disease.

<sup>k</sup>Data from 2 patients treated at 425 mg/m<sup>2</sup>, each with unknown response. <sup>l</sup>Data

from 2 patients treated at 680 mg/m<sup>2</sup>, each with stable disease. <sup>m</sup>Data from 6

patients treated at 680 mg/m<sup>2</sup>, each with progressive disease.

<sup>n</sup>Data from 2 patients treated at 680 mg/m<sup>2</sup>, each with an unknown response.

## Discussion

In this study, we have developed and validated a five-gene signature that robustly identifies Hh pathway activation and patients most likely to respond to Hh pathway–targeted therapy. Other studies identifying and analyzing gene signatures have shown impressive results; however, these studies often lacked rigorous data analysis and validation (26). Conversely, the gene signature, based on data from previous gene expression profiling studies, was independently selected and validated in separate settings. The five-gene Hh signature assay was optimized for FFPE tumor samples. The accuracy of the Hh signature model was demonstrated by a perfect concordance with the Hh status determined by standard Affymetrix profiling. Furthermore, tumor specimens from patients with relapsed medulloblastoma treated with the SMO inhibitor, sonidegib, in three phase I studies demonstrated a strong association between Hh positivity and tumor response (Table 3). Tumors from all 6 patients who achiev-

ed either a partial or complete tumor response were classified as Hh activated according to the five-gene Hh signature, whereas tumors from 35 nonresponders were Hh nonactivated. The underlying reason for lack of response in three patients with Hh-activated tumors is unknown but may be due to pathway activation downstream of SMO. Data from this study are consistent with profiling studies, suggesting that Hh-activated tumors are more prevalent in adults and young children ( $\geq$  3 years of age) than in older children (Table 3; refs. 6, 8). The low preponderance of Hh-activated tumors observed in children evaluated in this study may be attributed to their age as most children were older than 3 years of age. This observation may have a significant impact on studies designed to preselect children with Hh-activated medulloblastoma.

The clinical utility of a gene signature is largely dependent on the assay used to analyze it. Our procedure overcomes the limitations of standard profiling techniques, including insufficient sample

**Table 3. Summary of sonidegib activity in Hh pathway–activated medulloblastoma tumors from patients evaluated in three phase I trials<sup>a</sup>**

	Responders/Hh activated	Responders/non-Hh activated	Total
Adults	4/6 <sup>b</sup> (1 CR, 3 PR)	0/7	13
Children <sup>c</sup>	2/3 (2 CR, 1 PD)	0/34	37
PPV <sup>d</sup>		0.67 (0.30–0.93)	
NPV <sup>d</sup>		1.00 (0.91–1.00)	
Sensitivity		1.00 (0.54–1.00)	
Specificity		0.93 (0.81–0.99)	

Abbreviations: CR, complete response; NPV, negative predictive value; PD, progressive disease; PR, partial response; PPV, positive predictive value.

<sup>a</sup>These data represent a convenience sample. Thirty-one patients with medulloblastoma from the three studies did not have tumors evaluated with the five-gene signature assay.

<sup>b</sup>Includes one patient with stable disease.

<sup>c</sup>Most of the children evaluated were older than 3 years of age.

<sup>d</sup>Values are shown as estimate (Clopper Pearson exact 95% CIs; ref. 35).

quality and/or quantity, high cost, and inefficiency. Furthermore, because nucleic acids are degraded or modified during FFPE tissue processing, transcript analysis becomes difficult. To compensate, we developed a rigorous screening process to identify a group of genes that were still present and can be robustly analyzed in FFPE samples. Of note, analyses of serial tumor samples collected from several patients up to 10 years before treatment with sonidegib indicate that Hh pathway activation status is maintained from the initial diagnosis to the time of recurrence. However, in one patient with multiple tumor samples, Hh pathway activation status changed during the course of the disease, from Hh<sup>-</sup> to Hh<sup>b</sup>. Additional studies using the five-gene signature are necessary to confirm the ability to use archival tumor samples to preselect patients for future clinical studies of sonidegib.

The five-gene Hh signature identified in this study is composed primarily of genes not previously associated with canonical Hh signaling. One exception is the zinc finger transcription factor GLI1, which mediates transcriptional responses to Hh signaling (27). Upregulation of GLI1 has been observed in numerous malignancies, including basal cell carcinoma (BCC; ref. 28). Sonidegib exhibited exposure-dependent inhibition of GLI1 mRNA expression in normal skin, which correlated with changes in tumor samples in the phase I study described above and further validates the robustness of this assay (15). Other genes in the Hh signature that were upregulated include SHROOM2, which facilitates contractile network formation in endothelial cells, PDLIM3, which plays a role in muscle differentiation, and SPHK1, which catalyzes the phosphorylation of sphingosine to form sphingo-sine 1 phosphate (29–31). None of these genes has been previously associated with medulloblastoma, and their role, if any, in the pathogenesis of medulloblastoma is unclear. Conversely, OTX2, a transcription factor involved in early development of the central nervous system, is amplified and critical for the maintenance and progression of a subset of medulloblastomas (21%; ref. 32). OTX2 is differentially expressed in different medulloblastoma subsets and is highly expressed in medulloblastomas with amplification (21%; refs. 6, 32). However, it appears that OTX2 expression is low in Hh-activated medulloblastomas (6, 32).

In conclusion, the five-gene Hh signature is a robust tool for identifying patients with Hh pathway–activated medulloblastoma using FFPE tumor samples and can be used to optimize the risk-benefit of treatment with Hh pathway inhibitors. The techniques used in this study could be adapted for large-scale analysis of patient samples. Indeed, the five-gene Hh signature assay received an investigation device exemption from the United States Food and Drug Administration for use in a clinical trial (Novartis

Pharmaceuticals Corporation, documentation on file) and is currently being used to determine eligibility in a trial testing the efficacy of sonidegib in patients with Hh pathway–activated relapsed medulloblastoma (NCT01708174). This and future trials testing sonidegib in cancers with evidence of activated Hh signaling may serve to further validate the positive predictive value of the Hh signature and provide a clinical tool to guide treatment selection in patients with medulloblastoma. The five-gene Hh signature is also being tested in additional tumor types, including BCC; however, the results thus far are inconclusive as the clinical data are not yet mature.

#### Disclosure of Potential Conflicts of Interest

Y. Shou and D.D. Amakye were employees of Novartis. D.N. Robinson is an employee of and has ownership interest (including patents) in Novartis. K.L. Rose has ownership interest (including patents) in Novartis. Y. Ando reports receiving a commercial research grant and speakers bureau honoraria from Novartis. B. Georger is a consultant/advisory board member for Novartis. D.R. Hargrave is a consultant/advisory board member for Novartis. H.A. Tawbi, T.J. MacDonald, and M.W. Kieran are consultants/advisory board members for Novartis. No potential conflicts of interest were disclosed by the other authors.

#### Authors' Contributions

Conception and design: Y. Shou, D.M. Robinson, D.D. Amakye, K.L. Rose, Y.-J. Cho, T. Sharp, T.J. MacDonald, M.W. Kieran

Development of methodology: Y. Shou, D.M. Robinson, K.L. Rose, Y.-J. Cho, T. Sharp, R. Bandaru, M.W. Kieran

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): Y. Shou, K.L. Rose, Y.-J. Cho, K.L. Ligon, Y. Ando, B. Georger, F. Doz, D.M. Ashley, D.R. Hargrave, M. Casanova, H.A. Tawbi, J. Rodon, A.L. Thomas, A.C. Mita, T.J. MacDonald, M.W. Kieran

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): Y. Shou, D.M. Robinson, D.D. Amakye, Y.-J. Cho, K.L. Ligon, A.S. Haider, R. Bandaru, A.C. Mita, M.W. Kieran

Writing, review, and/or revision of the manuscript: Y. Shou, D.M. Robinson, D.D. Amakye, K.L. Rose, K.L. Ligon, A.S. Haider, R. Bandaru, Y. Ando, B. Georger, F. Doz, D.M. Ashley, D.R. Hargrave, M. Casanova, H.A. Tawbi, J. Rodon, A.L. Thomas, A.C. Mita, T.J. MacDonald, M.W. Kieran, Y.-J. Cho, T. Sharp

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): Y. Shou, D.M. Robinson, K.L. Ligon, H.A. Tawbi, T.J. MacDonald, M.W. Kieran

Study supervision: Y. Shou

#### Acknowledgments

The authors thank John Heath (Royal Children's Hospital in Melbourne, Australia), Eric Bouffet (Division of Haematology/Oncology at the Hospital for Sick Children in Toronto, Canada), Alba A. Brandes (Medical Oncology Department from Azienda USL in Bologna, Italy), and Julia Chisholm (The Royal Marsden Hospital in Surrey, UK) who provided patient samples. They

also thank Sha-Sha Wang and Feng Yang from Novartis Pharmaceuticals Corporation for technical support. Medical editorial assistance was provided by Jillian Brechbiel, PhD, and Karen Miller-Moslin, PhD.

### Grant Support

This study was supported by Novartis Pharmaceuticals Corporation for clinical studies and medical editorial support.

### A Five-Gene Hedgehog Signature in Medulloblastoma

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

### References

14. CBTRUS. CBTRUS statistical report: primary brain and central nervous system tumors diagnosed in the United States in 2004–2007. Available from: <http://www.cbtrus.org/2011-NPCR-SEER/WEB-0407-Report-3-3-2011.pdf>. Accessed October 2012.
15. Packer RJ, Vezina G. Management of and prognosis with medulloblastoma: therapy at a crossroads. *Arch Neurol* 2008;65:1419–24.
16. Packer RJ, Cogen P, Vezina G, Rorke LB. Medulloblastoma: clinical and biologic aspects. *Neuro Oncol* 1999;1:232–50.
17. Thompson MC, Fuller C, Hogg TL, Dalton J, Finkelstein D, Lau CC, et al. Genomics identifies medulloblastoma subgroups that are enriched for specific genetic alterations. *J Clin Oncol* 2006;24:1924–31.
18. Kool M, Koster J, Bunt J, Hasselt NE, Lakeman A, van Sluis P, et al. Integrated genomics identifies five medulloblastoma subtypes with distinct genetic profiles, pathway signatures and clinicopathological features. *PLoS One* 2008;3:e3088.
19. Northcott PA, Korshunov A, Witt H, Hielscher T, Eberhart CG, Mack S, et al. Medulloblastoma comprises four distinct molecular variants. *J Clin Oncol* 2011;29:1408–14.
20. Cho YJ, Tsherniak A, Tamayo P, Santagata S, Ligon A, Greulich H, et al. Integrative genomic analysis of medulloblastoma identifies a molecular subgroup that drives poor clinical outcome. *J Clin Oncol* 2011;29: 1424–30.
21. Taylor MD, Northcott PA, Korshunov A, Remke M, Cho YJ, Clifford SC, et al. Molecular subgroups of medulloblastoma: the current consensus. *Acta Neurochir (Wien)* 2012;123:465–72.
22. Lee Y, Miller HL, Jensen P, Hernan R, Connelly M, Wetmore C, et al. A molecular fingerprint for medulloblastoma. *Cancer Res* 2003;63:5428–37.
23. Slade I, Murray A, Hanks S, Kumar A, Walker L, Hargrave D, et al. Heterogeneity of familial medulloblastoma and contribution of germline PTCH1 and SUFU mutations to sporadic medulloblastoma. *Fam Cancer* 2011;10:337–42.
24. Teglund S, Toftgard R. Hedgehog beyond medulloblastoma and basal cell carcinoma. *Biochim Biophys Acta* 2010;1805:181–208.
25. Rubin LL, de Sauvage FJ. Targeting the Hedgehog pathway in cancer. *Nat Rev Drug Discov* 2006;5:1026–33.
26. Pan S, Wu X, Jiang J, Gao W, Wan Y, Cheng D, et al. Discovery of NVP-LDE225, a potent and selective smoothened antagonist. *ACS Med Chem Lett* 2010;1:130–4.
27. Buonamici S, Williams J, Morrissey M, Wang A, Guo R, Vattay A, et al. Interfering with resistance to smoothened antagonists by inhibition of the PI3K pathway in medulloblastoma. *Sci Transl Med* 2010;2:51ra70.
28. Rodon J, Tawbi H, Thomas A, Dummer R, Stoller R, Turtschi CP, et al. A phase 1, multicenter, open-label, first-in-human, dose-escalation study of the oral hedgehog inhibitor sonidegib (LDE225) in patients with advanced solid tumors. *Clin Cancer Res* 2014;20:1900–9.
29. Geoerger B, Aerts I, Casanova M, Chisholm J, Hargrave D, Leary SES, et al. A phase I/II study of LDE225, a smoothened (Smo) antagonist, in pediatric patients with recurrent medulloblastoma (MB) or other solid tumors. *J Clin Oncol* 2012 ASCO Ann Mtg Proc 2012;30:abstr 9519.
30. Lindstrom E, Shimokawa T, Toftgard R, Zaphiroopoulos PG. PTCH mutations: distribution and analyses. *Hum Mutat* 2006;27:215–9.
31. Kim K, Zakharkin SO, Allison DB. Expectations, validity, and reality in gene expression profiling. *J Clin Epidemiol* 2010;63:950–9.
44. ClinicalTrials.gov. [homepage on the Internet]. Dose finding and safety of oral LDE225 in patients with advanced solid tumors. Available from: <http://ClinicalTrials.gov/show/NCT00880308>. Accessed October 2012; last updated August 2014.
45. ClinicalTrials.gov. [homepage on the Internet]. A dose finding and safety study of oral LDE225 in children. Available from: <http://ClinicalTrials.gov/show/NCT01125800>. Accessed October 2012; last updated August 2014.
46. ClinicalTrials.gov. [homepage on the Internet]. An East Asian study of LDE225. Available from: <http://ClinicalTrials.gov/show/NCT01208831>. Accessed October 2012; last updated August 2014.
47. Tibshirani R. Regression shrinkage and selection via the lasso. *J R Stat Soc Series B Stat Methodol* 1996;58:267–88.
48. Hoerl A, Kennard R. Ridge regression: applications to nonorthogonal problems. *Technometrics* 1970;12:69–82.
49. Wu Z, Irizarry R, Gentleman R, Murillo F, Spencer F. A model-based background adjustment for oligonucleotide expression arrays. *J Am Stat Assoc* 2004;99:909–17.
50. Friedman J, Hastie T, Tibshirani R. Sparse inverse covariance estimation with the graphical lasso. *Biostatistics* 2008;9:432–41.
51. Castaldi PJ, Dahabreh IJ, Ioannidis JP. An empirical assessment of validation practices for molecular classifiers. *Brief Bioinform* 2011;12: 189–202.
52. Scales SJ, de Sauvage FJ. Mechanisms of Hedgehog pathway activation in cancer and implications for therapy. *Trends Pharmacol Sci* 2009;30: 303–12.
53. Bonifas JM, Pennypacker S, Chuang PT, McMahon AP, Williams M, Rosenthal A, et al. Activation of expression of hedgehog target genes in basal cell carcinomas. *J Invest Dermatol* 2001;116:739–42.
54. Pomies P, Pashmforoush M, Vegezzi C, Chien KR, Auffray C, Beckerle MC. The cytoskeleton-associated PDZ-LIM protein, ALP, acts on serum response factor activity to regulate muscle differentiation. *Mol Biol Cell* 2007;18: 1723–33.
55. Farber MJ, Rizaldy R, Hildebrand JD. Shroom2 regulates contractility to control endothelial morphogenesis. *Mol Biol Cell* 2011;22:795–805.
56. Van Brocklyn JR, Jackson CA, Pearl DK, Kotur MS, Snyder PJ, Prior TW. Sphingosine kinase-1 expression correlates with poor survival of patients with glioblastoma multiforme: roles of sphingosine kinase isoforms in growth of glioblastoma cell lines. *J Neuropathol Exp Neurol* 2005;64: 695–705.
57. Adamson DC, Shi Q, Wortham M, Northcott PA, Di C, Duncan CG, et al. OTX2 is critical for the maintenance and progression of Shh-independent medulloblastomas. *Cancer Res* 2010;70:181–91.
58. Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, et al. New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. *J Natl Cancer Inst* 2000;92:205–16.
59. Wen PY, Macdonald DR, Reardon DA, Cloughesy TF, Sorenson AG, Galanis E, et al. Updated response assessment criteria for high-grade gliomas: response assessment in Neuro-Oncology Working Group. *J Clin Oncol* 2010;28:1963–72.
60. Clopper CJ, Pearson ES. The use of confidence or fiducial limits illustrated in the case of the binomial. *Biometrika* 1934;26:404–13.

### **Anexo 3. Resumen de los datos del ensayo clínico “A Study of BYL719 in Adult Patients With Advanced Solid Malignancies, Whose Tumors Have an Alteration of the PIK3CA Gene” (ClinicalTrials.gov Identifier: NCT01219699)**

Datos presentados en:

AACR Annual Meeting 2012.

Fritsch CM, Schnell C, Chatenay-Rivauday C, et al. NVP-BYL719, a novel PI3K $\alpha$  selective inhibitor with all the characteristics required for clinical development as an anti-cancer agent. AACR Annual Meeting 2012;abstract 3748.

Huang A, Fritsch C, Wilson C, et al. Single agent activity of PIK3CA inhibitor BYL719 in a broad cancer cell line panel. [abstract]. AACR Annual Meeting 2012;abstract 3749.

Juric D, Rodon J, Gonzalez-Angulo AM, et al, BYL719, a next generation PI3K alpha specific inhibitor: preliminary safety, PK, and efficacy results from the first-in-human study. AACR annual meeting 2012; abstract CT-01.

AACR Annual Meeting 2013.

Rodon J, Juric J, Gonzalez-Angulo AM, et al. Towards defining the genetic framework for clinical response to treatment with BYL719, a PI3K $\alpha$ -specific inhibitor. AACR annual meeting 2013; abstract LB-65.

American Society of Clinical Oncology Annual Meeting 2014

Gonzalez-Angulo AM, Juric D, Argilés G, et al. Safety, pharmacokinetics, and preliminary activity of the  $\alpha$ -specific PI3K inhibitor BYL719: Results from the first-in-human study. ASCO annual meeting 2014; Abstract 2531

European Society for Medical Oncology Congress 2014

Juric D, Burris HA, Schuler M, et al. Phase I Study of the PI3K $\alpha$  Inhibitor Alpelisib (BYL719), as a Single Agent in Patients With Advanced Solid Tumors (aST). ESMO 2014; Abstract 451PD.

San Antonio Breast Cancer Symposium 2014

Janku F, Juric D, Cortes J, et al. Phase I study of the PI3K $\alpha$  inhibitor BYL719 plus fulvestrant in patients with PIK3CA-altered and wild type ER+/HER2- locally advanced or metastatic breast cancer. SABCS 2014; Abstract PD5-5

## **Summary report of the clinical trial "A Study of BYL719 in Adult Patients With Advanced Solid Malignancies, Whose Tumors Have an Alteration of the PIK3CA Gene" (ClinicalTrials.gov Identifier: NCT01219699)**

### **Introduction**

The phosphatidylinositol-3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) pathway is one of the most commonly activated signaling pathways in cancer.<sup>1</sup> Its activation is frequently the result of mutation or amplification of *PIK3CA*, the gene that encodes the catalytic subunit (p110 $\alpha$ ) of PI3K.<sup>2–5</sup> PI3K/AKT/mTOR pathway activation has been shown to promote tumor growth and progression, validating this as an attractive target for anticancer therapy.<sup>1,6–8</sup>

*PIK3CA* mutations have been observed in a variety of common solid tumors, including in 27–36% of breast,<sup>9,10</sup> 18% of colorectal,<sup>11</sup> 13% of head and neck,<sup>12</sup> and 12% of ovarian cancers.<sup>13</sup> Typically *PIK3CA* mutations are found in three main hot-spot clusters in the helical (E542K and E545K) and kinase domains (H1047R).<sup>14–16</sup> In preclinical models, these mutations lead to constitutive PI3K $\alpha$  activation, resulting in cellular transformation and tumorigenesis.<sup>14</sup> Preclinical models and early clinical data have suggested that targeting the PI3K/AKT/mTOR signaling pathway may be effective in cancers with *PIK3CA* mutations.<sup>17–21</sup>

A number of PI3K inhibitors are under clinical investigation, including pan-PI3K inhibitors, targeting all four isoforms of Class I PI3K, and isoform-specific PI3K inhibitors. Alpelisib (BYL719; Novartis Pharma AG, Basel, Switzerland) is an oral PI3K inhibitor that selectively targets the  $\alpha$ -isoform of Class I PI3K. PI3K $\alpha$  inhibitors may be effective in cancers that signal heavily through the  $\alpha$ -isoform, such as those with *PIK3CA* mutations and amplifications and those with mutation or overexpression of certain RTKs (e.g. HER2).<sup>22,23</sup> In comparison with pan-PI3K and dual PI3K/mTOR inhibitors, PI3K $\alpha$ -inhibitors may have an increased therapeutic window due to highly specific targeting of the  $\alpha$ -isoform of Class I PI3K. This may lead to an improved safety profile<sup>14,24</sup> and specific inhibition of the  $\alpha$ -isoform may also allow administration at therapeutic doses without being limited by off-target toxicity.<sup>14,24</sup>

In a variety of cancer cell lines, alpelisib has shown antitumor activity, especially in those harboring *PIK3CA* mutations,<sup>14,25,26</sup> and in tumor xenograft models with mutated or amplified *PIK3CA*.<sup>14</sup> The Cancer Cell Line Encyclopedia (CCLE), which catalogued the genetic and molecular profiles of 947 human cancer cell lines, revealed that alpelisib showed marked selective efficacy in *PIK3CA*-altered cell lines when compared with *PIK3CA*-wild-type cell lines. This selective efficacy was also observed when alpelisib was compared with pan-PI3K inhibitors.<sup>14</sup> Furthermore, in a screen of 34 *PIK3CA*-mutant cancer cell lines, 22 (64.7%) were sensitive to alpelisib compared with 100 (22.7%) out of 440 *PIK3CA* wild-type cell lines ( $p=1 \times 10^{-6}$ ; ( $EC_{50}$  and  $A_{max}$  cut-offs were 3.04  $\mu$ mol/L and –30%, respectively).<sup>14</sup>

Here we report the full analysis of the first-in-human dose-escalation and expansion study of single-agent alpelisib in patients with *PIK3CA*-altered advanced solid tumors or *PIK3CA*-altered or wild-type ER receptor-positive (ER+) breast cancer (NCT01219699).

## **Patients and methods**

### *Patient population*

Adult ( $\geq 18$  years) patients with histologically-confirmed, advanced unresectable solid tumors who had progressed (as per Response Evaluation Criteria in Solid Tumors version 1.0 [RECIST v1.0]) on their last line of therapy within 3 months before screening/baseline visit, or for whom no standard anticancer therapy existed, were eligible. The dose escalation arm enrolled patients with *PIK3CA*-altered (mutation or amplification) advanced solid tumors. The dose expansion arm also enrolled a sub-set of patients with *PIK3CA* wild-type ER+/HER2 negative locally advanced or metastatic breast cancer. Other key inclusion criteria included: availability of representative tumor sample for determination of *PIK3CA* gene status; presence of at least one measurable or non-measurable lesion as defined by RECIST v1.0; and fasting plasma glucose (FPG)  $<140$  mg/dl (7.8 mmol); and excluding those with clinically manifest diabetes mellitus [including history of gestational diabetes mellitus], or documented steroid-induced diabetes mellitus). Key exclusion criteria included: brain metastases (unless treated and free of attributable signs/symptoms in the absence of corticosteroid therapy); prior treatment with PI3K, AKT, or mTOR inhibitors and failure to benefit (patients who had shown clinical benefit from previous treatment with PI3K, AKT, or mTOR inhibitors were permitted); peripheral neuropathy NCI-CTC Grade  $\geq 3$ .

Approval was obtained from the ethics committees of participating institutions and regulatory authorities. All patients provided written informed consent and agreed to comply with the protocol. The study was conducted in accordance with the Declaration of Helsinki and guidelines for Good Clinical Practice as defined by the International Conference on Harmonization.

### *Study design and dose escalation*

This was a Phase IA, multicenter, open-label, dose-escalation study with a dose expansion arm, of single-agent alpelisib. The primary objective was to determine the maximum tolerated dose (MTD) or recommended Phase 2 dose (RP2D) of single-agent oral alpelisib (for two dosing regimens). Secondary objectives included assessment of safety and tolerability, pharmacokinetics (PK), and preliminary efficacy. Exploratory objectives included assessment of downstream effects of PI3K pathway inhibition and biomarkers of response.

Patients received oral alpelisib 30–450 mg once-daily (QD) or 120–200 mg twice-daily (BID) on a continuous schedule of 28-day cycles (Figure 2). Following MTD determination using a once-daily schedule, an expansion arm investigated additional patients at the MTD. A twice-daily schedule of alpelisib was investigated in a parallel study arm. Dose escalation was guided by an adaptive Bayesian logistic regression model with overdose control.<sup>27,28</sup> The MTD was defined as the highest dose of alpelisib that did not cause medically unacceptable dose-limiting toxicities (DLTs) during the first cycle of treatment in more than 33% of treated patients. Intra-patient dose escalation was not permitted during the first four cycles of treatment. Patients enrolling in the dose-expansion arm received alpelisib at the once-daily MTD. Treatment continued until disease progression, unacceptable toxicity, investigator's decision, or the patient's withdrawal of consent.

### *Safety and efficacy assessments*

Routine clinical and laboratory assessments, including hematology and biochemistry, were conducted at baseline, weekly until Day 28 of Cycle 2, and then every 2 weeks thereafter. Other safety assessments, including glucose monitoring, were carried out at baseline and then at regular intervals throughout the study. Adverse events (AE) were assessed continuously according to Common Toxicity for Adverse Events (CTCAE) v4.0, unless otherwise specified (i.e. hyperglycemia). Criteria for the grading of hyperglycemia were based on a modified version of the American Diabetes Association (ADA) accepted criteria.<sup>29</sup>

Tumor radiologic response was assessed by computerized tomography or magnetic resonance imaging according to RECIST v1.0 at screening (before start of therapy) on Day 28 of Cycle 2 and every 8 weeks thereafter.

#### *Biomarker and pharmacodynamic (PD) and PET assessments*

Tumor biopsies (fresh/archival) were collected from all patients prior to starting study treatment to assess *PIK3CA* gene status. Paired (pre- and post-treatment) fresh tumor samples were collected where possible to assess biomarkers that may be predictive of response including (but not limited to) *PTEN* alteration, *KRAS*, and *BRAF* mutations. Blood samples for glucose metabolism markers (FPG, insulin and C-peptide) were collected at baseline, pre-dose, and 2 and 4 hours post-dose on Day 2 and 9 of Cycle 1 and Day 2 of Cycle 2, and 4–6 hours post-dose on Day 28 of Cycle 2.

## **Results**

### *Patient characteristics and disposition*

Between October 2010 and March 2014, 132 patients with locally-assessed *PIK3CA*-altered advanced solid tumors or *PIK3CA*-altered or wild-type ER+ breast cancer, were enrolled across 11 clinical sites (Table 1). All patients had received prior antineoplastic therapy. In 42 (31.8%) patients, the last treatment prior to enrolment in the study was in the adjuvant or neoadjuvant setting.

One-hundred and six patients received once-daily alpelisib (30 mg [n=1], 60 mg [n=3], 90 mg [n=6], 180 mg [n=6], 270 mg [n=4], 300 mg [n=8], 350 mg [n=6], 400 mg [n=63], 450 mg [n=9] QD). Twenty-six patients received twice-daily alpelisib (120 mg [n=5], 150 mg [n=15], 200 mg [n=6] BID).

As of March 10, 2014, 122 patients had discontinued treatment; 99 (75.0%) due to disease progression, 18 (13.6%) due to AEs, 3 (2.3%) due to patient withdrawal of consent, and 2 (1.5%) due to death (unrelated to study drug).

### *Dose escalation and dose-limiting toxicities (DLTs)*

Of 76 patients evaluable during dose escalation, 9 (11.8%) DLTs were reported: 4 at 450 mg/QD (hyperglycemia\* [n=2], nausea [n=2]); all Grade 3); 1 at 150 mg/BID (Grade 3 hyperglycemia and hypophosphatemia); and 4 at 200 mg/BID (Grade 3 hyperglycemia [n=1] and Grade 4 hyperglycemia [n=3]). One case of Grade 3 hyperglycemia in a patient treated with alpelisib 450 mg/QD was not suspected to be related to study treatment. Two DLTs (Grade 3 intractable nausea and one case of Grade 4 hyperglycemia) led to hospitalization and permanent discontinuation of the study treatment; other DLTs were managed by interruption or adjustment of study treatment (and

concomitant medication as necessary). The MTDs of alpelisib for two dosing regimens were declared as 400 mg/QD and 150 mg/BID.

\*Grade 2 hyperglycemia (confirmed with a repeat FPG within 24 hours) that does not resolve to Grade 0 within 14 consecutive days (after initiation of glimepiride, glibenclamide or metformin) ≥ Grade 3 hyperglycemia (confirmed with a repeat FPG within 24 hours).<sup>29</sup>

#### *Safety and tolerability*

Median exposure to alpelisib was 11.9 (range: 0.4–98) weeks; 9.1 (range: 1–98) weeks in the 63 patients treated with 400 mg QD, and 13 (range 4–60.9) weeks in the 15 patients treated with 150 mg BID. Overall, single-agent alpelisib had a manageable toxicity profile. Across both treatment schedules, the most frequent AE observed and suspected to be study treatment-related was hyperglycemia, which was reported in 62 (47.0%) patients, and was Grade 3/4 in 31 (23.5%) patients (**Table 2**). In general, hyperglycemia was more frequently reported in patients receiving twice-daily alpelisib (40.0, 53.3, and 100% of patients in the 120, 150, and 200 mg/BID groups, respectively) and those receiving higher doses of alpelisib once daily (33.3, 50.8, and 66.7% of patients receiving 350, 400, and 450 mg/QD, respectively). Other frequent (>20%) all-grade AEs suspected to be study treatment-related were nausea (46.2%), diarrhea (37.9%), decreased appetite (37.1%), fatigue (28.8%), and vomiting (27.3%). With the exception of hyperglycemia, which is considered an ‘on-target’ effect of PI3K inhibition, Grade 3/4 AEs suspected to be related to study treatment were not commonly reported (0–3%). Hyperglycemia was a DLT in 7 patients; however in the overall patient population this was readily managed with oral anti-glycemic medications and generally responded to concomitant treatment. In most patients with hyperglycemia, alpelisib could be restarted with a dose reduction where needed. Hyperglycemia despite medical management was a reason for discontinuation in 6 (4.5%) patients. Skin toxicity (including rash, dermatitis, and other cutaneous hypersensitivities) suspected to be related to study treatment was observed in 46 (34.8%) patients receiving alpelisib. Rash associated with alpelisib was generally a maculo-papular eruption, which was mild-to-moderate in nature, and in some patients was associated with pruritus. Hypersensitivity was typically dose-related and developed early during treatment, with 37 (28.0%) cases being observed in the first cycle. Patients with rash were managed with concomitant antihistamine or corticosteroid therapy and dose interruptions or reductions were infrequent.

Sixteen (12.1%) patients experienced serious AEs\* suspected to be study-drug related. The most frequent all-grade serious AEs were: hyperglycemia, nausea, pneumonia, vomiting (each observed in 7 [5.3%] patients) and pyrexia (6 [4.5%] patients). There were 9 on-study deaths during the study; none were study-drug related.

\*SAE: medically important and significant adverse event, which requires inpatient hospitalization, results in persistent or significant disability/incapacity, or is fatal or life-threatening.

#### *PK analysis*

Alpelisib was rapidly absorbed, with median time to reach peak plasma concentrations ( $T_{max}$ ) on C1D1 approximately 2 hours at both MTDs. The median half-life of alpelisib was 7.5 (range: 4.6–27.1) hours at 400 mg/QD and 3.6 hours (range: 2.8–6.8 hours) at 150 mg/BID. The PK profiles of alpelisib were comparable across multiple time points (C1D1, C1D8, and C2D1) suggesting minimal drug

accumulation (Figure 2, Table I). Systemic exposure to once- and twice-daily alpelisib appears to be dose proportional within the range tested. Across the dose range tested, between-subject variability (mean coefficient of variation %) was moderate with 15–43% for both  $C_{\max}$  and  $AUC_{0-24}$  at steady state for the once-daily dosing regimen. For the twice-daily dosing regimen, between-subject variability was moderate to high with 26–54% for  $C_{\max}$  and  $AUC_{0-12}$  at steady state.

#### *Clinical activity*

Overall, 131 patients were evaluable for response. Partial responses (PRs) were reported in 7 (5.3%) patients (2 at 270 mg/QD, 1 at 350 mg/QD, 2 at 400 mg/QD, and 2 at 150 mg/BID) and 68 (51.9%) patients had stable disease (SD; Figure 3). In addition to the two PRs observed at each of the MTDs, SD was observed in 31 (of 62 evaluable patients) receiving alpelisib 400 mg/QD and 8 (of 15 evaluable patients) receiving alpelisib 150 mg/BID. The PRs were observed after 2–6 treatment cycles. The disease control rate (DCR), defined as complete responses + PRs + SD (for  $\geq 6$  weeks), was 53.2% (95% confidence interval [CI]: 40.1–66.0) in those treated with alpelisib 400 mg/QD and 66.7% (95% CI: 38.4–88.2) in those treated with alpelisib 150 mg/BID.

DCRs varied according to tumor type; in patients with head and neck cancer (n=12), the DCR was 75%, in patient with breast cancer, it was 62.9%, while in patients with colorectal cancer (CRC; n=35), it was 31.4%. At the MTDs, DCRs for patients with breast and head and neck cancers were: 60.9% at 400 mg/QD (n=23) and 66.7% at 150 mg/BID (n=3) in patients with breast cancer, and 62.5% at 400 mg/QD (n=8) in patients with head and neck cancer. No patients with head and neck cancer were treated with alpelisib 150 mg/BID. Of the 35 patients with breast cancer, one patient with ER+, PR+, HER2– breast cancer had a PR and 21 patients with breast cancer had SD: 11 with ER+/HER2– disease; seven with ER+/HER2+ disease; and two each in patients with ER-/HER2+ disease and patients with triple negative disease. No clinical activity (i.e. no CR, PR, or SD) was observed in the four patients with *PIK3CA*-wild-type breast cancer in the dose expansion arm.

Fifty-six patients showed some tumor shrinkage ('negative' best percentage change from baseline in sum of longest diameter [SLD]), including 14 showing >30% tumor shrinkage. Tumor shrinkage was seen across tumor types. Among ten\* patients with head and neck cancer, five showed some tumor shrinkage, including two with >30% tumor shrinkage; among 25\* patients with breast cancer, 15 showed some tumor shrinkage, including two with >30% tumor shrinkage and one with >50% tumor shrinkage (PR); among 32\* patients with CRC, 12 demonstrated tumor shrinkage, including one with ~60% tumor shrinkage (PR); and among 12\* patients with ovarian cancer, six showed tumor shrinkage, including one with ~40% tumor shrinkage.

\*excludes patients with no post-baseline assessment for target lesions or patients with only non-target lesions.

#### *Biomarker and PD analysis*

Based on the role of PI3K signaling in the control of glucose metabolism in both tumor and normal tissue, the PD effect of alpelisib on plasma glucose and tumor glucose uptake via FDG-PET changes was investigated. Figure 4 shows that there was a dose-dependent increase in the maximum change

of the FPG level in the first treatment cycle; these increases were most pronounced between the alpelisib 400 and 450 mg/QD dose levels and the 150 and 200 mg/BID dose levels, resulting in hyperglycemia as a DLT in 7 patients (as discussed) (22). This finding was corroborated by a dose-dependent decrease in the tumor uptake of FDG-PET (Figure 4d). In general, alpelisib administration was associated with dose-dependent increases in C-peptide and insulin from baseline during the first treatment cycle.

Sixty-two patients were evaluable for both efficacy data and next generation sequencing (NGS) analysis. Eighteen patients with CRC were evaluable; among these patients, one patient (treated with 150 mg/BID) with *PIK3CA*, *KRAS*, *APC*, and *SMAD4* mutations showed a PR (~60% tumor shrinkage as previously mentioned) (Figure 5a). Twelve patients with CRC had PD; 9 patients had *PIK3CA*, *TP53*, and *APC* mutations, and 7 of these 9 also had a concomitant *KRAS* mutation. Eleven of 18 evaluable patients with breast cancer experienced SD; 9 of whom had *PIK3CA* mutations, including 3 patients who also had *TP53* mutations (Figure 5b). Thirteen patients with “other tumor types” were evaluable and two PRs were observed; one in a patient with cervical cancer who had *PIK3CA* and *NOTCH1* mutations (~50% tumor shrinkage), and the other in a patient with endometrial cancer who had *PIK3CA* and *TP53* mutations and amplification of *ERBB2* (*HER2*) (~30% tumor shrinkage) (Figure 5e).

## Discussion

These results indicate that alpelisib has an acceptable safety profile at the MTDs (400 mg/QD and 150 mg/BID), in a population of patients including those with *PIK3CA*-altered advanced solid tumors, and those with *PIK3CA*-altered and wild-type ER+ breast cancer. The most common (>20%) AEs associated with alpelisib were largely on-target effects which are generally typical of those experienced with PI3K inhibitors, including hyperglycemia, gastrointestinal AEs, fatigue and rash. In particular, due to the central role of the PI3K/AKT/mTOR pathway in glucose homeostasis regulation, hyperglycemia is a common class effect associated with PI3K inhibitors.<sup>30</sup> In this study, hyperglycemia resolved quickly upon dose interruption and was typically fully managed with oral anti-diabetic treatment. Rash was well managed with concomitant antihistamine or corticosteroid therapy. The mechanism of alpelisib rash is unclear, but is thought to be a combination of hypersensitivity (i.e. adaptive immune-based mechanisms) and on-target inhibition of PI3K $\alpha$  (removing the skin's protection against UVA radiation).

PK/PD simulation demonstrating the dose-dependency of antitumor effects, predicts a difference between once- and twice-daily dosing regimens which is slightly in favor of the twice-daily regimen. This is due to greater time over estimated IC50/IC80 concentration required for the constant inhibition of PI3K $\alpha$  (and pathway).<sup>31</sup> However, as the once-daily regimen is better tolerated based on the safety data presented, only once-daily dosing was further pursued during clinical development. Alpelisib was rapidly absorbed with exposure to both dosing regimens increasing dose proportionally between 30 mg/QD and 450 mg/QD and between 120 mg/BID and 200 mg/BID. The PK profiles were comparable across multiple time-points, suggesting minimal drug accumulation.

Preliminary clinical activity of alpelisib was observed, with PRs observed at both the once- and twice-daily MTDs. In addition, there was evidence of antitumor activity in a variety of tumor types, including breast cancer and head and neck cancer. No antitumor activity was observed in the 4 patients with *PIK3CA*-wild-type breast cancer treated in the dose expansion arm. Despite small patient numbers, the DCR varied across different tumor types, with lower rates observed in CRC

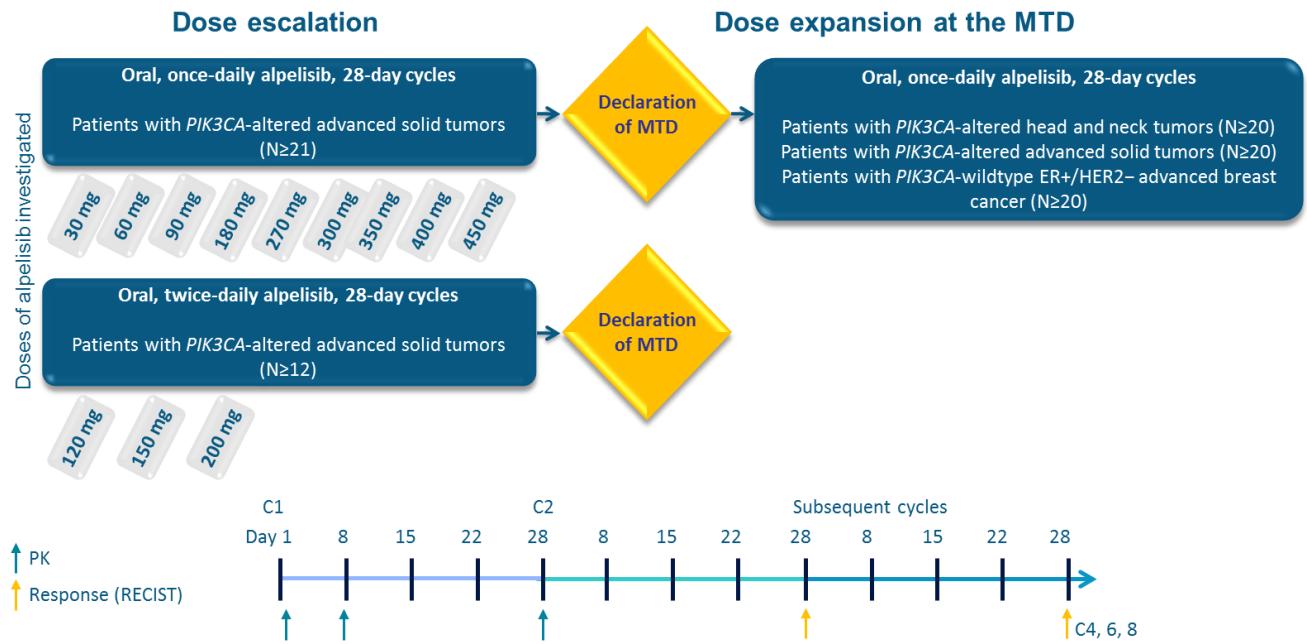
than in breast and head and neck cancer. This suggests that, despite the presence of *PIK3CA* alterations in all the treated tumor types, tumor lineage also plays a role in determining sensitivity to alpelisib and supports the further investigation of alpelisib in these tumor types. In addition, PD effects on plasma glucose suggest dose-dependent inhibition of PI3K pathway signaling.

This study is unique in that only patients with *PIK3CA* alterations were considered eligible from the first dose during dose escalation. The results support preclinical data from the CCLE which shows that *PIK3CA* mutation status enriches for alpelisib response,<sup>14</sup> and add to the evidence from early clinical studies showing that patients with *PIK3CA* mutations (albeit with a heterogeneous range of tumors) treated with PI3/AKT/mTOR inhibitors demonstrated a higher response rate than patients without mutations.<sup>20,21</sup> However, these data contrast with some observations with pan-PI3K and mTOR inhibitors whereby *PIK3CA* alterations have not been predictive of response. Further studies are required to characterize this and other predictors of response.

These results demonstrate the clinical safety and tolerability, favorable PK profile and early antitumor activity observed with alpelisib in patients with *PIK3CA*-altered advanced solid tumors and those with *PIK3CA*-altered ER+ breast cancer. Ongoing studies are underway to further assess the use of alpelisib as a single agent and in combination with endocrine therapy and other targeted agents, across a range of tumor types, including ER+ breast cancer and head and neck squamous cell carcinoma.

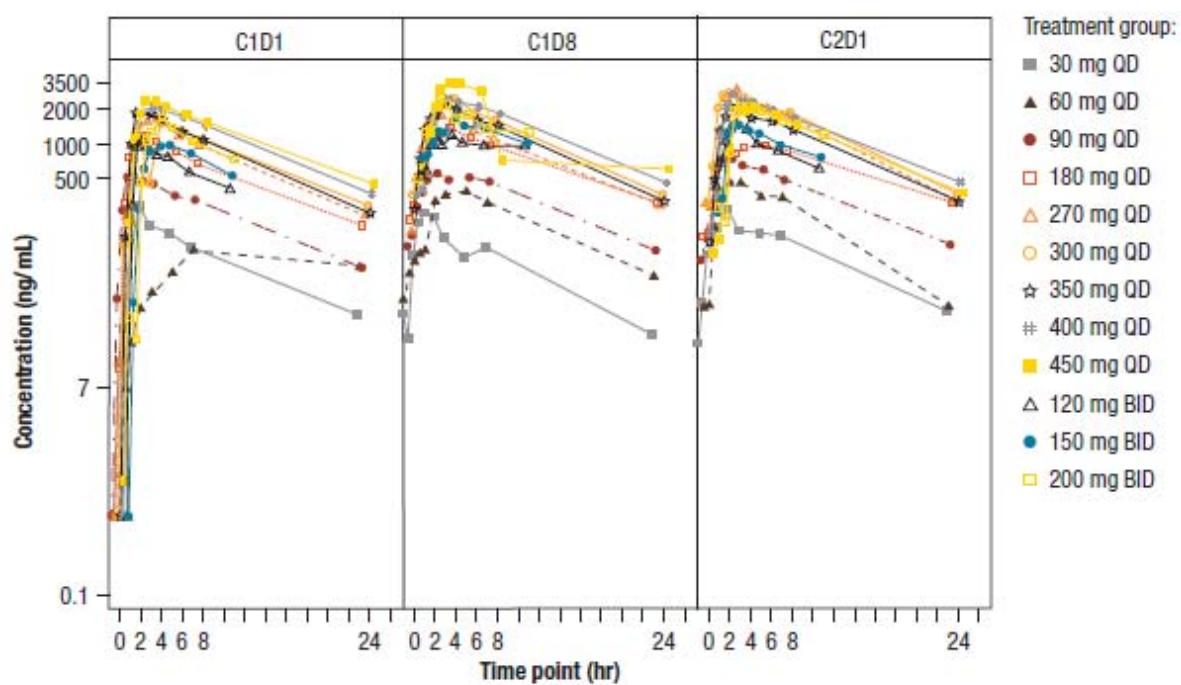
## Tables and figures

## **Figure 1. Study design**



C, cycle; ER+, estrogen receptor-positive; HER2-, human epidermal growth factor receptor 2-negative; MTD, maximum tolerated dose; PK, pharmacokinetics; RECIST, Response Evaluation Criteria In Solid Tumors.

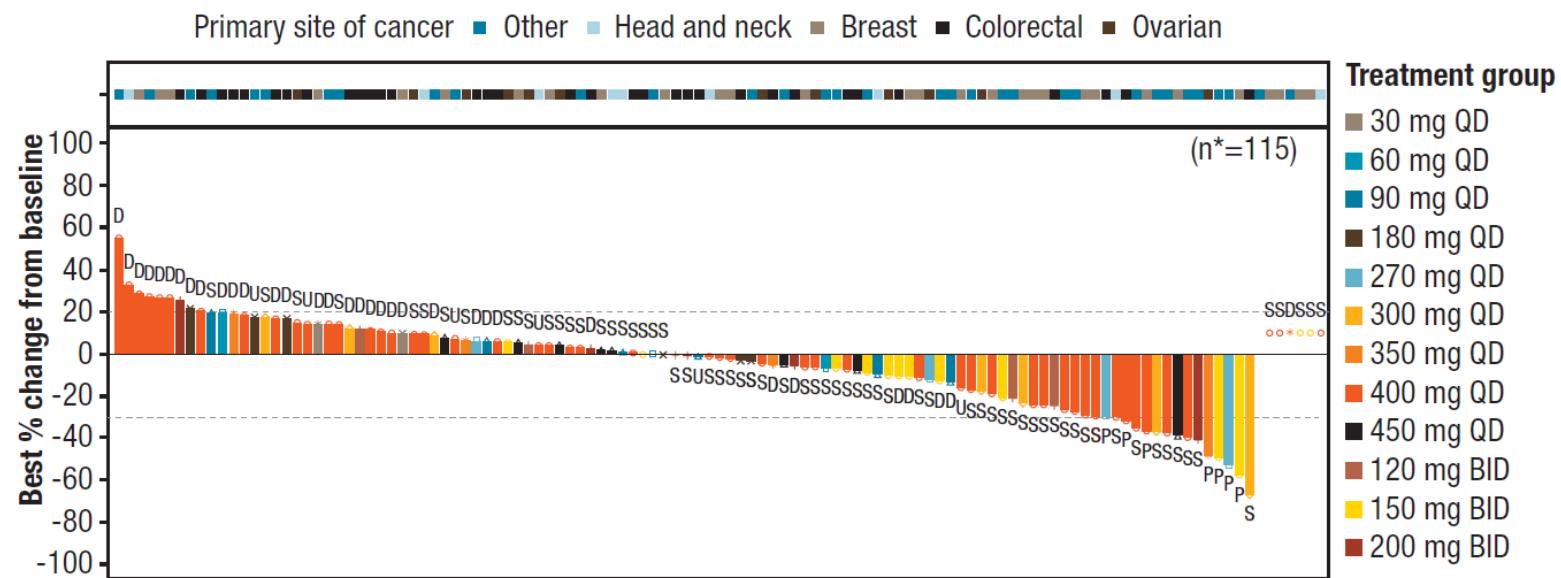
**Figure 2.** Geometric mean plasma concentration-time profiles of alpelisib



BID, twice-daily; C, cycle; D, day; Hr, hour; QD, once-daily

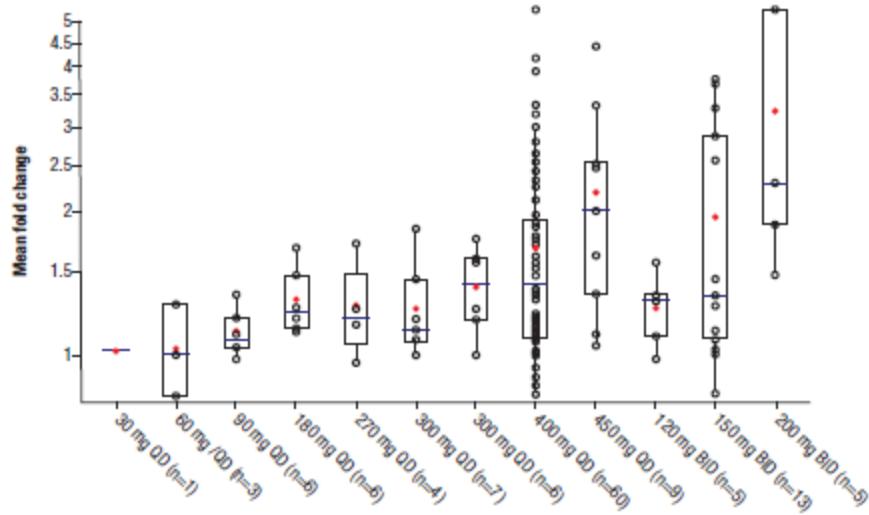
Data cut-off: March 10, 2014

**Figure 3.** Best percentage change in sum of longest diameters and best overall response according to primary site of cancer and treatment

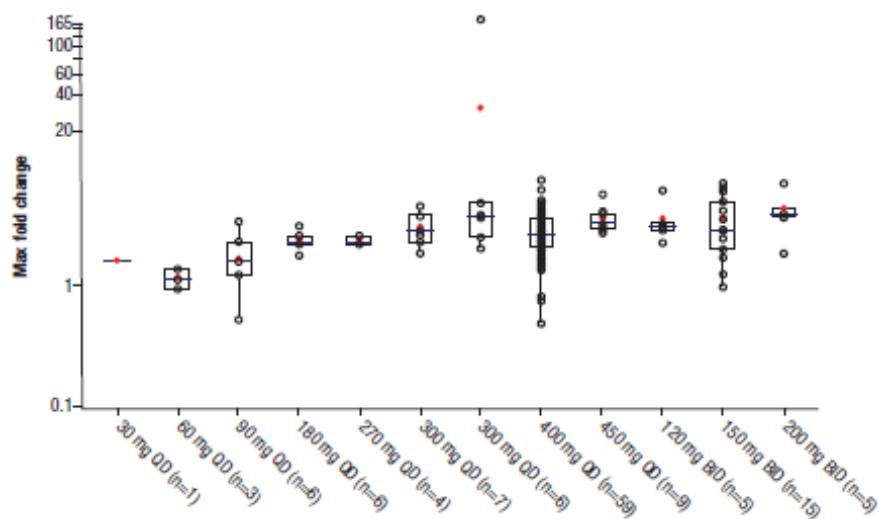


**Figure 4.** Maximum fold increase from baseline in fasting plasma glucose parameters, cycle 1, by treatment group

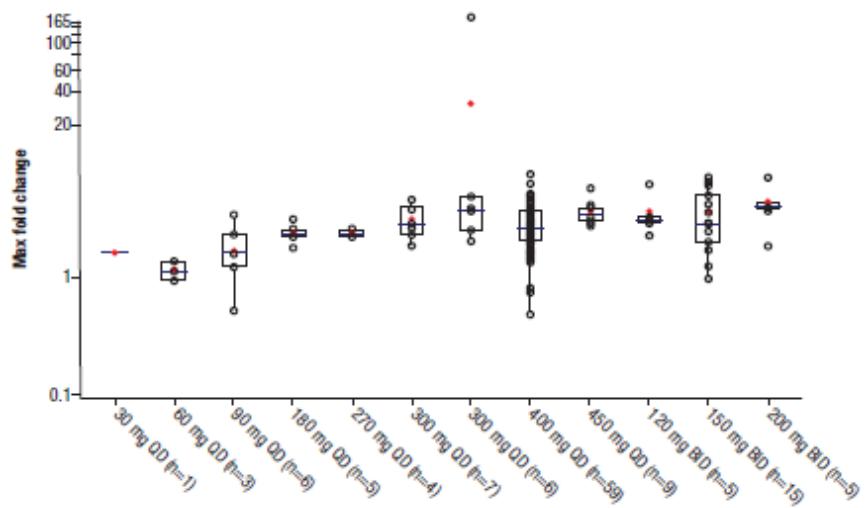
a. Plasma glucose



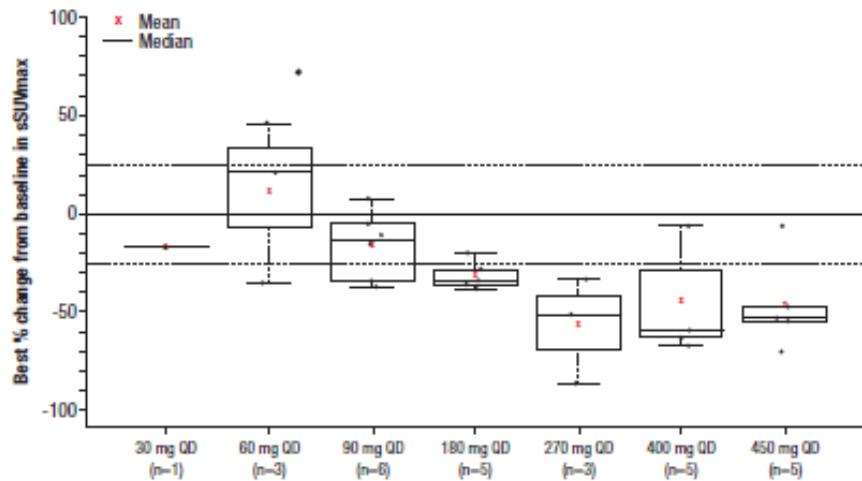
b. Insulin



c. C-peptide



d. Best percentage change from baseline in sSUVmax, by treatment group

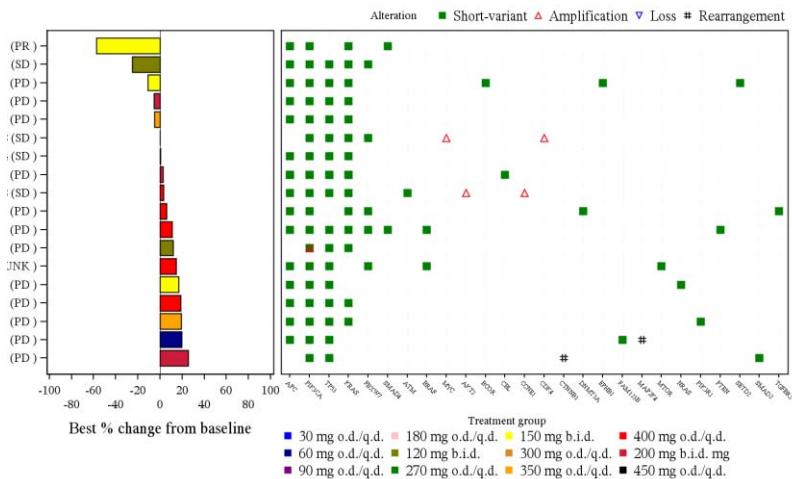


BID, twice-daily; QD, once-daily; sSUVmax, sum of maximum standardized uptake values

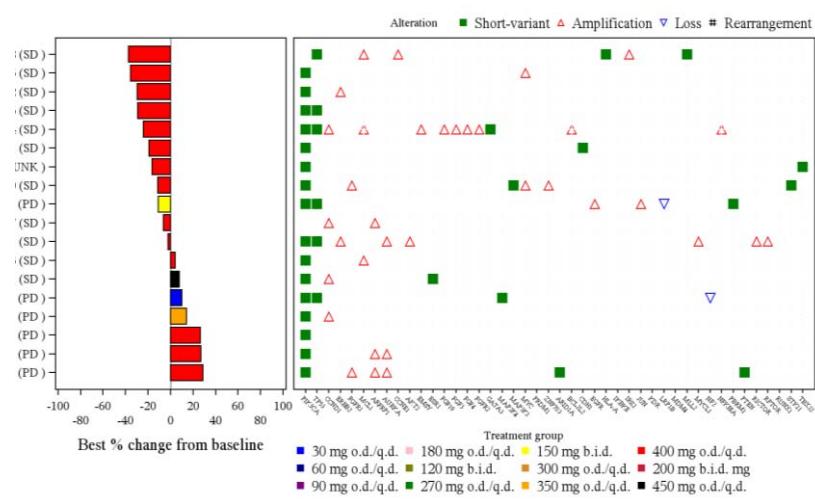
Data cut-off: March 10, 2014

**Figure 5.** Genetic alterations observed in tumor samples with known/likely functional significance using NGS analysis and best percentage change from baseline in sum of longest diameters as per investigator by treatment group

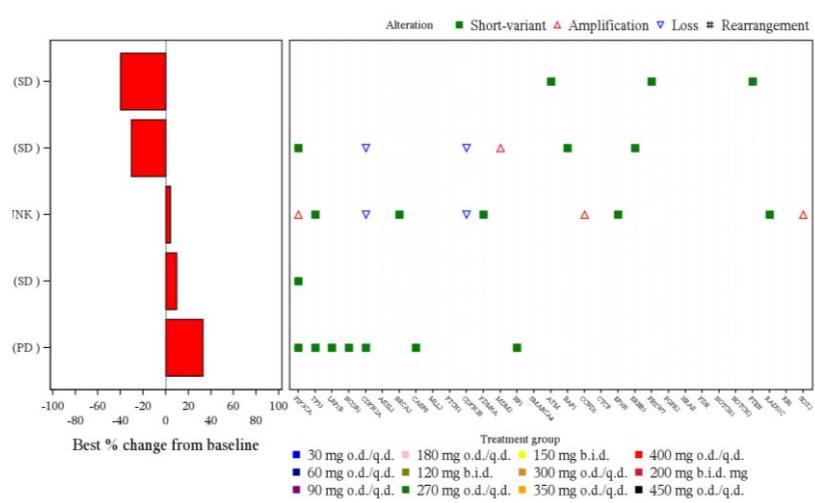
a. CRC



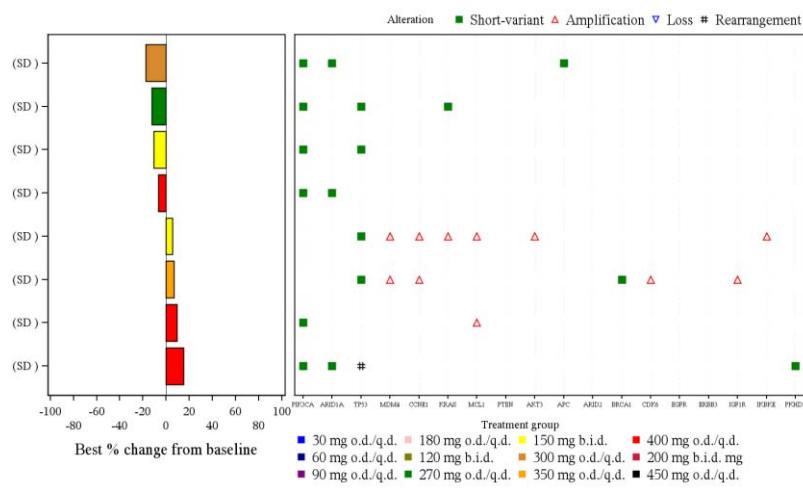
b. Breast cancer



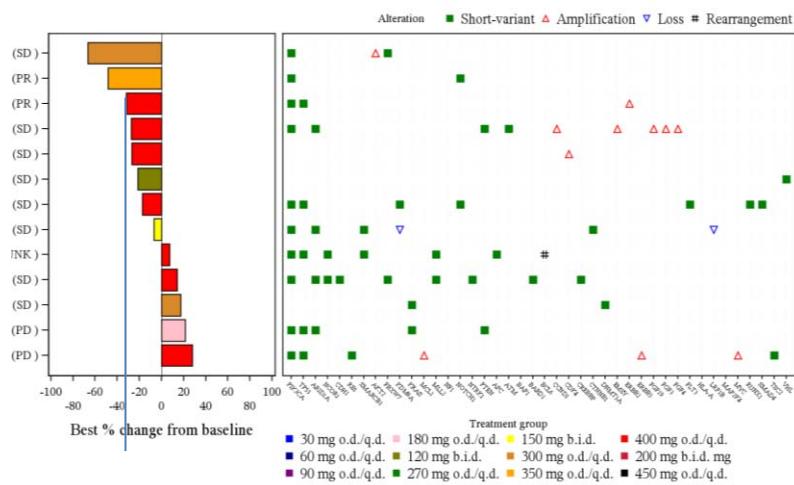
c. Head and neck cancer



#### d. Ovarian cancer



#### e. Other tumor types\*



BID, twice-daily; C, cycle; D, day; Hr, hour; QD, once-daily

\*Primary cancer sites classed as 'other' include: lung, cervix (both n=5), oral cavity, uterus, and endometrium (all n=3)

Data cut-off: March 10, 2014

**Table 1.** Patient characteristics at baseline

<b>Characteristic</b>	<b>Patients (N=132)</b>
Age, years	
Median range	59 (21–82)
Sex, n (%)	
Male	35 (26.5)
Female	97 (73.5)
ECOG Performance Status, n (%)*	
0	51 (38.6)
1	74 (56.1)
2	6 (4.5)
Primary cancer site, n (%)	
Breast	35 (26.5)
Head & neck	13 (9.8)
Colorectal	35 (26.5)
Ovarian	14 (10.6)
Other <sup>†</sup>	35 (26.5)
Time since initial diagnosis, n (%)	
<12 months	8 (6.1)
12–<36 months	56 (42.4)
>36 months	68 (51.5)
Median number of prior antineoplastic therapies (range)	4 (1–19)
PI3K molecular status	
Altered	123 (93.2)
Wild-type	5 (3.8)
Missing	4 (3.0)

ECOG, Eastern Cooperative Oncology Group

\*Data was missing for 1 patient

<sup>†</sup>Primary cancer sites classed as 'other' include: lung, cervix (both n=5), oral cavity, uterus, and endometrium (all n=3)

Data cut-off: March 10, 2014

**Table 2.** Adverse events ( $\geq 10\%$ ) suspected to be related to study treatment

Adverse event, n (%)	Grade	Once-daily doses					Twice-daily doses			All (N=132)
		$\leq 270$ mg* (n=20)	300 mg (n=8)	350 mg (n=6)	400 mg (n=63)	450 mg (n=9)	120 mg (n=5)	150 mg (n=15)	200 mg (n=6)	
Total	All	17 (85.0)	7 (87.5)	6 (100)	60 (95.2)	9 (100)	5 (100)	15 (100)	6 (100)	125 (94.7)
	3/4	2 (10.0)	1 (12.5)	3 (50.0)	30 (47.6)	6 (66.7)	2 (40.0)	6 (40.0)	6 (100)	56 (42.4)
Hyperglycemia	All	5 (25.0)	1 (12.5)	2 (33.3)	32 (50.8)	6 (66.7)	2 (40.0)	8 (53.3)	6 (100)	62 (47.0)
	3/4	0	1 (12.5)	0	16 (25.4)	3 (33.3)	1 (20.0)	5 (33.3)	5 (83.3)	31 (23.5)
Nausea	All	6 (30.0)	3 (37.5)	4 (66.7)	30 (47.6)	6 (66.7)	0	8 (53.3)	4 (66.7)	61 (46.2)
	3/4	0	0	0	1 (1.6)	2 (22.2)	0	0	0	3 (2.3)
Diarrhea	All	3 (15.0)	3 (37.5)	3 (50.0)	26 (41.3)	5 (55.6)	2 (40.0)	5 (33.3)	3 (50.0)	50 (37.9)
	3/4	0	0	0	2 (3.2)	0	0	0	1 (16.7)	3 (2.3)
Decreased appetite	All	4 (20.0)	3 (37.5)	3 (50.0)	24 (38.1)	5 (55.6)	1 (20.0)	5 (33.3)	4 (66.7)	49 (37.1)
	3/4	0	0	1 (16.7)	1 (1.6)	0	0	0	0	2 (1.5)
Fatigue	All	5 (25.0)	3 (37.5)	1 (16.7)	20 (31.7)	3 (33.3)	0	5 (33.3)	1 (16.7)	38 (28.8)
	3/4	0	0	0	0	1 (11.1)	0	0	0	2 (1.5)
Vomiting	All	6 (30.0)	2 (25.0)	2 (33.3)	19 (30.2)	3 (33.3)	0	1 (6.7)	3 (50.0)	36 (27.3)
	3/4	0	0	0	3 (4.8)	0	0	0	0	3 (2.3)
Stomatitis	All	0	3 (37.5)	1 (16.7)	10 (15.9)	2 (22.2)	1 (20.0)	5 (33.3)	2 (33.3)	24 (18.2)
	3/4	0	0	0	0	0	0	0	0	0
Dygesia	All	1 (5.0)	1 (12.5)	1 (16.7)	7 (11.1)	2 (22.2)	1 (20.0)	2 (13.3)	3 (50.0)	18 (13.6)
	3/4	0	0	0	0	0	0	0	0	0
Dyspepsia	All	3 (15.0)	2 (25.0)	1 (16.7)	6 (9.5)	0	1 (20.0)	2 (13.3)	2 (33.3)	17 (12.9)
	3/4	0	0	0	0	0	0	0	0	0
Rash	All	3 (15.0)	2 (25.0)	0	5 (7.9)	2 (22.2)	0	3 (20.0)	2 (33.3)	17 (12.9)
	3/4	0	0	0	0	0	0	0	0	0
Weight decrease	All	1 (5.0)	3 (37.5)	1 (16.7)	4 (6.3)	3 (33.3)	0	2 (13.3)	1 (16.7)	15 (11.4)
	3/4	0	0	0	0	0	0	0	0	0
Dry skin	All	1 (5.0)	3 (37.5)	2 (33.3)	5 (7.9)	0	0	2 (13.3)	1 (16.7)	14 (10.6)
	3/4	0	0	0	0	0	0	0	0	0

Maculopapular rash	All 3/4	0 0	0 0	1 (16.7) 0	9 (14.3) 3 (4.8)	1 (11.1) 0	0 0	2 (13.3) 0	1 (16.7) 0	14 (10.6) 3 (2.3)
--------------------	------------	--------	--------	---------------	---------------------	---------------	--------	---------------	---------------	----------------------

\*≤270 mg = 30, 60, 90, and 180 mg doses.

All AEs, with the exception of hyperglycemia, were defined according to NCI-CTCAE v4.0 criteria.

Data cut-off: March 10, 2014

## References

1. Liu P, Cheng H, Roberts TM, Zhao JJ. Targeting the phosphoinositide 3-kinase pathway in cancer. *Nat Rev Drug Discov* 2009; 8(8): 627-44.
2. Baselga J, Piccart M, Rugo H, et al. Assessment of genetic alteration using next-generation sequencing in postmenopausal women with hormone receptor-positive, HER2-negative advanced breast cancer: results from the BOLERO-2 phase III trial. AACR 2013 Abstract #4564.
3. De Roock W, Claes B, Bernasconi D, et al. Effects of KRAS, BRAF, NRAS, and PIK3CA mutations on the efficacy of cetuximab plus chemotherapy in chemotherapy-refractory metastatic colorectal cancer: a retrospective consortium analysis. *Lancet Oncol* 2010; 11(8): 753-62.
4. Velho S, Oliveira C, Ferreira A, et al. The prevalence of PIK3CA mutations in gastric and colon cancer. *Eur J Cancer* 2005; 41(11): 1649-54.
5. Oda K, Stokoe D, Taketani Y, McCormick F. High frequency of coexistent mutations of PIK3CA and PTEN genes in endometrial carcinoma. *Cancer Res* 2005; 65(23): 10669-73.
6. Saal LH, Johansson P, Holm K, et al. Poor prognosis in carcinoma is associated with a gene expression signature of aberrant PTEN tumor suppressor pathway activity. *Proc Natl Acad Sci U S A* 2007; 104(18): 7564-9.
7. Huang WC, Hung MC. Induction of Akt activity by chemotherapy confers acquired resistance. *J Formos Med Assoc* 2009; 108(3): 180-94.
8. Miller TW, Rexer BN, Garrett JT, Arteaga CL. Mutations in the phosphatidylinositol 3-kinase pathway: role in tumor progression and therapeutic implications in breast cancer. *Breast Cancer Res* 2011; 13(6): 224.
9. Cancer Genome Atlas Network. Comprehensive molecular portraits of human breast tumours. *Nature* 2012; 490(7418): 61-70.
10. Banerji S, Cibulskis K, Rangel-Escareno C, et al. Sequence analysis of mutations and translocations across breast cancer subtypes. *Nature* 2012; 486(7403): 405-9.
11. Cancer Genome Atlas Network. Comprehensive molecular characterization of human colon and rectal cancer. *Nature* 2012; 487(7407): 330-7.
12. Lui VW, Hedberg ML, Li H, et al. Frequent mutation of the PI3K pathway in head and neck cancer defines predictive biomarkers. *Cancer Discov* 2013; .
13. Levine DA, Bogomolniy F, Yee CJ, Lash A, Barakat RR, Borgen PI, Boyd J. Frequent mutation of the PIK3CA gene in ovarian and breast cancers. *Clin Cancer Res* 2005; 11(8): 2875-8.
14. Fritsch C, Huang A, Chatenay-Rivauday C, et al. Characterization of the novel and specific PI3Kalpha inhibitor NVP-BYL719 and development of the patient stratification strategy for clinical trials. *Mol Cancer Ther* 2014; .
15. Bachman KE, Argani P, Samuels Y, et al. The PIK3CA gene is mutated with high frequency in human breast cancers. *Cancer Biol Ther* 2004; 3(8): 772-5.
16. Saal LH, Holm K, Maurer M, et al. PIK3CA mutations correlate with hormone receptors, node metastasis, and ERBB2, and are mutually exclusive with PTEN loss in human breast carcinoma. *Cancer Res* 2005; 65(7): 2554-9.
17. Engelman JA, Chen L, Tan X, et al. Effective use of PI3K and MEK inhibitors to treat mutant Kras G12D and PIK3CA H1047R murine lung cancers. *Nat Med* 2008; 14(12): 1351-6.
18. Di Niclantonio F, Arena S, Tabernero J, et al. Deregulation of the PI3K and KRAS signalling pathways in human cancer cells determines their response to everolimus. *J Clin Invest* 2010; 120(8): 2858-66.
19. Janku F, Tsimberidou AM, Garrido-Laguna I, et al. PIK3CA mutations in patients with advanced cancers treated with PI3K/AKT/mTOR axis inhibitors. *Mol Cancer Ther* 2011; 10(3): 558-65.
20. Janku F, Wheler JJ, Westin SN, et al. PI3K/AKT/mTOR inhibitors in patients with breast and gynecologic malignancies harboring PIK3CA mutations. *J Clin Oncol* 2012; 30(8): 777-82.
21. Janku F, Wheler JJ, Naing A, et al. PIK3CA mutation H1047R is associated with response to PI3K/AKT/mTOR signaling pathway inhibitors in early-phase clinical trials. *Cancer Res* 2013; 73(1): 276-84.
22. Huang A, Fritsch C, Wilson C, et al. Single agent activity of PIK3CA inhibitor BYL719 in a broad cancer cell line panel. In: *Proceedings of the 103rd Annual Meeting of the American Association for Cancer Research; 2012 Mar 31-Apr 4; Chicago, Illinois. Philadelphia (PA): AACR* 2012; Abstract nr 3749.
23. Miller TW, Balko JM, Arteaga CL. Phosphatidylinositol 3-kinase and antiestrogen resistance in breast cancer. *J Clin Oncol* 2011; 29(33): 4452-61.
24. Jia S, Roberts TM, Zhao JJ. Should individual PI3 kinase isoforms be targeted in cancer? *Curr Opin Cell Biol* 2009; 21: 199-208.
25. Huang M, Shen A, Ding J, Geng M. Molecularly targeted cancer therapy: some lessons from the past decade. *Trends Pharmacol Sci* 2014; 35(1): 41-50.
26. Elkabets M, Vora S, Juric D, et al. mTORC1 inhibition is required for sensitivity to PI3K p110alpha inhibitors in PIK3CA-mutant breast cancer. *Sci Transl Med* 2013; 5(196): 196ra99.
27. Babb J, Rogatko A, Zacks S. Cancer phase I clinical trials: efficient dose escalation with overdose control. *Stat Med* 1998; 17(10): 1103-20.
28. Neuenschwander B, Branson M, Gsponer T. Critical aspects of the Bayesian approach to

- phase I cancer trials. *Stat Med* 2008; 27(13): 2420-39.
29. Inoue K, Kashima S, Ohara C, Matsumoto M, Akimoto K. Concordance of two diabetes diagnostic criteria using fasting plasma glucose and hemoglobin A1c: the Yupiter Medical Checkup Centre study. *PLoS One* 2012; 7(10): e47747.
30. Engelman JA, Luo J, Cantley LC. The evolution of phosphatidylinositol 3-kinases as regulators of growth and metabolism. *Nat Rev Genet* 2006; 7(8): 606-19.
31. De Buck SS, Jakab A, Boehm M, Bootle D, Juric D, Quadt C, Goggin TK. Population pharmacokinetics and pharmacodynamics of BYL719, a phosphoinositide 3-kinase antagonist, in adult patients with advanced solid malignancies. *Br J Clin Pharmacol* 2014; .

## Anexo 4 Estudios con Pilaralisib, Buparlisib y Sonidegib

### Estudios con Pilaralisib realizados tras el ensayo fase I aquí presentado (NCT00486135)

Título: Study of XL147 (SAR245408) in Combination With Trastuzumab or Paclitaxel and Trastuzumab in Subjects With Breast cancer Who Have Progressed on a Previous Trastuzumab-based Regimen

Patologías: Cáncer de mama|Neoplasmas de mama

Intervenciones: Fármaco: XL147 (SAR245408)|Biológico: trastuzumab|Fármaco: paclitaxel

URL: <http://ClinicalTrials.gov/show/NCT01042925>

Título: Study of XL147 (SAR245408) or XL765 (SAR245409) in Combination With Letrozole in Subjects With Breast cancer

Patologías: Cáncer de mama

Intervenciones: Fármaco: XL147 (SAR245408)|Fármaco: XL765 (SAR245409)|Fármaco: letrozole (Femara)

URL: <http://ClinicalTrials.gov/show/NCT01082068>

Título: Study of XL147 (SAR245408) in Advanced or Recurrent Cancer endometrial

Patologías: Cáncer endometrial|Neoplasmas del endometrio

Intervenciones: Fármaco: XL147 (SAR245408)

URL: <http://ClinicalTrials.gov/show/NCT01013324>

Título: Safety Study of XL147 (SAR245408), in Combination With Paclitaxel and Carboplatin in Adults With Solid tumors

Patologías: Cáncer|Cáncer de pulmón de células no pequeñas|Carcinoma endometrial |Carcinoma de ovario

Intervenciones: Fármaco: XL147(SAR245408),|Fármaco: paclitaxel|Fármaco: carboplatin

URL: <http://ClinicalTrials.gov/show/NCT00756847>

Título: Safety Study of XL147 (SAR245408) in Combination With Erlotinib in Adults With Solid tumors

Patologías: Cáncer|Cáncer pulmonar de células no pequeñas

Intervenciones: Fármaco: XL147 (SAR245408)|Fármaco: Erlotinib

URL: <http://ClinicalTrials.gov/show/NCT00692640>

Título: Exploratory Study of XL765 (SAR245409) or XL147 (SAR245408) in Subjects With Recurrent Glioblastoma Who Are Candidates for Surgical Resection

Patologías: Glioblastoma| Astrocitoma, Grado IV

Intervenciones: Fármaco: XL765 (SAR245409)|Fármaco: XL147 (SAR245408)

URL: <http://ClinicalTrials.gov/show/NCT01240460>

Título: Safety Study of XL647 and XL147 Administered in Combination Daily in Adults With Solid tumors

Patologías: Cáncer| Cáncer pulmonar de células no pequeñas |Cáncer de mama

Intervenciones: Fármaco: XL647|Fármaco: XL147

URL: <http://ClinicalTrials.gov/show/NCT00704392>

Título: Safety and Pharmacokinetics of SAR245408 Daily Oral in Patients With Solid tumors

Patologías: Neoplasma maligno  
Intervenciones: Fármaco: SAR245408  
URL: <http://ClinicalTrials.gov/show/NCT01392924>

Título: Oral SAR245408 (XL147) and Oral MSC1936369B in Patients With Locally Solid tumors  
Patologías: tumores sólidos  
Intervenciones: Fármaco: SAR245408 (XL147)|Fármaco: MSC1936369B  
URL: <http://ClinicalTrials.gov/show/NCT01357330>

**Estudios con Buparlisib realizados posteriormente al ensayo en fase I aquí presentado (NCT01068483)**

Título: BKM120 + Carboplatin + Paclitaxel for Patients With Solid tumors  
Patologías: Tumores sólidos  
Intervenciones: Fármaco: BKM120 days 1 - 21 plus paclitaxel + carboplatin|Fármaco: BKM120 (days 1 - 28, ) plus paclitaxel + carboplatin|Fármaco: BKM120 (days 1-21) + paclitaxel(day 1) + carboplatin (day 1)|Fármaco: BKM120, Paclitaxel + Carboplatin  
URL: <http://ClinicalTrials.gov/show/NCT01297452>

Título: Palliative Thoracic Radiotherapy Plus BKM120  
Patologías: Carcinoma, Cáncer pulmonar de células no pequeñas  
Intervenciones: Fármaco: BKM120  
URL: <http://ClinicalTrials.gov/show/NCT02128724>

Título: Combination of BKM120 and Bevacizumab in Refractory Solid tumors and Relapsed/ Glioblastoma Multiforme  
Patologías: Glioblastoma Multiforme  
Intervenciones: Fármaco: Bevacizumab|Fármaco: BKM120  
URL: <http://ClinicalTrials.gov/show/NCT01349660>

Título: A Trial of Irinotecan and BKM120 in Previously Treated Colorectal Cancer  
Patologías: Cáncer Colorrectal  
Intervenciones: Fármaco: Irinotecan|Fármaco: BKM120  
URL: <http://ClinicalTrials.gov/show/NCT01304602>

Título: A Trial of Gefitinib in Combination With BKM120 in Patients With non small-cell lung cancer, With Enrichment for Patients Whose Tumours Harbour Molecular Alterations of PI3K Pathway and Known to Overexpress EGFR  
Patologías: Cáncer pulmonar de células no pequeñas|Tumores sólidos  
Intervenciones: Fármaco: Gefitinib and BKM120  
URL: <http://ClinicalTrials.gov/show/NCT01570296>

Título: BKM120 in Metastatic Castration-resistant Prostate Cancer  
Patologías: Cáncer de próstata|Metastásico (Invasión de otras zonas del cuerpo)  
Intervenciones: Fármaco: BKM120  
URL: <http://ClinicalTrials.gov/show/NCT01385293>

Título: Trial of Erlotinib and BKM120 in Patients with non small-cell lung cancer Previously Sensitive to Erlotinib

Patologías: Cáncer pulmonar de células no pequeñas  
Intervenciones: Fármaco: BKM120 and Erlotinib  
URL: <http://ClinicalTrials.gov/show/NCT01487265>

Título: A Phase III Study of BKM120 With Fulvestrant in Patients With HR+,HER2-, AI Treated, Locally Advanced or Metastatic Breast Cancer Who Progressed on or After mTORi

Patologías: Metastásico Cáncer de mama HR+, HER2-  
Intervenciones: Fármaco: Fulvestrant|Fármaco: BKM120|Fármaco: BKM120 matching placebo  
URL: <http://ClinicalTrials.gov/show/NCT01633060>

Título: BKM120 and Fulvestrant for Treating Postmenopausal Patients With Estrogen Receptor-Positive Stage IV Breast cancer

Reclutamiento: Active, not recruiting  
Intervenciones: Fármaco: BKM120|Fármaco: Fulvestrant|Procedimiento: biopsy  
URL: <http://ClinicalTrials.gov/show/NCT01339442>

Título: PI3K Inhibitor BKM120, Carboplatin, and Pemetrexed Disodium in Treating Patients With Stage IV Non small-cell lung cancer

Patologías: Adenocarcinoma pulmonar| Cáncer de pulmón de células broncoalveolares|Carcinoma pulmonar de células grandes|Recurrent Cáncer pulmonar de células no pequeñas| Cáncer pulmonar de células no pequeñas en Fase IV

Intervenciones: Fármaco: PI3K inhibitor BKM120|Fármaco: pemetrexed disodium|Fármaco: carboplatin|Otros: laboratory biomarker analysis|Otros: pharmacological study|Procedimiento: quality-of-life assessment

URL: <http://ClinicalTrials.gov/show/NCT01723800>

Título: A Dose-finding Study of a Combination of Imatinib and BKM120 in the Treatment of 3rd Line GIST Patients

Patologías: GIST en 3ª línea

Intervenciones: Fármaco: STI571 + BKM120|Fármaco: STI571 + BKM120|Fármaco: STI571 + BKM120

URL: <http://ClinicalTrials.gov/show/NCT01468688>

Título: Pharmacodynamic Study of BKM120 in Breast cancer

Patologías: Cáncer de mama

Intervenciones: Fármaco: BKM120

URL: <http://ClinicalTrials.gov/show/NCT01513356>

Título: A Phase I Dose Escalation Study of BKM120 With Radiation Therapy and Temozolomide in Patients With Newly Diagnosed Glioblastoma

Patologías: Glioblastoma

Intervenciones: Fármaco: BKM120 + temozolomide|Fármaco: BKM120 +temozolomide with/without radiotherapy

URL: <http://ClinicalTrials.gov/show/NCT01473901>

Título: Phase I Study of the Oral PI3Kinase Inhibitor BKM120 or BYL719 and the Oral PARP Inhibitor Olaparib in Patients With Recurrent Triple Negative Breast cancer or High Grade Serous Ovarian cancer

Patologías: Cáncer ovárico|Cáncer de mama

Intervenciones: Fármaco: BKM120 and Olaparib|Fármaco: BYL719 and Olaparib

URL: <http://ClinicalTrials.gov/show/NCT01623349>

Título: A Study of the Experimental Fármaco BKM120 With Paclitaxel in Patients With HER2 Negative, Locally Advanced or Metastatic Breast Cancer With or Without PI3K Activation

Patologías: Cáncer de mama

Intervenciones: Fármaco: Paclitaxel | Fármaco: BKM120 matching placebo | Fármaco: BKM120

URL: <http://ClinicalTrials.gov/show/NCT01572727>

Título: Neoadjuvant BKM120 in High-risk Prostate Cancer

Patologías: Cáncer de próstata de alto riesgo

Intervenciones: Fármaco: BKM120

URL: <http://ClinicalTrials.gov/show/NCT01695473>

Título: PI3K Inhibitor BKM120 and Docetaxel in Treating Patients With Solid Tumor That is Locally advanced, Cannot Be Removed By Surgery or Metastatic.

Patologías: Tumor sólido sin especificar en adulto, específico del protocolo

Intervenciones: Fármaco: PI3K inhibitor BKM120 | Fármaco: docetaxel | Otros: pharmacological study | Otros: questionnaire administration | Otros: laboratory biomarker analysis

URL: <http://ClinicalTrials.gov/show/NCT01540253>

Título: Bevacizumab and BKM-120 in Patients With Renal Cell Carcinoma

Resultados del estudio: Resultados no disponibles

Patologías: Carcinoma de células renales

Intervenciones: Fármaco: BKM-120 Bevacizumab

URL: <http://ClinicalTrials.gov/show/NCT01283048>

Título: Study of BKM120 in Squamous Cell Carcinoma of Head and Neck

Patologías: Cáncer metastásico de células escamosas de cabeza y cuello | Cáncer recurrente de células escamosas de cabeza y cuello

Intervenciones: Fármaco: BKM120

URL: <http://ClinicalTrials.gov/show/NCT01527877>

Título: Phase Ib Study of BKM120 With Cisplatin and XRT in High Risk Locally advanced Squamous Cell Cáncer of Head and Neck

Patologías: Carcinoma de células escamosas de cabeza y cuello | Carcinoma orofaríngeo de células escamosas VPH positivo | Cáncer de la hipofaringe | carcinoma de células escamosas de cuello uterino invasivo precoz | Carcinoma de laringe | Cáncer de nasofaringe

Intervenciones: Fármaco: BKM120 | Fármaco: Cisplatino | Radiación: Radioterapia de intensidad modulada (IMRT)

URL: <http://ClinicalTrials.gov/show/NCT02113878>

Título: Cisplatin, Etoposide and PI3K Inhibitor BKM120 in Treating Patients With Advanced Solid Tumors or Small Cell Lung Cancer

Patologías: Cáncer de pulmón de células pequeñas en fase avanzada | Tumor sólido en adulto no especificado, específico del protocolo

Intervenciones: Fármaco: BKM120 | Fármaco: cisplatino | Fármaco: etopósido

URL: <http://ClinicalTrials.gov/show/NCT02194049>

Título: Study of BKM120 & Rituximab in Patients With Relapsed or Refractory Indolent B-Cell Lymphoma

**Patologías:** Linfoma de células B de la zona marginal extranodal del tejido linfoide asociado a mucosas | Linfoma de células B de la zona marginal extranodal | Linfoma folicular recurrente de grado 1 | Linfoma folicular recurrente de grado 2 | Linfoma folicular recurrente de grado 3 | Linfoma de células de manto recurrente | Linfoma de la zona marginal recurrente | Linfoma esplénico de la zona marginal | Macroglobulinemia de Waldenström  
**Intervenciones:** Fármaco: PI3K inhibitor BKM120 | Biológico: rituximab | Otros: Pharmacodynamics | Otros: estudios correlativos  
**URL:** <http://www.cancer.gov/clinicaltrials>

**Título:** Phase II Study of BKM120 for Subjects With Recurrent Glioblastoma  
**Reclutamiento:** Active, not recruiting  
**Intervenciones:** Fármaco: BKM120 | Fármaco: Surgery  
**URL:** <http://www.cancer.gov/clinicaltrials>

**Título:** A Study to Investigate Safety, Pharmacokinetics (PK) and Pharmacodynamics (PD) of BKM120 Plus GSK1120212 in Selected Advanced Solid Tumor Patients  
**Patologías:** Tumores sólidos avanzados y seleccionados  
**Intervenciones:** Fármaco: BKM120 | Fármaco: GSK1120212  
**URL:** <http://www.cancer.gov/clinicaltrials>

**Título:** Study to Assess Safety, Tolerability and Preliminary Efficacy of BKM120, PI3K Kinase Inhibitor, With Leukemias  
**Patologías:** Leucemia  
**Intervenciones:** Fármaco: BKM120 | Fármaco:  
**URL:** <http://www.cancer.gov/clinicaltrials>

**Título:** Phase Ib of Abiraterone Acetate Plus BEZ235 or BKM120 in Castration-resistant Prostate Cancer (CRPC) Patients  
**Patologías:** Cáncer de próstata resistente a la castración  
**Intervenciones:** Fármaco: BEZ235 | Fármaco: BKM120 | Fármaco: BEZ235 | Fármaco: BKM120 | Fármaco:  
**URL:** <http://www.cancer.gov/clinicaltrials>

**Título:** Phase III Study of BKM120/Placebo With Fulvestrant in Postmenopausal Patients With Hormone Receptor Positive HER2-negative Locally Advanced Breast Cancer Refractory to Aromatase Inhibitor  
**Patologías:** Cáncer de mama  
**Intervenciones:** Fármaco: BKM120 Matching placebo | Fármaco: Fulvestrant | Fármaco: BKM120 | Fármaco:  
**URL:** <http://www.cancer.gov/clinicaltrials>

**Título:** A Trial of BKM120 (a PI3K Inhibitor) in Patients With Triple Negative Breast Cancer  
**Patologías:** Cáncer de mama  
**Intervenciones:** Fármaco: BKM120 | Fármaco:  
**URL:** <http://www.cancer.gov/clinicaltrials>

**Título:** BKM120 for Patients With PI3K-activated Tumors  
**Patologías:** Tumores con activación de la vía de PI3K  
**Intervenciones:** Fármaco: BKM120 | Fármaco:  
**URL:** <http://www.cancer.gov/clinicaltrials>

Título: Safety and Efficacy of BKM120 and Lapatinib in HER2+/PI3K-activated, Trastuzumab-resistant Breast cancer

Patologías: Cáncer de mama

Intervenciones: Fármaco: BKM120 + lapatinib

URL: <http://Ensayos clínicos. ID. del gobierno>

Título: BKM120 + mFOLFOX6 in Advanced Solid TumorsWith Expansion Cohort Pancreatic Cancer

Patologías: Tumores sólidos avanzados|Cáncer colorrectal metastásico| Cáncer de páncreas metastásico

Intervenciones: Fármaco: BKM120|Fármaco: mFOLFOX6

URL: <http://Ensayos clínicos. ID. del gobierno>

Título: Paclitaxel and BKM120 Before Surgery in Treating Patients With Stage II or III Estrogen Receptor-Positive and HER2-Negative Breast Cancer

Patologías: Neoplasias de mama

Intervenciones: Fármaco: paclitaxel|Fármaco: BKM120|Fármaco:

URL: <http://Ensayos clínicos. ID. del gobierno>

Título: PhIB BKM120 or BEZ235+Endocrine Treatment in Post-Menopausal Patients With Hormone Receptor + Breast Cancer

Patologías: Cáncer de mama

Intervenciones: Fármaco: BEZ235|Fármaco: BKM 120|Fármaco: Letrozole|Fármaco: BKM120|Fármaco:

URL: <http://Ensayos clínicos. ID. del gobierno>

Título: BKM120 in Advanced , Metastatic, or Recurrent Endometrial Cancer.

Patologías: Cáncer endometrial

Intervenciones: Fármaco: BKM120|Fármaco:

URL: <http://Ensayos clínicos. ID. del gobierno>

Título: A Trial of Oral BEZ235 and BKM120 in Combination With Paclitaxel With or Without Trastuzumab

Patologías: Tumores sólidos metastásicos o localmente avanzados,

Intervenciones: Fármaco: BEZ235 + paclitaxel|Fármaco: BKM120 + paclitaxel|Fármaco: BEZ235 + paclitaxel + trastuzumab|Fármaco: BKM120 + paclitaxel + trastuzumab

URL: <http://Ensayos clínicos. ID. del gobierno>

Título: BKM120 as Second-line Therapy for Endometrial Cancer.

Patologías: Cáncer endometrial avanzado

Intervenciones: Fármaco: BKM120|Fármaco:

URL: <http://Ensayos clínicos. ID. del gobierno>

Título: Study of LEE011 With Fulvestrant and BYL719 or BKM120 in Advanced Breast Cancer

Reclutamiento: Recruiting

Resultados del estudio: Resultados no disponibles

Patologías: Cáncer de mama

Intervenciones: Fármaco: LEE011|Fármaco: BYL719|Fármaco: fulvestrant|Fármaco: BKM120

URL: <http://ClinicalTrials.gov/show/NCT02088684>

Título: BKM120 in Esophageal Squamous Cell Carcinoma After Failure of First Line Chemotherapy

Patologías: Cáncer de esófago

Intervenciones: Fármaco: BKM120

URL: <http://ClinicalTrials.gov/show/NCT01806649>

Título: Safety and Efficacy of BKM120 in Combination With Trastuzumab in Patients With Relapsing HER2 Overexpressing Breast Cancer Who Have Previously Failed Trastuzumab

Patologías: Cáncer de mama metastásico|Trastuzumab|BKM120|HER2+

Intervenciones: Fármaco: BKM120

URL: <http://ClinicalTrials.gov/show/NCT01132664>

Título: Safety, Pharmacokinetics and Pharmacodynamics of BKM120 Plus MEK162 in Selected Advanced Solid Tumor Patients

Reclutamiento: Activo, no reclutando

Resultados del estudio: resultados no disponibles

Patologías: Tumores sólidos avanzados|Tumores sólidos seleccionados

Intervenciones: Fármaco: BKM120 + MEK162

URL: <http://ClinicalTrials.gov/show/NCT01363232>

Título: Phase II, Open Label, Non-randomized, Trial of BKM120 for Metastatic or Locally Advanced Cervical Cancer

Patologías: Tratamiento de cáncer cervical metastásico o localmente avanzado

Intervenciones: Fármaco: BKM120

URL: <http://ClinicalTrials.gov/show/NCT01613677>

Título: Cabazitaxel and BKM120 in Patients With Metastatic Castrate-Resistant Prostate Cancer (mCRPC) Previously Treated With Docetaxel

Reclutamiento: Withdrawn

Resultados del estudio: resultados no disponibles

Patologías: Cáncer de próstata avanzado

Intervenciones: Fármaco: BKM 120|Fármaco: Cabazitaxel

URL: <http://ClinicalTrials.gov/show/NCT02035124>

Título: A Phase I Study of BKM120 in Adult Chinese Patients With Advanced Solid tumors

Patologías: Cáncer de mama avanzado, Carcinomas avanzados con histología de células escamosas

Intervenciones: Fármaco: BKM120

URL: <http://ClinicalTrials.gov/show/NCT01626209>

Título: P13Kinase Inhibitor BKM120 in Combination With Panitumumab in Patients With advanced RAS-Wild Type Colorectal Cancer.

Patologías: Cáncer colorrectal metastásico

Intervenciones: Fármaco: BKM120|Fármaco: Panitumumab

URL: <http://ClinicalTrials.gov/show/NCT01591421>

Título: PI3K Inhibitor BKM120 and Cetuximab in Treating Patients With Recurrent or Metastastatic Head and Neck Cancer

Patologías: Carcinoma metastásico escamoso de cuello con cáncer de células escamosas primario oculto|cáncer escamoso metastásico de cuello recurrente con cáncer primario

oculto|cáncer recurrente de glándulas salivares| Carcinoma recurrente de células escamosas de hipofaringe| Carcinoma recurrente de células escamosas de la laringe| Carcinoma recurrente de células escamosas de labio y cavidad bucal| Carcinoma recurrente de células escamosas de nasofaringe| Carcinoma recurrente de células escamosas de orofaringe| Carcinoma recurrente de células escamosas de seno paranasal y la cavidad nasal| Carcinoma verrugoso recurrente de laringe| Carcinoma verrugoso recurrente de cavidad bucal| carcinoma de células escamosas de las glándulas salivares| carcinoma de células escamosas de la hipofaringe fase IV|carcinoma de células escamosas de nasofaringe fase IV|Cáncer de las glándulas salivares fase IV|carcinoma de células escamosas de laringe, fase IV|carcinoma de células escamosas de labio y cavidad oral, fase IV|carcinoma de células escamosas de la orofaringe, fase IV|carcinoma de células escamosas de seno paranasal y cavidad nasal, fase IV|Carcinoma verrugoso de laringe, fase IV |Carcinoma verrugoso de cavidad bucal, fase IV|cáncer de las glándulas salivares, fase IVB|carcinoma de células escamosas de laringe, fase IVB| carcinoma de células escamosas de labio y cavidad bucal, fase IVB| carcinoma de células escamosas de orofaringe, fase IVB| carcinoma de células escamosas de seno paranasal y cavidad nasal, fase IVB|carcinoma verrugoso de la laringe, fase IVB|carcinoma verrugoso de la cavidad oral, fase IVB|Cáncer de las glándulas salivares, fase IVC| carcinoma de células escamosas de labio y cavidad bucal, fase IVC|carcinoma de células escamosas de orofaringe, fase IVC| carcinoma de células escamosas del seno paranasal y cavidad nasal, fase IVC|carcinoma verrugoso de laringe, fase IVC|carcinoma verrugoso de la cavidad oral, fase IVC|Cáncer de lengua.

URL: <http://ClinicalTrials.gov/show/NCT01816984>

Título: NeoPHOEBe: Neoadjuvant Trastuzumab + BKM120 in Combination With Weekly Paclitaxel in HER2-positive Primary Breast cancer

Patologías: HER2-positivo, recién diagnosticado, Cáncer de mama primario, terapia neoadyuvante, Trastuzumab

Intervenciones: Fármaco: BKM120|Fármaco: Trastuzumab|Fármaco: Paclitaxel|Fármaco: Placebo

URL: <http://ClinicalTrials.gov/show/NCT01816594>

Título: BKM120 For Triple Negative Breast cancer

Patologías: Cáncer de mama

Intervenciones: Fármaco: BKM120

URL: <http://ClinicalTrials.gov/show/NCT01790932>

Título: Buparlisib (BKM120) In Patients With Recurrent/Refractory Primary Central Nervous System Lymphoma (PCNSL) and Recurrent/Refractory Secondary Central Nervous System Lymphoma (SCNSL)

Patologías: linfoma|linforma del sistema nervioso central primario|Recurrente/ linforma del sistema nervioso central refractario

Intervenciones: Fármaco: Buparlisib (BKM120)

URL: <http://ClinicalTrials.gov/show/NCT02301364>

Título: Study of Efficacy and Safety of Buparlisib (BKM120) Plus Paclitaxel Versus Placebo Plus Paclitaxel in Head and Neck Cancer Previously Pre-treated With a Platinum Therapy

Patologías: Carcinoma de células escamosas de cabeza y cuello metastásico o recurrente previamente tratado.

Intervenciones: Fármaco: Paclitaxel|Fármaco: Buparlisib|Fármaco: Buparlisib Placebo

URL: <http://ClinicalTrials.gov/show/NCT01852292>

Título: BKM120+Abiraterone Acetate for CRPC

Patologías: Cáncer de próstata

Intervenciones: Fármaco: BKM120|Fármaco: Abiraterone|Fármaco: Prednisone

URL: <http://ClinicalTrials.gov/show/NCT01741753>

Título: Activity and Safety Study of BKM120 in monotherapy in Patient With Head and Neck Cancer

Patologías: Neoplasias de cabeza y cuello |Metástasis de neoplasia|Enfermedad recurrente|Enfermedad en progresión

Intervenciones: Fármaco: BKM120

URL: <http://ClinicalTrials.gov/show/NCT01737450>

Título: BKM120 Combined With Vemurafenib (PLX4032) in BRAFV600E/K Mutant Melanoma

Patologías: Melanoma Metastásico con BRAF Mutante

Intervenciones: Fármaco: BKM120 Combined with Vemurafenib (PLX4032)

URL: <http://ClinicalTrials.gov/show/NCT01512251>

Título: Study of BKM120 or BYL719 and Capecitabine in Patients With Breast cancer

Patologías: Cáncer de mama metastásico

Intervenciones: Fármaco: BKM120|Fármaco: Capecitabine|Fármaco: BYL719|Fármaco: Trastuzumab|Fármaco: Lapatinib

URL: <http://ClinicalTrials.gov/show/NCT01300962>

Título: A Phase I Study of BKM120 and Everolimus in Solid Malignancies

Patologías: Tumores sólidos

Intervenciones: Fármaco: BKM120|Fármaco: Everolimus

URL: <http://ClinicalTrials.gov/show/NCT01470209>

Título: Capecitabine + BKM120 TNBC BC Brain Metastasis

Patologías: Cáncer de mama triple negativo | Metástasis en el cerebro|Cáncer de mama

Intervenciones: Fármaco: BKM120|Fármaco: capecitabine

URL: <http://ClinicalTrials.gov/show/NCT02000882>

Título: Phase Ib, Dose Escalation Study of Oral LDE225 in Combination With BKM120 in Patients With advanced tumors

Patologías: Dose Escalation|Safety|Preliminary Efficacy| Tumores sólidos avanzados| Cáncer de mama metastásico | Adenocarcinoma pancreático avanzado | Cáncer colorrectal metastásico | Glioblastoma Multiforme recurrente| Cáncer gástrico|Cáncer de unión gastroesofágica | Cáncer de mama metastásico triple negativo | Cáncer de mama receptor hormonal positivo (ER+/PR+, y Her2-)

Intervenciones: Fármaco: LDE225|Fármaco: BKM120

URL: <http://ClinicalTrials.gov/show/NCT01576666>

Título: Safety and Efficacy of BKM120 in Patients With non-small cell lung cancer

Patologías: Cáncer pulmonar de células no pequeñas

Intervenciones: Fármaco: BKM120 or Docetaxel|Fármaco: BKM120 or Docetaxel or Pemetrexed

URL: <http://ClinicalTrials.gov/show/NCT01297491>

Título: A Study of BKM120 in Adult Japanese Patients With Advanced solid tumors

Patologías: Tumor sólido avanzado

Intervenciones: Fármaco: BKM120

URL: <http://ClinicalTrials.gov/show/NCT01283503>

Título: Phase I BKM120/Abraxane in Tumores sólidos, Expansion Phase Recurrent Endometrial or ovarian cancer.

Patologías: Cáncer ovárico|Cáncer endometrial| Cáncer ovárico recurrente| Cáncer endometrial recurrente

Intervenciones: Fármaco: BKM120|Fármaco: Nabpaclitaxel

URL: <http://ClinicalTrials.gov/show/NCT02117817>

Título: Safety and Efficacy of INC280 and Buparlisib (BKM120) in Patients With Recurrent Glioblastoma

Patologías: c-MET Inhibitor; PI3K Inhibitor, PTEN Mutations, Homozygous Deletion of PTEN or PTEN Negative by IHC, INC280, BKM120, Buparlisib; Glioblastoma recurrente

Intervenciones: Fármaco: INC280|Fármaco: Buparlisib

URL: <http://ClinicalTrials.gov/show/NCT01870726>

Título: A Study of BKM120 (Buparlisib) in Relapsed or Refractory Thymomas

Patologías: Timoma

Intervenciones: Fármaco: BKM120

URL: <http://ClinicalTrials.gov/show/NCT02220855>

Título: BKM120 in Cancers With PIK3CA Activating Mutations

Patologías: Cáncer de pulmón|Cáncer de mama| Cáncer Colorrectal |Colangiocarcinoma|Tumores sólidos

Intervenciones: Fármaco: BKM120

URL: <http://ClinicalTrials.gov/show/NCT01501604>

Título: Pharmacokinetic Study of BKM120 in Subjects With Hepatic Impairment

Patologías: Insuficiencia hepática

Intervenciones: Fármaco: BKM120

URL: <http://ClinicalTrials.gov/show/NCT01727128>

Título: LGX818 in Combination With Agents (MEK162; BKM120; LEE011; BGJ398; INC280) in BRAF Melanoma

Patologías: Melanoma

Intervenciones: Fármaco: LGX818|Fármaco: MEK162|Fármaco: LEE011|Fármaco: BGJ398|Fármaco: BKM120|Fármaco: INC280

URL: <http://ClinicalTrials.gov/show/NCT01820364>

Título: Safety and Efficacy of BKM120 in Relapsed and Refractory NHL

Patologías: Linfoma difuso de células B, linfoma de células de manto, linfoma folicular

Intervenciones: Fármaco: BKM120

URL: <http://ClinicalTrials.gov/show/NCT01693614>

Título: A Study to Find the Maximum Tolerated Dose of the Experimental Combination of INC424 and BKM120 in Patients With Primary or Secondary Myelofibrosis

Patologías: Mielofibrosis

Intervenciones: Fármaco: INC424 | Fármaco: BKM120

URL: <http://ClinicalTrials.gov/show/NCT01730248>

Título: GINECO-EN102b - BKM120 as Monotherapy in the Treatment of Initial or Recurrent endometrial cancer

Patologías: Cáncer endometrial

Intervenciones: Fármaco: BKM120

URL: <http://ClinicalTrials.gov/show/NCT01397877>

Título: Safety and Efficacy of Buparlisib (BKM120) in Patients With Untreated Squamous non-small lung cancer

Patologías: Cáncer pulmonar de células no microcíticas

Intervenciones: Fármaco: BKM120 | Fármaco: placebo | Fármaco: Carboplatin | Fármaco: Paclitaxel

URL: <http://ClinicalTrials.gov/show/NCT01820325>

Título: LGX818 and MEK162 in Combination With a Third Agent (BKM120, LEE011, BGJ398 or INC280) in Advanced BRAF Melanoma

Patologías: Melanoma

Intervenciones: Fármaco: LGX818 | Fármaco: MEK162 | Fármaco: LEE011 | Fármaco: BGJ398 | Fármaco: BKM120 | Fármaco: INC280

URL: <http://ClinicalTrials.gov/show/NCT02159066>

Título: Phase II Study of Buparlisib + Docetaxel in Advanced or Metastatic Squamous non small-cell lung Cancer (NSCLC) Patients

Patologías: Cáncer pulmonar de células no pequeñas

Intervenciones: Fármaco: Buparlisib | Fármaco: Buparlisib matching placebo | Fármaco: Docetaxel

URL: <http://ClinicalTrials.gov/show/NCT01911325>

Título: A Phase II Study With BYL719 in Premenopausal Patients With Locally Advanced breast Cancer

Patologías: Cáncer de mama premenopáusico | Inhibición de la vía PI3K

Intervenciones: Fármaco: BYL719 | Fármaco: BKM120 | Fármaco: Control

URL: <http://ClinicalTrials.gov/show/NCT02058381>

Título: Buparlisib in Treating Patients With Relapsed or Refractory Non-Hodgkin Lymphoma

Patologías: Recurrent Adult Linfoma difuso de células B | Linfoma folícular recurrente de grado 3 | Linfoma recurrente de células de manto

Intervenciones: Fármaco: buparlisib | Otros: laboratory biomarker analysis | Otros: questionnaire administration

URL: <http://ClinicalTrials.gov/show/NCT01719250>

Título: Buparlisib, Gemcitabine Hydrochloride, and Cisplatin in Treating Patients With advanced solid tumors

Patologías: Cáncer pulmonar recurrente de células no pequeñas | Cáncer pulmonar de células no pequeñas fase IIIA | Cáncer pulmonar de células no pequeñas fase IIIB | Cáncer pulmonar de células no pequeñas fase IV | tumor sólido en adultos sin especificar, Protocol Specific

Intervenciones: Fármaco: buparlisib|Fármaco: gemcitabine hydrochloride|Fármaco: cisplatin|Otros: pharmacological study|Otros: laboratory biomarker analysis|Otros: questionnaire administration  
URL: <http://ClinicalTrials.gov/show/NCT01971489>

Título: Erismodegib and Buparlisib in Treating Patients With advanced basalcell carcinomas  
Patologías: Basalioma |Síndrome de Basalioma nevoide|Cáncer de piel recurrente  
Intervenciones: Fármaco: buparlisib|Fármaco: erismodegib|Otros: laboratory biomarker analysis|Otros: questionnaire administration  
URL: <http://ClinicalTrials.gov/show/NCT02303041>

Título: Study of Letrozole With or Without BYL719 or Buparlisib, for the Neoadjuvant Treatment of Postmenopausal Women with breast cancer.  
Patologías: Cáncer de mama  
Intervenciones: Fármaco: BYL719|Fármaco: BKM120|Fármaco: Placebo  
URL: <http://ClinicalTrials.gov/show/NCT01923168>

Título: Pharmacokinetic Study of Buparlisib in Subjects With Renal dysfunction  
Patologías: Insuficiencia renal  
Intervenciones: Fármaco: Buparlisib  
URL: <http://ClinicalTrials.gov/show/NCT02048787>

Título: Phase Ib/II Study of Buparlisib Plus Carboplatin or Lomustine in Patients With Recurrent Glioblastoma Multiforme  
Patologías: Glioblastoma Multiforme recurrente (rGBM)  
Intervenciones: Fármaco: buparlisib|Fármaco: carboplatin|Fármaco: lomustine|Fármaco: placebo  
URL: <http://ClinicalTrials.gov/show/NCT01934361>

#### **Estudios con Sonidegib realizados posteriormente al estudio aquí presentado (NCT00880308)**

Título: A Pre-surgical Study of LDE225 in Men With High-risk Localized Prostate Cancer  
Patologías: Cáncer de próstata  
Intervenciones: Fármaco: LDE225  
URL: <http://ClinicalTrials.gov/show/NCT02111187>

Título: Effect of Hepatic Impairment on LDE225..  
Patologías: función hepática normal,|insuficiencia hepática  
Intervenciones: Fármaco: LDE225  
URL: <http://ClinicalTrials.gov/show/NCT01764776>

Título: Gemcitabine + Nab-paclitaxel With LDE-225 (Hedgehog Inhibitor) as Neoadjuvant Therapy for Pancreatic Adenocarcinoma  
Patologías: Adenocarcinoma pancrático resecable  
Intervenciones: Fármaco: LDE-225|Fármaco: Gemcitabine|Fármaco: nab-paclitaxel  
URL: <http://ClinicalTrials.gov/show/NCT01431794>

Título: Phase I Trial of LDE225 for Steroid-refractory Chronic GVHD After Allogeneic HSCT  
Patologías: Enfermedad de injerto contra huésped|Transplante alogénico de células madre

Intervenciones: Fármaco: LDE225  
URL: <http://ClinicalTrials.gov/show/NCT02086513>

Título: Phase IB/II Trial of LDE225 and Paclitaxel in Recurrent Ovarian Cancer  
Patologías: Cáncer ovárico recurrente | Resistente al platino  
Intervenciones: Fármaco: LDE225  
URL: <http://ClinicalTrials.gov/show/NCT02195973>

Título: LDE225 for Patients With PTCH1 or SMO Mutated Tumors  
Patologías: Tumores sólidos y hematológicos con PTCH1 o SMO activado  
Intervenciones: Fármaco: LDE225  
URL: <http://ClinicalTrials.gov/show/NCT02002689>

Título: Study With LDE225 in Combination With Docetaxel in Triple Negative (TN) Advanced Breast cancer (ABC) Patients  
Patologías: Cáncer de mama avanzado  
Intervenciones: Fármaco: LDE225 | Fármaco: Docetaxel  
URL: <http://ClinicalTrials.gov/show/NCT02027376>

Título: Safety/Efficacy Study of LDE225 (Sonidegib) Plus Bortezomib in Patients With Relapsed or Relapsed/Refractory Multiple Myeloma  
Patologías: Mieloma múltiple  
Intervenciones: Fármaco: LDE225 | Fármaco: Bortezomib  
URL: <http://ClinicalTrials.gov/show/NCT02254551>

Título: Dose-escalation, and Safety Study of LDE225 and Gemcitabine in Locally Advanced or Metastatic Pancreatic Cancer Patients  
Patologías: Cáncer de páncreas  
Intervenciones: Fármaco: LDE225+gemcitabine  
URL: <http://ClinicalTrials.gov/show/NCT01487785>

Título: LDE225 and Paclitaxel in Tumores sólidos  
Patologías: Tumor sólido avanzado | Cáncer ovárico  
Intervenciones: Fármaco: LDE225 | Fármaco: Paclitaxel  
URL: <http://ClinicalTrials.gov/show/NCT01954355>

Título: A Pilot Study of a Hedgehog Pathway Inhibitor (LDE-225) in Surgically Resectable Pancreatic Cancer  
Patologías: Cáncer de páncreas resecable  
Intervenciones: Fármaco: LDE-225  
URL: <http://ClinicalTrials.gov/show/NCT01694589>

Título: Phase I Study to Evaluate the Effect of LDE225 on the Pharmacokinetics of Bupropion and Warfarin in Patients  
Patologías: Tumor sólido avanzado  
Intervenciones: Fármaco: LDE225 | Fármaco: Wafarin | Fármaco: Bupropion  
URL: <http://ClinicalTrials.gov/show/NCT01769768>

Título: LDE225 + Everolimus in Advanced Gastroesophageal Adenocarcinoma  
Patologías: Esophageal Cancer

Intervenciones: Fármaco: Everolimus | Fármaco: LDE 225  
URL: <http://ClinicalTrials.gov/show/NCT02138929>

Título: Study of Efficacy and Safety of LDE225 in Adult Patients With Relapsed/Refractory Acute Leukemia

Patologías: leucemias agudas

Intervenciones: Fármaco: LDE225

URL: <http://ClinicalTrials.gov/show/NCT01826214>

Título: A Phase I Dose Finding and Safety Study of Oral LDE225 in Children and a Phase II Portion to Assess Preliminary Efficacy in Recurrent or Refractory MB

Patologías: Meduloblastoma,| rhabdомiosarcoma,| Neuroblastoma,| Hepatoblastoma,| glioma de alto grado| Astrocytoma

Intervenciones: Fármaco: LDE225

URL: <http://ClinicalTrials.gov/show/NCT01125800>

Título: Combination of the Hedgehog Inhibitor, LDE225, With Etoposide and Cisplatin in the First-Line Treatment of Patients With Extensive Stage Small Cell Lung Cancer (ES-SCLC)

Patologías: Cáncer de pulmón

Intervenciones: Fármaco: LDE225, Etoposide and Cisplatin

URL: <http://ClinicalTrials.gov/show/NCT01579929>

Título: LDE225 in Treating Patients With Stage II-III Estrogen Receptor- and HER2-Negative Breast Cancer

Patologías: Neoplasias de mama

Intervenciones: Fármaco: Erismodegib | Fármaco: Placebo

URL: <http://ClinicalTrials.gov/show/NCT01757327>

Título: An East Asian Study of LDE225

Patologías: Tumores sólidos avanzados | Meduloblastoma | Basalioma

Intervenciones: Fármaco: LDE225

URL: <http://ClinicalTrials.gov/show/NCT01208831>

Título: A Trial to Evaluate the Safety, Local Tolerability, Pharmacokinetics and Pharmacodynamics of LDE225 on Skin Basaliomas in Gorlin Syndrome Patients

Patologías: Tratamiento de los basaliomas (BCCs) en pacientes con el síndrome de Gorlin

Intervenciones: Fármaco: Placebo | Fármaco: LDE225 | Fármaco: LDE225 0.25% | Fármaco: LDE225 0.75%

URL: <http://ClinicalTrials.gov/show/NCT00961896>

Título: To Evaluate the Safety, Local Tolerability, PK and PD of LDE225 on Sporadic Superficial and Nodular Skin Basaliomas(sBCC)

Patologías: Sporadic Superficial and Nodular Skin Basaliomas

Intervenciones: Fármaco: LDE225 | Fármaco: Vehicle

URL: <http://ClinicalTrials.gov/show/NCT01033019>

Título: A Phase Ib/II Dose-finding Study to Assess the Safety and Efficacy of LDE225 + INC424 in Patients With MF

Patologías: Primary Myelofibrosis | Thrombocythemia, Essential | Thrombocytosis | Myeloproliferative Disorders | Bone Marrow Diseases | Hematologic Diseases | Blood Coagulation Disorders | Blood Platelet Disorders | Hemorrhagic Disorders  
Intervenciones: Fármaco: LDE225 | Fármaco: INC424  
URL: <http://ClinicalTrials.gov/show/NCT01787552>

Título: Pilot LDE225 in Locally Advanced or Metastatic BCC + Previously Tx Non-LDE225 Smoothened Inhibitors

Patologías: Basalioma  
Intervenciones: Fármaco: LDE225  
URL: <http://ClinicalTrials.gov/show/NCT01529450>

Título: Nilotinib and LDE225 in the Treatment of Chronic or Accelerated Phase Myeloid Leukemia in Patients Who Developed Resistance to Prior Therapy

Patologías: Philadelphia Chromosome Positive Chronic Myelogenous Leukemia  
Intervenciones: Fármaco: Nilotinib + LDE225  
URL: <http://ClinicalTrials.gov/show/NCT01456676>

Título: A Phase II Study of Efficacy and Safety in Patients With Locally Advanced or Metastatic Basalioma

Patologías: Basalioma  
Intervenciones: Fármaco: LDE225  
URL: <http://ClinicalTrials.gov/show/NCT01327053>

Título: Erismodegib and Lenalidomide After Stem Cell Transplant in Treating Patients With Multiple Myeloma

Patologías: Refractory Multiple Myeloma  
Intervenciones: Fármaco: erismodegib | Fármaco: lenalidomide  
URL: <http://ClinicalTrials.gov/show/NCT02086552>

Título: LDE225 in Patients With Advanced or Metastatic Hepatocellular Carcinoma and Child-Pugh A Cirrhosis

Patologías: Hepatocellular Carcinoma | Cirrhosis  
Intervenciones: Fármaco: LDE225  
URL: <http://ClinicalTrials.gov/show/NCT02151864>

Título: A Phase II Study of Oral LDE225 in Patients With Hedge-Hog (Hh)-Pathway Activated Relapsed Medulloblastoma (MB)

Patologías: Medulloblastoma  
Intervenciones: Fármaco: LDE225  
URL: <http://ClinicalTrials.gov/show/NCT01708174>

Título: LDE225 + Docetaxel/Prednisone for Adv/Met Castrate Resistant Prostate Cancer w/ Disease Progression After Docetaxel

Patologías: Prostate Cancer  
Intervenciones: Fármaco: Docetaxel | Fármaco: Prednisone | Fármaco: LDE225  
URL: <http://ClinicalTrials.gov/show/NCT02182622>

Título: Phase Ib, Dose Escalation Study of Oral LDE225 in Combination With BKM120 in Patients With Advanced solid tumors

**Patologías:** Dose Escalation|Safety|Preliminary Efficacy|Avanzado Tumores sólidos|Metastásico Cáncer de mama| Adenocarcinoma pancreático avanzado | Cáncer colorrectal metastásico |Recurrent Glioblastoma Multiforme recurrente|Cáncer Gástrico |Cáncer de la unión gastroesofágica | Cáncer de mama metastásico triple negativo | Cáncer de mama metastásico receptor hormonal positivo (ER+/PR+, y Her2-)  
**Intervenciones:** Fármaco: LDE225|Fármaco: BKM120  
**URL:** <http://ClinicalTrials.gov/show/NCT01576666>

**Título:** Phase I/II Study of LDE225 With Gemcitabine and Nab-paclitaxel in Patients With Pancreatic Cancer

**Patologías:** Cáncer de páncreas

**Intervenciones:** Fármaco: gemcitabine and nab paclitaxel

**URL:** <http://ClinicalTrials.gov/show/NCT02358161>

**Título:** Azacitidine and Erismodegib in Treating Patients With Myeloid Malignancies

**Patologías:** Leucemia megacarioblástica aguda en adultos| leucemia monoblástica aguda en adultos| leucemia monocítica en adultos| leucemia mieloide aguda con Inv(16)(p13.1q22); CBFB-MYH11| leucemia mieloide aguda en adultos con maduración| leucemia mieloide aguda en adultos con t(16;16)(p13.1;q22); CBFB-MYH11| leucemia mieloide aguda en adultos con t(8;21)(q22;q22); RUNX1-RUNX1T1| leucemia mieloide aguda en adultos con t(9;11)(p22;q23); MLL3-MLL| leucemia mieloide aguda en adultos sin maduración| leucemia mielomonocítica aguda en adultos| leukemia promielocítica aguda en adultos con t(15;17)(q22;q12); PML-RARA| eritroleucemia en adultos| eritroleucemia pura en adultos| leucemia mieloide aguda relacionada con agentes alquilantes|leukemia mielomonocítica crónica|síndrome mielodisplásico de novo| trombocitemia esencial| policitemia vera|syndrome mielodisplásica tratada anteriormente|mielofibrosis primaria|leukemia mieloide aguda recurrente en adultos| leukemia mieloide aguda en adultos no tratados

**Intervenciones:** Fármaco: Azacitidine|Fármaco: Erismodegib|Otros: Quality-of-Life Assessment|Otros: Laboratory Biomarker Analysis

**URL:** <http://ClinicalTrials.gov/show/NCT02129101>

**Título:** Efficacy, Safety and Pharmacokinetics of Oral LDE225 in Treatment of Patients With Nevvoid Basalioma Syndrome

**Patologías:** Basalioma|Síndrome de Gorlin |

**Intervenciones:** Fármaco: LDE225|Fármaco: Placebo

**URL:** <http://ClinicalTrials.gov/show/NCT01350115>

**Título:** LDE225 With Fluorouracil, Leucovorin, Oxaliplatin, and Irinotecan for Untreated Advanced Pancreatic Cancer

**Patologías:** Cáncer de páncreas

**Intervenciones:** Fármaco: LDE225; Fluorouracil; Leucovorin; Oxaliplatin; Irinotecan

**URL:** <http://ClinicalTrials.gov/show/NCT01485744>

**Título:** A Biomarker Study to Identify Predictive Signatures of Response to LDE225 (Hedgehog Inhibitor) In Patients With Resectable Pancreatic Cancer

**Patologías:** Pancreatic Ductal Adenocarcinoma

**Intervenciones:** Fármaco: LDE225

**URL:** <http://ClinicalTrials.gov/show/NCT01911416>

Título: Erismodegib and Buparlisib in Treating Patients With Advanced or Metastatic Basalioma

Patologías: Basalioma | síndrome de Gorlin|Cáncer de piel recurrente

Intervenciones: Fármaco: buparlisib|Fármaco: erismodegib|Otros: laboratory biomarker analysis|Otros: questionnaire administration

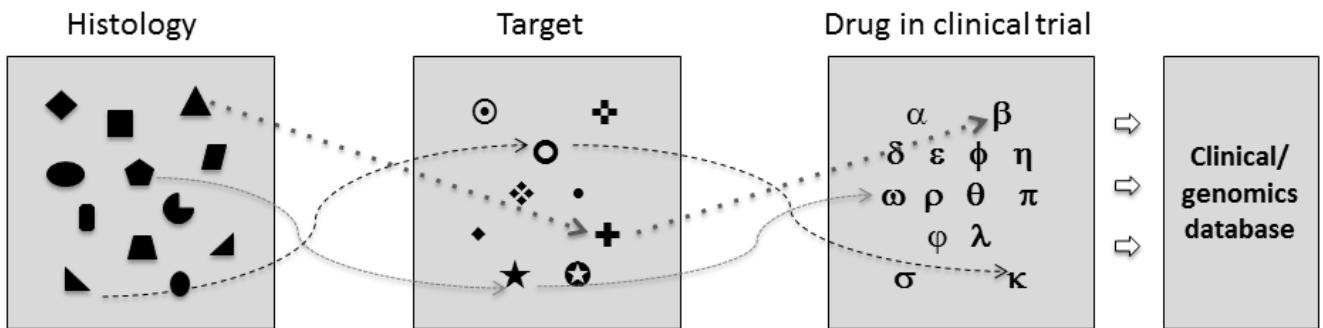
URL: <http://ClinicalTrials.gov/show/NCT02303041>

## Anexo 5

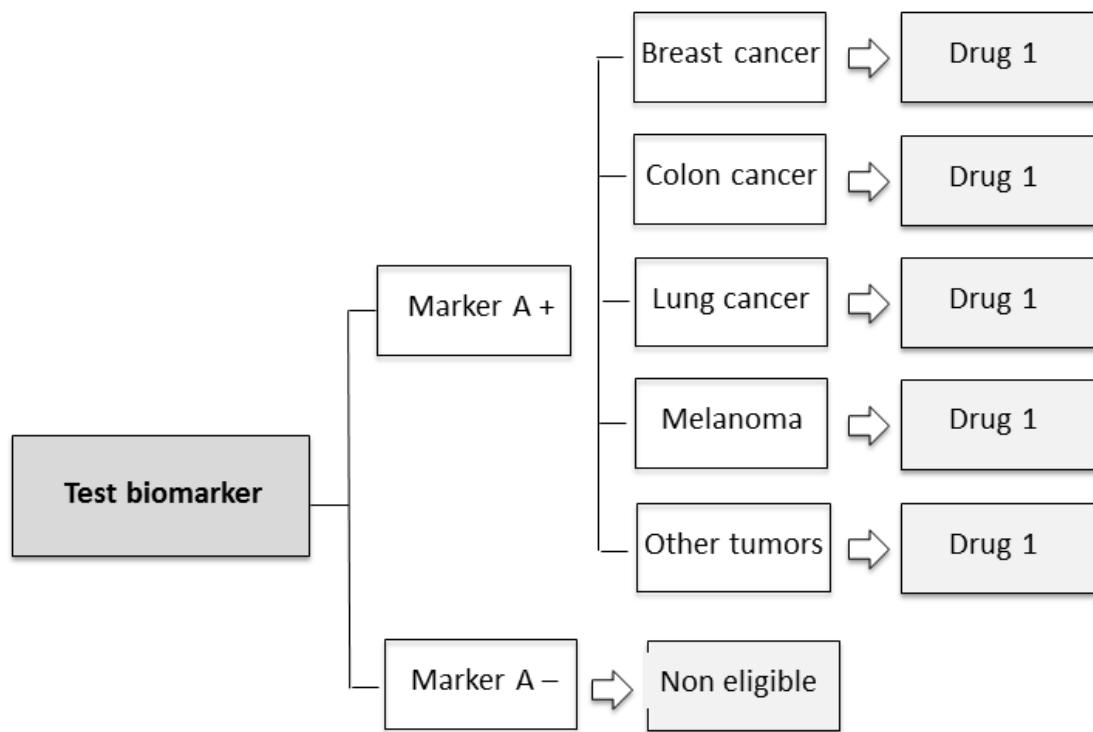
**Tabla 14. Estudios genómicos en oncología disponibles en la Unitat de Investigació en Terapia Molecular.**

Trial	Drug	Mechanism	Alteration	Trial design
DS160C00001	AZD9291	EGFR inh	EGFR T790 mutation	Resistance trial
CEGF816X2101	EGF816	EGFRmut-TKI	EGFR T790 mutation	Resistance trial
CINC280X2102	INC280	c-MET inh	MET amplification	Nested clinical trial to VHIO's Prescreening program
CINC280X2104	INC280+cetuxi	c-MET inh	MET amplification	Nested clinical trial to VHIO's Prescreening program
CINC280X2105C	EGF816+ INC280	EGFR mut-TKI, C-METinh	MET amplification	Resistance trial
CINC280X2202	INC280+gefitinib	c-MET inh	EGFR mutations AND MET amplification	Resistance trial
CINC280X2204	INC280+BKM120	c-MET inh+PI3K inh	PTEN mutation	Nested clinical trial to VHIO's Prescreening program
CMEK162X2109	MEK162+BYL719	MEKinh+PI3kinh	KRAS/NRAS mutation	Nested clinical trial to VHIO's Prescreening program
IGF-MC-JCA	LY3039478	Notch inh	NOTCH 1-4 amplification	Nested clinical trial to VHIO's Prescreening program
TED11449	SAR125844	MET inh	MET amplification	Nested clinical trial to VHIO's Prescreening program
42756493EDI1001	JNJ-42756493	pFGFR Tkinh	FGFR1-3 mutation, amplification, traslocation	Nested clinical trial to VHIO's Prescreening program
CBGJ398X2101	BGJ398	anti FGFR	FGFR1-3 mutation, amplification, traslocation	Nested clinical trial to VHIO's Prescreening program
CFGF401X2101	FGF401	FGFR4 inh	FGFR4 mutation	Nested clinical trial to VHIO's Prescreening program
CL1-49076-001	S 49076	MET inh (AXL,FGFR1 y2)	MET amplification	Nested clinical trial to VHIO's Prescreening program
Debio 1347-101	Debio1347(CH5183284)	anti FGFR	FGFR1-3 mutation, amplification, traslocation	Nested clinical trial to VHIO's Prescreening program
FGF117360	GSK3052230/pacli/carbo/docetaxel	anti FGFR	FGFR1-3 mutation, amplification, traslocation	Nested clinical trial to VHIO's Prescreening program
PRN1371-001	PRN1371	panFGFR Kinase Inhibitor	FGFR1-3 mutation, amplification, traslocation	Nested clinical trial to VHIO's Prescreening program
TPU-TAS-120-101	Tas120	anti FGFR	FGFR1-3 mutation, amplification, traslocation	Nested clinical trial to VHIO's Prescreening program
200919	GSK2118436	BRAF inh	BRAF mutation	Nested clinical trial to VHIO's Prescreening program
BRF114144	GSK2118436; GSK1120212	BRAF inh; MEK inh	BRAF mutation	Basket trial
BRF117019	BRAF inhibitor (dabrafenib); GSK2118436 and MEK Inhibitor (trametinib); GSK1120212	BRAF & MEK inh	BRAF mutation	Nested clinical trial to VHIO's Prescreening program
CMEK162X2110	LGX818 + MEK162+LEO01	RAFk inh + MEK inh+CDK4/6 inh	BRAF mutation	Resistance trial
GO29030	MEHD7945A + GDC0973	anti HER3/EGFr + MEK inh	KRAS mutation	Nested clinical trial to VHIO's Prescreening program
MEK116833	GSK1120212, GSK2118436, panitumumab	MEK inh, BRAF inh, AB anti EGFr	BRAF mutation	Nested clinical trial to VHIO's Prescreening program
MO28072	vemurafenib+cetuxi	Braf inh	BRAF mutation	Basket trial
CLGK974X2101	LGK974	porcupina inh	RNF43, ZNRF3 mutation, RSP01-2 traslocation	Nested clinical trial to VHIO's Prescreening program
CWNT974X2102	WNT974(LGK974); LGX818+cetuxi	porcupine inh; RAFk inh	RNF43, ZNRF3 mutation, RSP01-2 traslocation	Nested clinical trial to VHIO's Prescreening program
CBGJ398X2102	BGJ398+ BYL719	FGR-R pan-inh; PI3k inh	PIK3CA mutation	Nested clinical trial to VHIO's Prescreening program
CCLR457X2101	CLR457	PI3K inh	PIK3CA, PTEN, EGFR mutations, MET, HER2 amplification	Nested clinical trial to VHIO's Prescreening program
GO27802	GDC-0032+docetaxel o paclitaxel	PI3k inh	PIK3CA mutation or amplification	Nested clinical trial to VHIO's Prescreening program
ISX-MC-JDA	LY3009120	panRaf con actividad en HRAS, KIT, Variantes BRAF no-V600e y KRAS	KRAS, HRAS, NRAS, BRAF, ARAF mutation	Nested clinical trial to VHIO's Prescreening program
INK1117-001	INK1117	PI3kinh	PIK3CA mutation	Nested clinical trial to VHIO's Prescreening program
PMT 4979g	GDC0032+endocrine treatment	PI3kinh	PIK3CA mutation or amplification	Nested clinical trial to VHIO's Prescreening program
CHDM201X2101	HDM201	HDM2-p53 inh	HDM2 amplification	Nested clinical trial to VHIO's Prescreening program
CHDM201X2103C	HDM201+ LEE201	HDM2 inh + CDK inh	HDM2 amplification (enrichment)	Nested clinical trial to VHIO's Prescreening program
CLOP628X2101	LOP628	anti -cKit humanized IgG1/k antibody conjugated	KIT mutation (enrichment)	Nested clinical trial to VHIO's Prescreening program
IDH305X2101	IDH305	IDH1 mut inhibitor	IDH1 mutation	Nested clinical trial to VHIO's Prescreening program
LOXO-TRK-14001	Loxo Oncology	TRKA-C inh	ALK, ROS, NTK1-3 mutation, traslocation	Nested clinical trial to VHIO's Prescreening program
PUMA-NER 5201	Neratinib	panERB inhibitor	HER2, HER3 mutation	Basket trial
RXDX-101-01	RXDX-101	pan-trk, ROS1, and ALK inhibitor	NTK1-3 mutation, traslocation	Nested clinical trial to VHIO's Prescreening program
MK-3475-028	MK3475	PD1 inh	PDL1	Basket trial
MO29518	MPDL3280A	Basket trial PDL1	PDL1	Basket trial
CLDK378X2102	LDK378, AUY922	ALK inh, HSP90 inh	ALK traslocation	Resistance trial
Winther	all available targeted therapies	Personalized therapy	All actionable alterations	Trial in Personalized Medicine (n-of-1)

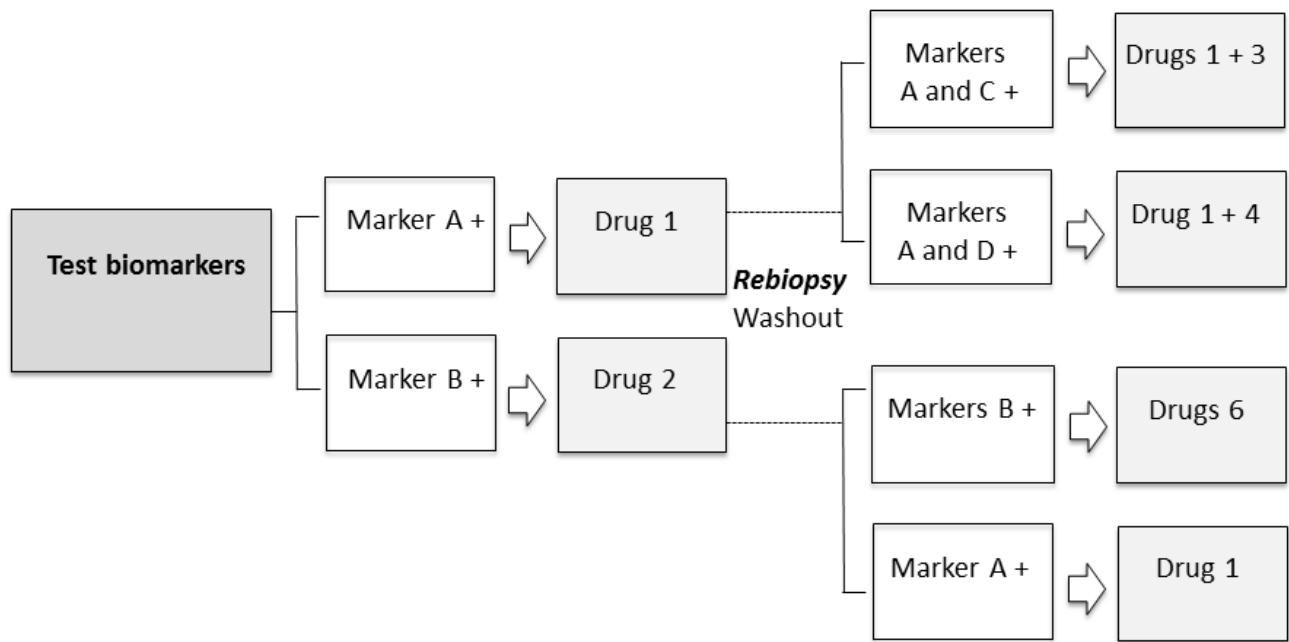
**Figura 10A**



**Figura 10B**



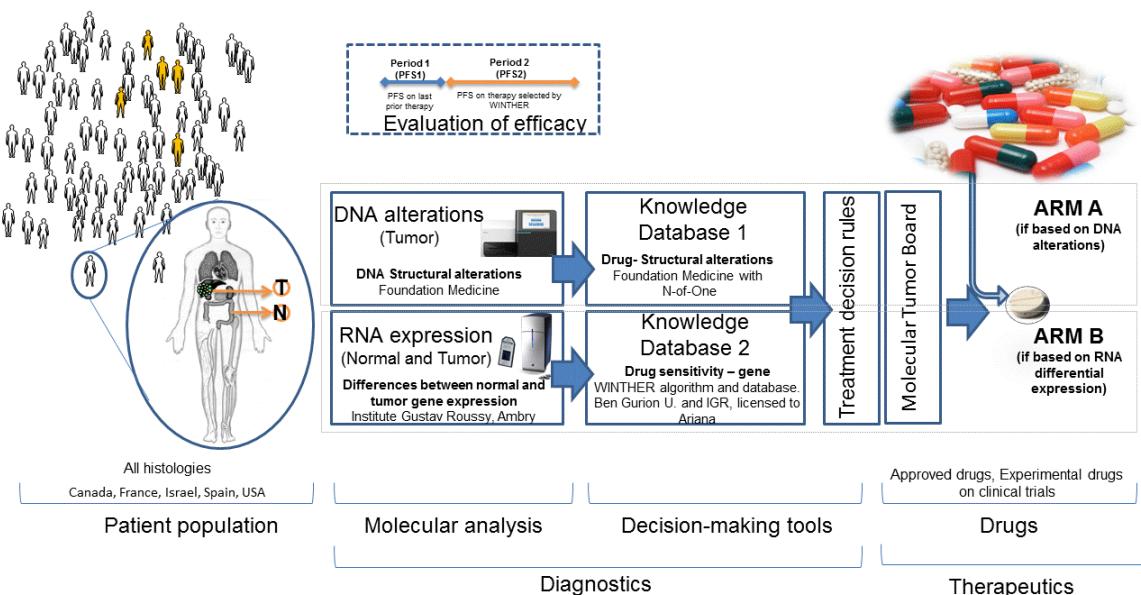
**Figura 10C**



**Figura 10D**



**Figura 10E**



**Figura 10.** Tipos de ensayos clínicos en Medicina Genómica, disponibles en UITM, clasificado según su diseño

- Bases de datos clínicos y genómicos y estudios longitudinales de cohortes con estudios de casos y controles anidados
- Estudios independientes de histología (histology-agnostic), específicos de alteración
- Estudios de resistencia:
- Diseño de ensayo clínico “N-de-1”
- El estudio WINTHER

## 7. Bibliografia

1. Hanahan D, Weinberg RA. The hallmarks of cancer. *Cell*. 2000;100(1):57-70. Epub 2000/01/27.
2. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell*. 2011;144(5):646-74. Epub 2011/03/08.
3. Kim R, Emi M, Tanabe K. Cancer immunoediting from immune surveillance to immune escape. *Immunology*. 2007;121(1):1-14. Epub 2007/03/28.
4. Hoeijmakers JH. DNA damage, aging, and cancer. *N Engl J Med*. 2009;361(15):1475-85. Epub 2009/10/09.
5. Holland AJ, Cleveland DW. Boveri revisited: chromosomal instability, aneuploidy and tumorigenesis. *Nature reviews Molecular cell biology*. 2009;10(7):478-87. Epub 2009/06/24.
6. Benz CC, Yau C. Ageing, oxidative stress and cancer: paradigms in parallax. *Nature reviews Cancer*. 2008;8(11):875-9. Epub 2008/10/25.
7. Mills RE, Walter K, Stewart C, Handsaker RE, Chen K, Alkan C, et al. Mapping copy number variation by population-scale genome sequencing. *Nature*. 2011;470(7332):59-65.
8. Cancer Genome Atlas Research N. Comprehensive genomic characterization defines human glioblastoma genes and core pathways. *Nature*. 2008;455(7216):1061-8. Epub 2008/09/06.
9. Hudson TJ, Anderson W, Artez A, Barker AD, Bell C, Bernabe RR, et al. International network of cancer genome projects. *Nature*. 2010;464(7291):993-8.
10. Forbes SA, Bhamra G, Bamford S, Dawson E, Kok C, Clements J, et al. The Catalogue of Somatic Mutations in Cancer (COSMIC). Current protocols in human genetics / editorial board, Jonathan L Haines [et al]. 2008;Chapter 10:Unit 10 1. Epub 2008/04/23.
11. McDermott U, Sharma SV, Dowell L, Greninger P, Montagut C, Lamb J, et al. Identification of genotype-correlated sensitivity to selective kinase inhibitors by using high-throughput tumor cell line profiling. *Proc Natl Acad Sci U S A*. 2007;104(50):19936-41.
12. Hahn WC, Weinberg RA. Modelling the molecular circuitry of cancer. *Nature reviews Cancer*. 2002;2(5):331-41. Epub 2002/06/05.
13. Luo J, Solimini NL, Elledge SJ. Principles of cancer therapy: oncogene and non-oncogene addiction. *Cell*. 2009;136(5):823-37. Epub 2009/03/10.
14. Ciriello G, Miller ML, Aksoy BA, Senbabaoglu Y, Schultz N, Sander C. Emerging landscape of oncogenic signatures across human cancers. *Nat Genet*. 2013;45(10):1127-33. Epub 2013/09/28.
15. Weinstein IB. Cancer. Addiction to oncogenes--the Achilles heel of cancer. *Science (New York, NY)*. 2002;297(5578):63-4. Epub 2002/07/06.
16. Felsher DW, Bishop JM. Reversible tumorigenesis by MYC in hematopoietic lineages. *Mol Cell*. 1999;4(2):199-207. Epub 1999/09/17.
17. Chin L, Tam A, Pomerantz J, Wong M, Holash J, Bardeesy N, et al. Essential role for oncogenic Ras in tumour maintenance. *Nature*. 1999;400(6743):468-72. Epub 1999/08/10.
18. Huettner CS, Zhang P, Van Etten RA, Tenen DG. Reversibility of acute B-cell leukaemia induced by BCR-ABL1. *Nat Genet*. 2000;24(1):57-60. Epub 1999/12/30.
19. Shirasawa S, Furuse M, Yokoyama N, Sasazuki T. Altered growth of human colon cancer cell lines disrupted at activated Ki-ras. *Science (New York, NY)*. 1993;260(5104):85-8. Epub 1993/04/02.
20. Kohno T, Ichikawa H, Totoki Y, Yasuda K, Hiramoto M, Nammo T, et al. KIF5B-RET fusions in lung adenocarcinoma. *Nat Med*. 2012;18(3):375-7. Epub 2012/02/14.

21. Banerji S, Cibulskis K, Rangel-Escareno C, Brown KK, Carter SL, Frederick AM, et al. Sequence analysis of mutations and translocations across breast cancer subtypes. *Nature*. 2012;486(7403):405-9. Epub 2012/06/23.
22. Palanisamy N, Ateeq B, Kalyana-Sundaram S, Pflueger D, Ramnarayanan K, Shankar S, et al. Rearrangements of the RAF kinase pathway in prostate cancer, gastric cancer and melanoma. *Nat Med*. 2010;16(7):793-8. Epub 2010/06/08.
23. Garnett MJ, Edelman EJ, Heidorn SJ, Greenman CD, Dastur A, Lau KW, et al. Systematic identification of genomic markers of drug sensitivity in cancer cells. *Nature*. 2012;483(7391):570-5. Epub 2012/03/31.
24. Yang W, Soares J, Greninger P, Edelman EJ, Lightfoot H, Forbes S, et al. Genomics of Drug Sensitivity in Cancer (GDSC): a resource for therapeutic biomarker discovery in cancer cells. *Nucleic Acids Res*. 2013;41(Database issue):D955-61. Epub 2012/11/28.
25. Barretina J, Caponigro G, Stransky N, Venkatesan K, Margolin AA, Kim S, et al. The Cancer Cell Line Encyclopedia enables predictive modelling of anticancer drug sensitivity. *Nature*. 2012;483(7391):603-7. Epub 2012/03/31.
26. Chapman PB, Hauschild A, Robert C, Haanen JB, Ascierto P, Larkin J, et al. Improved survival with vemurafenib in melanoma with BRAF V600E mutation. *The New England journal of medicine*. 2011;364(26):2507-16. Epub 2011/06/07.
27. Gelmon KA, Tischkowitz M, Mackay H, Swenerton K, Robidoux A, Tonkin K, et al. Olaparib in patients with recurrent high-grade serous or poorly differentiated ovarian carcinoma or triple-negative breast cancer: a phase 2, multicentre, open-label, non-randomised study. *Lancet Oncol*. 2011;12(9):852-61.
28. Kwak EL, Bang YJ, Camidge DR, Shaw AT, Solomon B, Maki RG, et al. Anaplastic lymphoma kinase inhibition in non-small-cell lung cancer. *The New England journal of medicine*. 2010;363(18):1693-703. Epub 2010/10/29.
29. Horstmann E, McCabe MS, Grochow L, Yamamoto S, Rubinstein L, Budd T, et al. Risks and benefits of phase 1 oncology trials, 1991 through 2002. *The New England journal of medicine*. 2005;352(9):895-904. Epub 2005/03/05.
30. Demetri GD, von Mehren M, Blanke CD, Van den Abbeele AD, Eisenberg B, Roberts PJ, et al. Efficacy and safety of imatinib mesylate in advanced gastrointestinal stromal tumors. *N Engl J Med*. 2002;347(7):472-80. Epub 2002/08/16.
31. Hudis CA. Trastuzumab--mechanism of action and use in clinical practice. *N Engl J Med*. 2007;357(1):39-51.
32. Aggarwal S. Targeted cancer therapies. *Nature reviews Drug discovery*. 2010;9(6):427-8. Epub 2010/06/02.
33. Parulekar WR, Eisenhauer EA. Phase I trial design for solid tumor studies of targeted, non-cytotoxic agents: theory and practice. *Journal of the National Cancer Institute*. 2004;96(13):990-7. Epub 2004/07/09.
34. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). *Eur J Cancer*. 2009;45(2):228-47. Epub 2008/12/23.
35. Choi H, Charnsangavej C, Faria SC, Macapinlac HA, Burgess MA, Patel SR, et al. Correlation of computed tomography and positron emission tomography in patients with metastatic gastrointestinal stromal tumor treated at a single institution with imatinib mesylate: proposal of new computed tomography response criteria. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2007;25(13):1753-9. Epub 2007/05/02.
36. Wolchok JD, Hoos A, O'Day S, Weber JS, Hamid O, Lebbe C, et al. Guidelines for the evaluation of immune therapy activity in solid tumors: immune-related response criteria.

- Clinical cancer research : an official journal of the American Association for Cancer Research. 2009;15(23):7412-20. Epub 2009/11/26.
37. Gomez-Roca C, Koscielny S, Ribrag V, Dromain C, Marzouk I, Bidault F, et al. Tumour growth rates and RECIST criteria in early drug development. *Eur J Cancer*. 2011;47(17):2512-6. Epub 2011/07/19.
38. Institute NC. Common Terminology Criteria for Adverse Events v4.0 In: NCI N, DHHS., editor. Bethesda: NIH.
39. Dienstmann R, Brana I, Rodon J, Tabernero J. Toxicity as a biomarker of efficacy of molecular targeted therapies: focus on EGFR and VEGF inhibiting anticancer drugs. *The oncologist*. 2011;16(12):1729-40. Epub 2011/12/03.
40. Tsimberidou A, Iskander N, Hong D. Personalized medicine in a phase I clinical trials program: The M. D. Anderson Cancer Center Initiative. *J Clin Oncol*. 2011; 29:(suppl; abstr CRA2500).
41. Shaw AT, Kim DW, Mehra R, Tan DS, Felip E, Chow LQ, et al. Ceritinib in ALK-rearranged non-small-cell lung cancer. *N Engl J Med*. 2014;370(13):1189-97. Epub 2014/03/29.
42. Garcia VM, Cassier PA, de Bono J, Garraway LA, Janne PA, Gerlinger M, et al. Parallel anticancer drug development and molecular stratification to qualify predictive biomarkers: dealing with obstacles hindering progress. *Cancer discovery*. 2011;1(3):207-12.
43. Rodon J, Saura C, Dienstmann R, Vivancos A, Ramon y Cajal S, Baselga J, et al. Molecular prescreening to select patient population in early clinical trials. *Nat Rev Clin Oncol*. 2012;9(6):359-66. Epub 2012/04/05.
44. Yap TA, Sandhu SK, Workman P, de Bono JS. Envisioning the future of early anticancer drug development. *Nature reviews Cancer*. 2010;10(7):514-23.
45. Simon R, Roychowdhury S. Implementing personalized cancer genomics in clinical trials. *Nature reviews Drug discovery*. 2013;12(5):358-69. Epub 2013/05/01.
46. de Gramont A, Watson S, Ellis LM, Rodon J, Tabernero J, Hamilton SR. Pragmatic issues in biomarker evaluation for targeted therapies in cancer. *Nat Rev Clin Oncol*. 2015;12(4):197-212. Epub 2014/11/26.
47. Carden CP, Sarker D, Postel-Vinay S, Yap TA, Attard G, Banerji U, et al. Can molecular biomarker-based patient selection in Phase I trials accelerate anticancer drug development? *Drug discovery today*. 2010;15(3-4):88-97. Epub 2009/12/08.
48. Dancey JE, Bedard PL, Onetto N, Hudson TJ. The genetic basis for cancer treatment decisions. *Cell*. 2012;148(3):409-20. Epub 2012/02/07.
49. Garrido-Laguna I, Hidalgo M, Kurzrock R. The inverted pyramid of biomarker-driven trials. *Nat Rev Clin Oncol*. 2011;8(9):562-6. Epub 2011/08/03.
50. Bachman KE, Argani P, Samuels Y, Silliman N, Ptak J, Szabo S, et al. The PIK3CA gene is mutated with high frequency in human breast cancers. *Cancer biology & therapy*. 2004;3(8):772-5.
51. Von Hoff DD, Stephenson JJ, Jr., Rosen P, Loesch DM, Borad MJ, Anthony S, et al. Pilot study using molecular profiling of patients' tumors to find potential targets and select treatments for their refractory cancers. *J Clin Oncol*. 2010;28(33):4877-83.
52. Tsimberidou AM, Iskander NG, Hong DS, Wheler JJ, Falchook GS, Fu S, et al. Personalized medicine in a phase I clinical trials program: the MD anderson cancer center initiative. *Clinical cancer research : an official journal of the American Association for Cancer Research*. 2012;18(22):6373-83. Epub 2012/09/12.
53. Courtney KD, Corcoran RB, Engelman JA. The PI3K pathway as drug target in human cancer. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2010;28(6):1075-83. Epub 2010/01/21.

54. Engelman JA, Luo J, Cantley LC, Knight ZA, Gonzalez B, Feldman ME, et al. The evolution of phosphatidylinositol 3-kinases as regulators of growth and metabolism. *Nature reviews Genetics*. 2006;7(8):606-19.
55. Foukas LC, Claret M, Pearce W, Okkenhaug K, Meek S, Peskett E, et al. Critical role for the p110alpha phosphoinositide-3-OH kinase in growth and metabolic regulation. *Nature*. 2006;441(7091):366-70. Epub 2006/04/21.
56. Cairns RA, Harris IS, Mak TW, Chandarlapaty S, Sawai A, Scaltriti M, et al. Regulation of cancer cell metabolism. *Nature reviews Cancer*. 2011;11(2):85-95.
57. Manning BD, Cantley LC. AKT/PKB signaling: navigating downstream. *Cell*. 2007;129(7):1261-74. Epub 2007/07/03.
58. Agoulnik IU, Hodgson MC, Bowden WA, Ittmann MM. INPP4B: the new kid on the PI3K block. *Oncotarget*. 2011;2(4):321-8. Epub 2011/04/14.
59. Bendell JC, Rodon J, Burris HA, de Jonge M, Verweij J, Birle D, et al. Phase I, dose-escalation study of BKM120, an oral pan-Class I PI3K inhibitor, in patients with advanced solid tumors. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2012;30(3):282-90. Epub 2011/12/14.
60. Burris H, Rodon J, Sharma S, Herbst RS, Tabernero J, Infante JR, et al. First-in-human phase I study of the oral PI3K inhibitor BEZ235 in patients (pts) with advanced solid tumors. *J Clin Oncol*. 2010;28(15s (suppl) ):abstr 3005.
61. Moreno Garcia V, Baird R, Shah K, Basu B, Tunariu N, Blanco M, editors. A phase I study evaluating GDC-0941, an oral phosphoinositide-3 kinase (PI3K) inhibitor, in patients with advanced solid tumors or multiple myeloma. . 2011 ASCO Annual meeting 2011; Chicago, IL.
62. Carver BS, Chapinski C, Wongvipat J, Hieronymus H, Chen Y, Chandarlapaty S, et al. Reciprocal feedback regulation of PI3K and androgen receptor signaling in PTEN-deficient prostate cancer. *Cancer cell*. 2011;19(5):575-86. Epub 2011/05/18.
63. Engelman JA, Chen L, Tan X, Crosby K, Guimaraes AR, Upadhyay R, et al. Effective use of PI3K and MEK inhibitors to treat mutant Kras G12D and PIK3CA H1047R murine lung cancers. *Nat Med*. 2008;14(12):1351-6. Epub 2008/11/26.
64. Zhao L, Vogt PK. Helical domain and kinase domain mutations in p110alpha of phosphatidylinositol 3-kinase induce gain of function by different mechanisms. *Proc Natl Acad Sci U S A*. 2008;105(7):2652-7. Epub 2008/02/13.
65. Samuels Y, Wang Z, Bardelli A, Silliman N, Ptak J, Szabo S, et al. High frequency of mutations of the PIK3CA gene in human cancers. *Science (New York, NY)*. 2004;304(5670):554. Epub 2004/03/16.
66. Kang S, Bader AG, Vogt PK. Phosphatidylinositol 3-kinase mutations identified in human cancer are oncogenic. *Proc Natl Acad Sci U S A*. 2005;102(3):802-7. Epub 2005/01/14.
67. O'Brien C, Wallin JJ, Sampath D, GuhaThakurta D, Savage H, Punnoose EA, et al. Predictive biomarkers of sensitivity to the phosphatidylinositol 3' kinase inhibitor GDC-0941 in breast cancer preclinical models. *Clinical cancer research : an official journal of the American Association for Cancer Research*. 2010;16(14):3670-83. Epub 2010/05/11.
68. Sangai T, Akcakanat A, Chen H, Tarco E, Wu Y, Do KA, et al. Biomarkers of Response to Akt Inhibitor MK-2206 in Breast Cancer. *Clinical cancer research : an official journal of the American Association for Cancer Research*. 2012;18(20):5816-28. Epub 2012/08/31.
69. Tanaka H, Yoshida M, Tanimura H, Fujii T, Sakata K, Tachibana Y, et al. The selective class I PI3K inhibitor CH5132799 targets human cancers harboring oncogenic PIK3CA mutations. *Clin Cancer Res*. 2011;17(10):3272-81.
70. Pasca di Magliano M, Hebrok M. Hedgehog signalling in cancer formation and maintenance. *Nature reviews Cancer*. 2003;3(12):903-11. Epub 2004/01/23.
71. Teglund S, Toftgard R. Hedgehog beyond medulloblastoma and basal cell carcinoma. *Biochimica et biophysica acta*. 2010;1805(2):181-208. Epub 2010/01/21.

72. Sekulic A, Migden MR, Oro AE, Dirix L, Lewis KD, Hainsworth JD, et al. Efficacy and safety of vismodegib in advanced basal-cell carcinoma. *N Engl J Med.* 2012;366(23):2171-9. Epub 2012/06/08.
73. Pan S, Wu X, Jiang J, Gao W, Wan Y, Cheng D, et al. Discovery of NVP-LDE225, a Potent and Selective Smoothened Antagonist. *ACS medicinal chemistry letters.* 2010;1(3):130-4. Epub 2010/06/10.
74. Chen H, Tu H, Meng ZQ, Chen Z, Wang P, Liu LM. K-ras mutational status predicts poor prognosis in unresectable pancreatic cancer. *Eur J Surg Oncol.* 2010;36(7):657-62.
75. Leach FS, Tokino T, Meltzer P, Burrell M, Oliner JD, Smith S, et al. p53 Mutation and MDM2 amplification in human soft tissue sarcomas. *Cancer Res.* 1993;53(10 Suppl):2231-4.
76. Eichhorn PJ, Gili M, Scaltriti M, Serra V, Guzman M, Nijkamp W, et al. Phosphatidylinositol 3-kinase hyperactivation results in lapatinib resistance that is reversed by the mTOR/phosphatidylinositol 3-kinase inhibitor NVP-BEZ235. *Cancer Res.* 2008;68(22):9221-30. Epub 2008/11/18.
77. Serra V, Markman B, Scaltriti M, Eichhorn PJ, Valero V, Guzman M, et al. NVP-BEZ235, a dual PI3K/mTOR inhibitor, prevents PI3K signaling and inhibits the growth of cancer cells with activating PI3K mutations. *Cancer Res.* 2008;68(19):8022-30. Epub 2008/10/03.
78. Isakoff SJ, Baselga J. Trastuzumab-DM1: building a chemotherapy-free road in the treatment of human epidermal growth factor receptor 2-positive breast cancer. *J Clin Oncol.* 2011;29(4):351-4.
79. Nowell PC. The clonal evolution of tumor cell populations. *Science (New York, NY).* 1976;194(4260):23-8. Epub 1976/10/01.
80. Coyle VM, Johnston PG. Genomic markers for decision making: what is preventing us from using markers? *Nat Rev Clin Oncol.* 2010;7(2):90-7. Epub 2009/12/17.
81. Raynaud FI, Eccles S, Clarke PA, Hayes A, Nutley B, Alix S, et al. Pharmacologic characterization of a potent inhibitor of class I phosphatidylinositide 3-kinases. *Cancer Res.* 2007;67(12):5840-50. Epub 2007/06/19.
82. Brachmann SM, Hofmann I, Schnell C, Fritsch C, Wee S, Lane H, et al. Specific apoptosis induction by the dual PI3K/mTor inhibitor NVP-BEZ235 in HER2 amplified and PIK3CA mutant breast cancer cells. *Proc Natl Acad Sci U S A.* 2009;106(52):22299-304. Epub 2009/12/17.
83. Roper J, Richardson MP, Wang WV, Richard LG, Chen W, Coffee EM, et al. The dual PI3K/mTOR inhibitor NVP-BEZ235 induces tumor regression in a genetically engineered mouse model of PIK3CA wild-type colorectal cancer. *PLoS One.* 2011;6(9):e25132. Epub 2011/10/04.
84. Weigelt B, Warne PH, Downward J. PIK3CA mutation, but not PTEN loss of function, determines the sensitivity of breast cancer cells to mTOR inhibitory drugs. *Oncogene.* 2011;30(29):3222-33. Epub 2011/03/02.
85. Stemke-Hale K, Gonzalez-Angulo AM, Lluch A, Neve RM, Kuo WL, Davies M, et al. An integrative genomic and proteomic analysis of PIK3CA, PTEN, and AKT mutations in breast cancer. *Cancer Res.* 2008;68(15):6084-91. Epub 2008/08/05.
86. Thomas RK, Baker AC, Debiasi RM, Winckler W, Laframboise T, Lin WM, et al. High-throughput oncogene mutation profiling in human cancer. *Nature genetics.* 2007;39(3):347-51. Epub 2007/02/13.
87. Santarpia L, Qi Y, Stemke-Hale K, Wang B, Young EJ, Booser DJ, et al. Mutation profiling identifies numerous rare drug targets and distinct mutation patterns in different clinical subtypes of breast cancers. *Breast Cancer Res Treat.* 2012;134(1):333-43. Epub 2012/04/28.
88. Wang Y, Carlton VE, Karlin-Neumann G, Sapolsky R, Zhang L, Moorhead M, et al. High quality copy number and genotype data from FFPE samples using Molecular Inversion Probe (MIP) microarrays. *BMC medical genomics.* 2009;2:8. Epub 2009/02/21.
89. Sidhu SS, Egile C, Malfilatre M, Lefranc C, Ruffin Y, Ma J, et al. Anti-tumor activity of pimasertib in combination with SAR245409 or SAR245408 in human primary colorectal cancer

- xenograft models bearing PI3K/KRas and KRas mutations. AACR 104th Annual Meeting 2013;; Washington, DC: AACR; 2013.
90. Maira SM, Pecchi S, Huang A, Burger M, Knapp M, Sterker D, et al. Identification and characterization of NVP-BKM120, an orally available pan-class I PI3-kinase inhibitor. *Mol Cancer Ther*. 2012;11(2):317-28. Epub 2011/12/23.
91. Won HH, Scott SN, Brannon AR, Shah RH, Berger MF. Detecting somatic genetic alterations in tumor specimens by exon capture and massively parallel sequencing. *Journal of visualized experiments : JoVE*. 2013(80):e50710. Epub 2013/11/07.
92. Gerlinger M, Rowan AJ, Horswell S, Larkin J, Endesfelder D, Gronroos E, et al. Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. *The New England journal of medicine*. 2012;366(10):883-92. Epub 2012/03/09.
93. Tenbaum SP, Ordonez-Moran P, Puig I, Chicote I, Arques O, Landolfi S, et al. beta-catenin confers resistance to PI3K and AKT inhibitors and subverts FOXO3a to promote metastasis in colon cancer. *Nat Med*. 2012;18(6):892-901. Epub 2012/05/23.
94. Adzhubei I, Jordan DM, Sunyaev SR. Predicting functional effect of human missense mutations using PolyPhen-2. Current protocols in human genetics / editorial board, Jonathan L Haines [et al]. 2013;Chapter 7:Unit7 20. Epub 2013/01/15.
95. Johannessen CM, Reczek EE, James MF, Brems H, Legius E, Cichowski K. The NF1 tumor suppressor critically regulates TSC2 and mTOR. *Proc Natl Acad Sci U S A*. 2005;102(24):8573-8. Epub 2005/06/07.
96. Thomas L, Richards M, Mort M, Dunlop E, Cooper DN, Upadhyaya M. Assessment of the potential pathogenicity of missense mutations identified in the GTPase-activating protein (GAP)-related domain of the neurofibromatosis type-1 (NF1) gene. *Human mutation*. 2012;33(12):1687-96. Epub 2012/07/19.
97. Busaidy NL, Farooki A, Dowlati A, Perentesis JP, Dancey JE, Doyle LA, et al. Management of Metabolic Effects Associated With Anticancer Agents Targeting the PI3K-Akt-mTOR Pathway. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2012;30(23):2919-28. Epub 2012/07/11.
98. Knight ZA, Gonzalez B, Feldman ME, Zunder ER, Goldenberg DD, Williams O, et al. A pharmacological map of the PI3-K family defines a role for p110alpha in insulin signaling. *Cell*. 2006;125(4):733-47. Epub 2006/05/02.
99. Van Cutsem E, Tejpar S, Vanbekevoort D, Peeters M, Humbert Y, Gelderblom H, et al. Intrapatient Cetuximab Dose Escalation in Metastatic Colorectal Cancer According to the Grade of Early Skin Reactions: The Randomized EVEREST Study. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2012;30(23):2861-8. Epub 2012/07/04.
100. Hong DS, Bowles DW, Falchook GS, Messersmith WA, George GC, O'Bryant CL, et al. A multicenter phase I trial of PX-866, an oral irreversible phosphatidylinositol 3-kinase inhibitor, in patients with advanced solid tumors. *Clinical cancer research : an official journal of the American Association for Cancer Research*. 2012;18(15):4173-82. Epub 2012/06/14.
101. Shapiro GI, Rodon J, Bedell C, Kwak EL, Baselga J, Brana I, et al. Phase I safety, pharmacokinetic, and pharmacodynamic study of SAR245408 (XL147), an oral pan-class I PI3K inhibitor, in patients with advanced solid tumors. *Clin Cancer Res*. 2014;20(1):233-45. Epub 2013/10/30.
102. Yan Y, Wagle M-C, Punnoose E, Musib L, Budha N, Nannini N. A first-in-human trial of GDC-0068: A novel, oral, ATP-competitive Akt inhibitor, demonstrates robust suppression of the Akt pathway in surrogate and tumor tissues. . *Mol Cancer Ther*. 2011;10 (11):Supplement 1, Abstract B154.
103. Crean S, Boyd DM, Sercus B, Lahn M. Safety of multi-targeted kinase inhibitors as monotherapy treatment of cancer: a systematic review of the literature. *Current drug safety*. 2009;4(2):143-54. Epub 2009/05/16.

104. Laird A, Sillman A, Sun B, Mengistab A, Wu B, Chu F, et al., editors. Evaluation of peripheral blood cells and hair as surrogate tissues for clinical trial pharmacodynamic assessment of XL147 and XL765, inhibitors of the PI3K signaling pathway. 2008 EORTC-NCI-AACR Annual Meeting; 2008; Geneva, Switzerland.
105. Williams R, Baker AF, Ihle NT, Winkler AR, Kirkpatrick L, Powis G. The skin and hair as surrogate tissues for measuring the target effect of inhibitors of phosphoinositide-3-kinase signaling. *Cancer Chemother Pharmacol*. 2006;58(4):444-50. Epub 2006/02/18.
106. Vander Heiden MG, Cantley LC, Thompson CB. Understanding the Warburg effect: the metabolic requirements of cell proliferation. *Science (New York, NY)*. 2009;324(5930):1029-33. Epub 2009/05/23.
107. Tanaka H, Yoshida M, Tanimura H, Fujii T, Sakata K, Tachibana Y, et al. The selective class I PI3K inhibitor CH5132799 targets human cancers harboring oncogenic PIK3CA mutations. *Clinical cancer research : an official journal of the American Association for Cancer Research*. 2011;17(10):3272-81. Epub 2011/05/12.
108. Juric D, Rodon J, Gonzalez-Angulo A, Burris H, Bendell J, Berlin J, editors. BYL719, a next generation PI3K alpha specific inhibitor: Preliminary safety, PK, and efficacy results from the first-in-human study. . 2012 AACR Annual meeting 2012; Chicago, IL.
109. Vasudevan KM, Barbie DA, Davies MA, Rabinovsky R, McNear CJ, Kim JJ, et al. AKT-independent signaling downstream of oncogenic PIK3CA mutations in human cancer. *Cancer cell*. 2009;16(1):21-32. Epub 2009/07/04.
110. Edgar KA, Wallin JJ, Berry M, Lee LB, Prior WW, Sampath D, et al. Isoform-specific phosphoinositide 3-kinase inhibitors exert distinct effects in solid tumors. *Cancer Res*. 2010;70(3):1164-72.
111. Morrow CJ, Gray A, Dive C. Comparison of phosphatidylinositol-3-kinase signalling within a panel of human colorectal cancer cell lines with mutant or wild-type PIK3CA. *FEBS letters*. 2005;579(23):5123-8. Epub 2005/09/10.
112. Elkabets M, Vora S, Juric D, Morse N, Mino-Kenudson M, Muranen T, et al. mTORC1 inhibition is required for sensitivity to PI3K p110alpha inhibitors in PIK3CA-mutant breast cancer. *Sci Transl Med*. 2013;5(196):196ra99. Epub 2013/08/02.
113. Ibrahim YH, Garcia-Garcia C, Serra V, He L, Torres-Lockhart K, Prat A, et al. PI3K inhibition impairs BRCA1/2 expression and sensitizes BRCA-proficient triple-negative breast cancer to PARP inhibition. *Cancer discovery*. 2012;2(11):1036-47.
114. Carracedo A, Ma L, Teruya-Feldstein J, Rojo F, Salmena L, Alimonti A, et al. Inhibition of mTORC1 leads to MAPK pathway activation through a PI3K-dependent feedback loop in human cancer. *The Journal of clinical investigation*. 2008;118(9):3065-74. Epub 2008/08/30.
115. Chandarlapaty S, Sawai A, Scaltriti M, Rodrik-Outmezguine V, Grbovic-Huezo O, Serra V, et al. AKT inhibition relieves feedback suppression of receptor tyrosine kinase expression and activity. *Cancer cell*. 2011;19(1):58-71. Epub 2011/01/11.
116. Ilic N, Utermark T, Widlund HR, Roberts TM. PI3K-targeted therapy can be evaded by gene amplification along the MYC-eukaryotic translation initiation factor 4E (eIF4E) axis. *Proc Natl Acad Sci U S A*. 2011;108(37):E699-708. Epub 2011/08/31.
117. Liu P, Cheng H, Santiago S, Raeder M, Zhang F, Isabella A, et al. Oncogenic PIK3CA-driven mammary tumors frequently recur via PI3K pathway-dependent and PI3K pathway-independent mechanisms. *Nat Med*. 2011;17(9):1116-20. Epub 2011/08/09.
118. Muellner MK, Uras IZ, Gapp BV, Kerzendorfer C, Smida M, Lechtermann H, et al. A chemical-genetic screen reveals a mechanism of resistance to PI3K inhibitors in cancer. *Nature chemical biology*. 2011;7(11):787-93. Epub 2011/09/29.
119. Rodrik-Outmezguine VS, Chandarlapaty S, Pagano NC, Poulikakos PI, Scaltriti M, Moskate E, et al. mTOR kinase inhibition causes feedback-dependent biphasic regulation of AKT signaling. *Cancer discovery*. 2011;1(3):248-59. Epub 2011/12/06.

120. Serra V, Scaltriti M, Prudkin L, Eichhorn PJ, Ibrahim YH, Chandarlapaty S, et al. PI3K inhibition results in enhanced HER signaling and acquired ERK dependency in HER2-overexpressing breast cancer. *Oncogene*. 2011;30(22):2547-57. Epub 2011/02/01.
121. Rodon J, Juric J, Gonzalez-Angulo AM, Bendell J, Berlin J, Bootle D, et al., editors. Towards defining the genetic framework for clinical response to treatment with BYL719, a PI3Kalpha-specific inhibitor. 2013 AACR Annual meeting 2013; Chicago, IL.
122. Kumari A, Ermilov AN, Allen BL, Bradley RM, Dlugosz AA, Mistretta CM. Hedgehog pathway blockade with the cancer drug LDE225 disrupts taste organs and taste sensation. *Journal of neurophysiology*. 2015;113(3):1034-40. Epub 2014/11/14.
123. LoRusso PM, Rudin CM, Reddy JC, Tibes R, Weiss GJ, Borad MJ, et al. Phase I trial of hedgehog pathway inhibitor vismodegib (GDC-0449) in patients with refractory, locally advanced or metastatic solid tumors. *Clin Cancer Res*. 2011;17(8):2502-11.
124. Jimeno A, Weiss GJ, Miller WH, Jr., Gettinger S, Eigl BJ, Chang AL, et al. Phase I study of the Hedgehog pathway inhibitor IPI-926 in adult patients with solid tumors. *Clin Cancer Res*. 2013;19(10):2766-74. Epub 2013/04/12.
125. COSMIC Database [database on the Internet]. Available from: <http://sanger.ac.uk/genetics/GCP/cosmic/>.
126. Cho YJ, Tsherniak A, Tamayo P, Santagata S, Ligon A, Greulich H, et al. Integrative genomic analysis of medulloblastoma identifies a molecular subgroup that drives poor clinical outcome. *J Clin Oncol*. 2011;29(11):1424-30. Epub 2010/11/26.
127. Northcott PA, Korshunov A, Witt H, Hielscher T, Eberhart CG, Mack S, et al. Medulloblastoma comprises four distinct molecular variants. *J Clin Oncol*. 2011;29(11):1408-14. Epub 2010/09/09.
128. Thompson MC, Fuller C, Hogg TL, Dalton J, Finkelstein D, Lau CC, et al. Genomics identifies medulloblastoma subgroups that are enriched for specific genetic alterations. *J Clin Oncol*. 2006;24(12):1924-31. Epub 2006/03/29.
129. Robinson G, Parker M, Kranenburg TA, Lu C, Chen X, Ding L, et al. Novel mutations target distinct subgroups of medulloblastoma. *Nature*. 2012;488(7409):43-8. Epub 2012/06/23.
130. Gajjar A, Stewart CF, Ellison DW, Kaste S, Kun LE, Packer RJ, et al. Phase I study of vismodegib in children with recurrent or refractory medulloblastoma: a pediatric brain tumor consortium study. *Clin Cancer Res*. 2013;19(22):6305-12. Epub 2013/10/01.
131. Senderowicz AM, Pfaff O. Similarities and differences in the oncology drug approval process between FDA and European Union with emphasis on in vitro companion diagnostics. *Clin Cancer Res*. 2014;20(6):1445-52. Epub 2014/03/19.
132. Dienstmann R, Rodon J, Barretina J, Tabernero J. Genomic medicine frontier in human solid tumors: prospects and challenges. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2013;31(15):1874-84. Epub 2013/04/17.
133. Kool M, Jones DT, Jager N, Northcott PA, Pugh TJ, Hovestadt V, et al. Genome sequencing of SHH medulloblastoma predicts genotype-related response to smoothened inhibition. *Cancer cell*. 2014;25(3):393-405. Epub 2014/03/22.
134. Guillard S, Clarke PA, Te Poele R, Mohri Z, Bjerke L, Valenti M, et al. Molecular pharmacology of phosphatidylinositol 3-kinase inhibition in human glioma. *Cell cycle (Georgetown, Tex)*. 2009;8(3):443-53.
135. Rodon J, Dienstmann R, Serra V, Tabernero J, Whittaker SR, Theurillat JP, et al. Development of PI3K inhibitors: lessons learned from early clinical trials. *Nature reviews Clinical oncology*. 2013;10(3):143-53.
136. Buonomici S, Williams J, Morrissey M, Wang A, Guo R, Vattay A, et al. Interfering with resistance to smoothened antagonists by inhibition of the PI3K pathway in medulloblastoma. *Sci Transl Med*. 2010;2(51):51ra70. Epub 2010/10/01.

137. Dienstmann R, Rodon J, Tabernero J. Biomarker-driven patient selection for early clinical trials. *Curr Opin Oncol.* 2013;25(3):305-12. Epub 2013/03/16.
138. De Mattos-Arruda L, Rodon J. Pilot studies for personalized cancer medicine: focusing on the patient for treatment selection. *Oncologist.* 2013;18(11):1180-8. Epub 2013/10/19.
139. Dienstmann R, Rodon J, Tabernero J. Optimal design of trials to demonstrate the utility of genomically-guided therapy: Putting Precision Cancer Medicine to the test. *Molecular oncology.* 2014. Epub 2014/08/02.
140. Roychowdhury S, Iyer MK, Robinson DR, Lonigro RJ, Wu YM, Cao X, et al. Personalized oncology through integrative high-throughput sequencing: a pilot study. *Sci Transl Med.* 2011;3(111):111ra21.
141. Forbes SA, Bindal N, Bamford S, Cole C, Kok CY, Beare D, et al. COSMIC: mining complete cancer genomes in the Catalogue of Somatic Mutations in Cancer. *Nucleic Acids Res.* 2011;39(Database issue):D945-50.
142. Garcia VM, Cassier PA, de Bono J. Parallel anticancer drug development and molecular stratification to qualify predictive biomarkers: dealing with obstacles hindering progress. *Cancer discovery.* 2011;1(3):207-12. Epub 2012/05/16.
143. Gerlinger M, Horswell S, Larkin J, Rowan AJ, Salm MP, Varela I, et al. Genomic architecture and evolution of clear cell renal cell carcinomas defined by multiregion sequencing. *Nature genetics.* 2014;46(3):225-33. Epub 2014/02/04.
144. Jakobsen JN, Sorensen JB. Intratumor heterogeneity and chemotherapy-induced changes in EGFR status in non-small cell lung cancer. *Cancer Chemother Pharmacol.* 2012;69(2):289-99. Epub 2011/12/02.
145. Vakiani E, Janakiraman M, Shen R, Sinha R, Zeng Z, Shia J, et al. Comparative genomic analysis of primary versus metastatic colorectal carcinomas. *J Clin Oncol.* 2012;30(24):2956-62. Epub 2012/06/06.
146. Sun C, Wang L, Huang S, Heynen GJ, Prahallas A, Robert C, et al. Reversible and adaptive resistance to BRAF(V600E) inhibition in melanoma. *Nature.* 2014;508(7494):118-22. Epub 2014/03/29.
147. Das Thakur M, Salangsang F, Landman AS, Sellers WR, Pryer NK, Levesque MP, et al. Modelling vemurafenib resistance in melanoma reveals a strategy to forestall drug resistance. *Nature.* 2013;494(7436):251-5. Epub 2013/01/11.
148. Misale S, Yaeger R, Hobor S, Scala E, Janakiraman M, Liska D, et al. Emergence of KRAS mutations and acquired resistance to anti-EGFR therapy in colorectal cancer. *Nature.* 2012;486(7404):532-6.
149. Douillard JY, Ostoros G, Cobo M, Ciuleanu T, Cole R, McWalter G, et al. Gefitinib treatment in EGFR mutated caucasian NSCLC: circulating-free tumor DNA as a surrogate for determination of EGFR status. *J Thorac Oncol.* 2014;9(9):1345-53. Epub 2014/08/15.