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Recent advances in the pharmacological targeting of ubiquitin-regulating enzymes in cancer



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ABSTRACT

As a post-translational modification that has pivotal roles in protein degradation, ubiquitination ensures that intracellular proteins act in a precise spatial and temporal manner to regulate diversified cellular processes. Perturbation of the ubiquitin system contributes directly to the onset and progression of a wide variety of diseases, including various subtypes of cancer. This highly regulated system has been for years an active research area for drug discovery that is exemplified by several approved drugs. In this review, we will provide an update of the main breakthrough scientific discoveries that have been leading the clinical development of ubiquitin-targeting therapies in the last decade, with a special focus on E1 and E3 modulators. We will further discuss the unique challenges of identifying new potential therapeutic targets within this ubiquitous and highly complex machinery, based on available crystallographic structures, and explore chemical approaches by which these challenges might be met.

1. Introduction: aberrant ubiquitin signaling and cancer

The ubiquitin-proteasome system (UPS), which elementary components are the 26 S proteasome and the small ubiquitin molecule, is a major protein degradation system that regulates a number of cellular functions that are critical to eukaryote cell homeostasis, such as cell cycle [1], apoptosis [2], DNA damage [3] and immune functions [4]. Ubiquitination refers to the enzymatic post-translational modification (PTM) in which the ubiquitin protein is covalently attached to cellular proteins. This process takes place in three steps, ending with the ubiquitin union to lysine residues on the target protein [5]. The core enzymes regulating this process are the ubiquitin-activating enzyme (UAE or E1), the ubiquitin-conjugating enzyme (UBC or E2), and the ubiquitin ligase (E3) [6]. There are two known E1 activating enzyme genes (UBA1 (UBE1) and UBA6 (UBE6)), about 50 E2 enzymes, and more than 600 E3 ligases encoded in the human genome [7]. As E3 enzymes ultimately determine the target of ubiquitination and the specificity of substrate recognition, they mediate either proteasomal degradation or non-degradative signaling and therefore play a critical role in the functionality of the UPS and in the regulation of key intracellular players in both physiological and malignant settings [8-10].

Structurally, E3s can be divided into 'really interesting new gene' (RING) class, the 'homologous to E6-AP carboxy-terminus' (HECT) class which forms a thioester bond with ubiquitin and then conjugates it to the substrate, and the 'RING-between-RING' (RBR) class which have a RING1-in-between RING-RING2 motif [11].

Together with ubiquitin ligase activity, deubiquitination is also a complex and dynamic process in which enzymes known as deubiquitinases (DUBs) are responsible for removing ubiquitin from a substrate. In this process, the isopeptide bond between ubiquitin and its substrate can be cleaved by the specific DUBs to produce monoubiquitin for recycling [12]. More than 100 DUBs have been discovered so far, classified into five different subfamilies based on the presence of conserved catalytic domains: the ubiquitin-specific proteases (USP), ovarian tumor domain (OTU), Machado–Joseph domain (MJD), ubiquitin-C terminal hydrolases (UCH), and the Jab1/MPN (JAMM) metalloproteases [13]. Thanks to the balanced action of both ubiquitin ligases and deubiquitinases, cells are able to adapt their proteome in response to a variety of cellular and environmental factors.

Accumulating evidence indicates that cancer cells are highly

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Antitumoral activity

Protumoral activity



Fig. 1. Impact of the ubiquitin system in tumorigenesis. Insulin receptor substrate 1 (IRS-1), Phosphatidylinositol 4,5-bisphosphate (PIP2), Phosphatidylinositol (3,4,5)-trisphosphate (PIP3), Phosphoinositide 3-kinase (PI3K), Phosphatase and tensin homolog (PTEN), Protein kinase B (AKT), Tumor necrosis factor receptor (TNFR), Receptor-interacting protein kinase 1 (RIPK1), cellular inhibitor of apoptosis protein-1/2 (cIAP1/2), TNF Receptor Associated Factor 2/6 (TRAF2/6), NF-κB essential modulator (NEMO), linear ubiquitin chain assembly complex (LUBAC), nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha (IKBα), Skp1-cullin 1-F-box β-Transducin Repeat-Containing Protein (SCFβ-TrCP), X-linked inhibitor of apoptosis protein (XIAP), WW domain-containing protein 1/2 (WWP1/2), Ovarian tumor proteases domain-containing deubiquitinase with linear linkage specificity (OTULIN), Ubiquitin-specific proteases 7/10/36/37 (USP7/ 10/36/37), F-box/WD repeat-containing protein 7 (FBXW7), S-phase kinase associated protein 2 (SKP2), Cereblon (CRBN), G1 To S Phase Transition 1 (GSPT1), Ikaros (IKZF1), Aiolos (IKZF3), Tripartite Motif Containing 24 (TRIM24), Mouse double minute 2 homolog (MDM2), Mitochondrial E3 ubiquitin protein ligase 1 (MULAN), carboxy-terminus of Hsc70 interacting protein (CHIP). [Created with BioRender.com].

dependent on a functional UPS system for tumor initiation, tumor metabolism, and survival. Indeed, UPS dysregulation and the consequent aberrant activation or deactivation of signaling pathways are common hallmarks of several cancer subtypes [14]. Interestingly, dysregulated ubiquitin ligases and deubiquitinase activities and consequent augmentation (gain of function) o impairment (loss of function) activity of target proteins has been associated with the initiation and progression of multiple cancer subtypes [15].

Depending on the substrate specificity and on the affected signaling pathway, ubiquitin ligases and DUBs can act as either tumor promoters or tumor suppressors (Fig. 1). Regulators of the cell cycle are among the main factors affected by aberrancies in the UPS pathway. For instance, the cyclin-dependent kinase (CDK) inhibitor p27^{KIP1} is expressed at high levels in quiescent cells; nevertheless, its expression levels decrease considerably in tumor cells due to its proteasome-mediated degradation [16]. Together with cyclin E/A/CDK2 complexes and the E3 S-phase kinase-associated protein 2 (SKP2), p27^{KIP1} also constitutes a regulatory network engaged in a bidirectional crosstalk with the c-MYC proto-oncogene, being the stability and the role on cell cycle regulation and senescence of this latter dictated by phosphorylation and ubiquitylation [17]. SKP2 belongs to the SCF (Skp1/cullin/F-box) family, the

largest group of E3 ubiquitin ligases whose substrates are generally key players in regulating vital cellular processes such as DNA replication and cell cycle. Dysregulation of this ligase family usually leads to cancer [18]. The intracellular accumulation of c-MYC, together with the activation of the mechanistic target of rapamycin (mTOR)-dependent translation, is also regulated at the early stage of transformation by F-box/WD repeat-containing protein 7 (FBW7), another E3 ubiquitin ligase with a key tumor suppressor activity in cancer development and which mutation-associated loss of function is frequently found in malignant cells [19,20]. It is worth noting that FBW7-mediated degradation of c-MYC is also tightly dependent on the overexpression of the nucleolar DUB USP36, found in a subset of human breast and lung cancers and linked to the overexpression of the proto-oncogene [21]. Finally, c-MYC is also regulated at transcriptional level by the transcription factors Ikaros and Aiolos, found overexpressed in hyperdiploid multiple myeloma (MM), and which proteasome-dependent degradation is regulated by cereblon (CRBN), the substrate receptor of the CRL4^{CRBN} E3 ubiquitin ligase [22].

Besides cell cycle, overexpression of the tumor suppressor p53 - which protects cells from genomic insults and regulates several target genes that take part in cell-cycle control, apoptosis, senescence, and

DNA repair [23,24] - is associated with somatic mutations of TP53 gene that occur at rates from 38% to 50% in ovarian, esophageal, colorectal, head and neck, larynx, and lung cancers, and detected in about 5% of primary leukemia, sarcoma, testicular cancer, malignant melanoma, and cervical cancer [25]. These high expression levels are accompanied by deregulation of a p53-regulating E3 ubiquitin ligase, murine double minute 2 (MDM2 or HDM2 for humans), which binds to the transactivation domain of p53, impairing its transcriptional activity and mediating its transport from the nucleus to the cytoplasm for its degradation [26,27]. The DUB that counteracts MDM2-induced p53 nuclear export and degradation is USP10, a cytoplasmic ubiquitin-specific protease that is stabilized after DNA damage by an ataxia-telangiectasia mutated (ATM)-dependent process followed by its translocation to the nucleus where it activates p53 [28]. Importantly, USP10 is frequently found under-expressed in renal cell carcinoma [29]. Furthermore, tripartite motif containing 24 (TRIM24), the E3 ubiquitin ligase that negatively regulates p53 via a conserved RING domain, is depleted in human breast cancer cells where its loss favors p53-dependent apoptosis [30].

Another crucial process regulating the cell fate that can be deregulated in cancer due to UPS improper activity is apoptosis signaling. Among the antiapoptotic factors found to be accumulated due to improper degradation in malignant cells, the BCL-2 family member myeloid cell leukemia 1 (MCL-1) exerts a crucial role in promoting cancer cell survival and resistance to chemotherapy and is regulated by the E3s tripartite motif containing 17 (TRIM17), MULE and FBW7 [31–33]. The stabilization of BCL-2-like protein 11 (BIM), a pro-apoptotic member of the BCL-2 family linking stress-induced signals to the apoptotic executioners and which defective expression can be found for instance in lung cancer and B-cell lymphoma [34,35], is regulated by the UPS mediated by MAPK/ERK signaling [36].

A crucial regulator of malignant cell survival and tumor progression is protein kinase B, (PKB) also known as AKT, which stability and activity are negatively regulated by the mitochondrial E3 MULAN (MUL1/ GIDE/MAPL) and by the DUB cylindromatosis (CYLD), respectively [37]. While MULAN expression is lost in head and neck cancer [38], CYLD deficiency has been shown to promote cancer cell proliferation, survival, glucose uptake, and tumoral growth in mice models of prostate tumor [39]. AKT signaling is also affected by the intracellular accumulation of the ubiquitin ligase X-linked inhibitor of apoptosis (XIAP/-BIRC4) which ubiquitinates the main negative regulator of the AKT pathway, i.e. phosphatase and tensin homolog deleted on chromosome 10 (PTEN), directing this latter to degradation and modulating the sensitivity of ovarian cancer cell to platinum [40]. In chronic lymphocytic leukemia (CLL), the DUB USP7 controls the nuclear pool of PTEN, both in TP53-wild type and -null environment, and, in this model, PTEN acts as the main tumor suppressive mediator along the USP7-PTEN axis in a p53-dispensable manner [41]. USP7 is also found overexpressed in prostate cancer, where it is associated with PTEN nuclear exclusion [42].

The linear ubiquitin chain assembly complex (LUBAC) is composed by Heme-oxidized IRP2 ubiquitin ligase-1 L (HOIL-1 L), HOIL-1 Linteracting protein (HOIP), and Shank-associated RH domain interactor (SHARPIN), being the function of HOIP controlled by the DUBS OTULIN and CYLD. While the dysregulation of the LUBAC-mediated linear ubiquitination pathway has been linked to the development of cancer, the ubiquitination of NF- κ B-essential modulator (NEMO) by this system is critical for genotoxic NF- κ B activation and for the protection of tumor cells from DNA damage-induced cell death [43,44].

Tumor necrosis factor alpha (TNF α) is an inflammatory cytokine that plays significant roles in both immunity and cancer [45] and which signaling mediated by different cell death complexes can be restricted by ubiquitin hydrolases or DUBs, namely A20/TNFAIP3, CYLD, and OTU deubiquitinase with linear linkage specificity (Otulin) [46,47] (Fig. 1).

2. Therapeutic targeting of the ubiquitin machinery in cancer patients

Data obtained during the last decades on the regulation and functions of the ubiquitin system have accelerated dramatically our knowledge of the ubiquitin code. Given the multiple alterations regarding the expression and/or activity of several ubiquitin-regulating enzymes in numerous malignancies, these latter have attracted extreme attention for the treatment of cancer in the last decades and increasing number of inhibitors targeting specific components of the UPS are continuously being studied and developed [48]. Beside proteasome inhibitors, which have already shown remarkable usefulness in the clinical management of different subtypes of cancers, especially lymphoid neoplasms [49], several inhibitors targeting either E3 ligases or DUBS are currently at different development stages in clinical studies.

2.1. Development of E3 ligase and deubiquitinase small molecule inhibitors

Deregulation of E3 enzymes is frequently associated with poor prognosis in different cancer subtypes, conferring a logical interest in their therapeutic targeting. A vivid example of this interest is exemplified with the preclinical development of MDM2 inhibitors as a novel approach to cancer therapy [50], although this process has been relatively slow. Indeed, since the publication of MDM2 crystal structure in 1996, multiple molecules able to interrupt p53-MDM2 binding have been described, but it was only in 2004 that the cis-imidazoline analogues known as Nutlins (Nutlin-1, -2, and -3) showed selectivity against p53 and potent anti-cancer activity [51-53]. Among these molecules, Nutlin-3 showed a potent antitumoral activity in mouse xenograft models of human cancer, retaining the wild-type (wt) p53 [51]. In addition, it has been shown in vitro that Nutlin-3 disrupts the interaction between MDM2 and the p53 homologue p73 and activates the transcription factor E2F-1, together with the hypoxia-inducible factor-1 α (HIF-1 α), therefore producing consistent p53-independent effects, although at higher doses [54–56].

Supporting the notion that toxicity of conventional chemotherapeutics can be reduced by means of non-genotoxic agents able to activate p53, *ex vivo* experiments in patient samples retaining wt p53 show that Nutlin-3 synergizes with doxorubicin and 1- β -Darabinofuranosylcytosine (Ara-C) in acute myeloid leukemia (AML) and doxorubicin, chlorambucil, and fludarabine in CLL, amongst others [57].

These encouraging results with Nutlins paved the way to the development of new small molecule MDM2 inhibitors such as MI-219, which was shown to disrupt p53-MDM2 binding, leading to p53 activation and suppression of tumour cell growth in both in vitro and in vivo models of lung cancer [58]. Administration of the compound activated a p53-dependent response in tumor tissue and in cell lines as indicated by p53 accumulation, and led to a strong antitumoral activity in xenograft models of human cancer with wt p53, without evidence of toxicity [59, 60].

CGM097 is a substituted dihydroisoquinolinone derivative designed to mimic crucial hydrophobic interactions of p53 within the MDM2 pocket at Phe19, Trp23, and Leu26 [61]. *In vitro*, CGM097 is able to block the proliferation of primary AML blasts and AML cell lines with wt p53, to reduce the growth of AML xenografted tumors, and to improve the survival in different mouse models of AML patient-derived xenografts [62,63].

Milademetan is a dispiropyrrolidine-based inhibitor able to reactivate p53 signaling in cancer cells with wt p53, and with remarkable antitumor effect in in vitro and in vivo models of AML, B-cell non-Hodgkin lymphoma (B-NHL), and other non-hematological cancers [64,65].

APG-115 is a spirooxindole-derived inhibitor with improved stability when compared to the HDM2 blocking agent SAR405838, and with demonstrated capacity to block the proliferation of AML malignant cells

Table 1

E1, E3, or DUB modulators currently under clinical development.

Structure	Compound	Mechanism of Action	Clinical trial stage	NCT number	References
E1 modulators					
F ₃ C-S	TAK-243	UAE inhibition by Ub-TAK-243 complex formation	Phase 1 in AML, CML, MS	NCT03816319	[88,89]
$ \begin{array}{c} $	CC-122 (Avadomide)	Increases CRL4 ^{CRBN} binding to IKZF1/3	Phase 1/2 in NHL	NCT03310619	[90]
	CGM097	MDM2 inhibitor	Phase 1 in solid tumors	NCT01760525	[91]
	CC-220 (Iberdomide)	Increases CRL4 ^{CRBN} binding to IKZF1/3	Phase 1/2 in MM	NCT04564703	[92]
OF OH	APG-115	MDM2 inhibitor	Phase 1/2 AML and CML	NCT04358393	[93]
Q O Br	Tasisulam	degradation of RBM39	Phase 3 in melanoma	NCT01006252	[94]
	Indisulam	degradation of RBM39	Phase 2 in colorectal cancer	NCT00165867	[95]
	NSC-339004	degradation of RBM39	Phase 2 in lung cancer	NCT00008372	[96]
	DS-3032 (Milademetan)	MDM2 inhibitor	Phase 1 in AML	NCT03671564	[97]
DUB modulators	VLX1570	USP14 > UCH37 inhibitor	Phase 1/2 in MM	NCT02372240	[98]
N+ 0- 0″					

and to impair the spread of AML disease in mice [66].

Regarding the SCF family, since the F-box determines the specificity of these complexes, most small molecule inhibitors have been developed against the corresponding residues. Oridonin, a diterpenoid isolated from *Rabdosia rubescens* [67], promotes the deubiquitination and degradation of c-MYC and triggers apoptosis in leukemia and lymphoma cells [68]. Other molecules like ZL25 and compound A directly target SKP2, resulting first in cellular senescence and lastly in p27^{KIP1}-mediated cell death and cell cycle arrest [67,69]. Erioflorin is another natural compound that stabilizes the tumor suppressor programmed cell death 4 (PDCD4) by blocking its degradation, therefore repressing NF-κB pathway activity, and reducing the proliferation rate of cancer cells [70]. GS143 is a β-TrCP1 ligase inhibitor which also blocks NF-kB signaling by inhibiting the ubiquitination and proteasomal degradation of the intracellular inhibitor of NF-κB (IκBα) [71].

Among the different DUBs that are being deeply evaluated as possible targets for anticancer therapy, counteracting the activity of USP9X, USP14, UCH37, and USP5 using the inhibitor WP1130 has been shown to trigger apoptosis by promoting p53-dependent signalling and by deregulating the anti-apoptotic MCL-1 [19]. P53 may also be stabilized in vitro by the small molecule inhibitor HBX41108 [72], which impairs USP7-mediated deubiquitination, thus promoting the degradation of the p53 negative regulator, MDM2. In parallel, the selective USP7 inhibitor P5091 not only evokes apoptosis but can also overcome bortezomib resistance in in vitro models of MM and exert a synergistic effect in vivo when co-administered with the glucocorticoid dexamethasone and/or the immunomodulatory drug (IMiD) lenalidomide [73]. Another interesting player in the stability of p53 is OTU Domain-containing ubiquitin aldehyde-binding protein 1 (OTUB1) which, similarly to USP7, stabilizes p53 both in vitro and in vivo by non-canonical inhibition of MDM2-mediated ubiquitination [74]. Specifically, OTUB1 suppresses UBCH5, the E2 partner of MDM2. The understanding of OTUB1 function in the regulation of p53 activity, has opened the door to the study of possible small molecules that disrupt the interaction between MDM2 and UBCH5, inhibiting both its p53-dependent and p53-independent oncogenic properties [75].

2.2. Clinical activity of E1-targeting drugs and E3 modulators

2.2.1. UBA1 inhibitors

Prior to the recent elucidation of UBA1 crystal structure, different strategies were carried out to modulate the activity of the E1 enzyme, thanks to the close similarity between human UBA1 and related proteins [76–78]. Among the first modulators described, the adenosine sulfamate and first-in-class UBA1 inhibitor, TAK-243 (MLN7243), is the only drug that has entered into clinical evaluation (Table 1) [77]. This compound, designed on the structural basis of the NEDD8-activtating enzyme (NAE) inhibitor pevonedistat [79], is active against UBA1, and in lesser extent against UBA6 and NAE [77]. In vitro, TAK-243 treatment leads to reduce levels of mono- and poly-ubiquitylated proteins and to the accumulation of several ubiquitin-regulated proteins, including p53, c-JUN, c-MYC, MCL-1, and XIAP, and to apoptotic cell death consequent to DNA damage and cell cycle arrest at G1 and/or G2/M phase [77,80-83]. Another important mechanism involved in the antitumor effect of TAK-243 in different disease models is the induction of proteotoxic stress at the level of the endoplasmic reticulum (ER), leading to activation of the unfolded protein response (UPR) [77,80,81,83,84]. The pleiotropic effects of TAK-243 towards multiple signaling pathways beside cell cycle and ER stress [80], supported its efficacy and selectivity in a wide range of solid tumors including cutaneous squamous cell carcinoma, small-cell lung cancer, pancreatic cancer, and glioblastoma [77,83-86], but also in several life-threatening hematological conditions, like AML, MM, CLL and a set of indolent or aggressive B-cell lymphoma [77,80-82,87].

Based on these preclinical data and considering the favorable toxicological profile of the molecule, TAK-243 entered into clinical evaluation with patients with either advanced solid or hematological tumors (Table 1). A first phase 1, open-label dose escalation study (NCT02045095) established the maximum tolerated dose (MTD) in 29 patients with solid cancers. Although the efficacy of the compound was demonstrated histologically by the detection of decreased ploy-ubiquitinated chains, this trial highlighted serious advert events (AEs) in > 30% of the patients. A subsequent phase 2 study is currently enrolling patients undergoing relapsed or refractory (R/R) AML, myelodysplastic syndrome (MDS), or chronic myelomonocytic leukemia (CMML) (NCT03816319). Beside UBA1 expression and ubiquitylation status, the development of biomarkers that may allow monitoring patient response to TAK-243 in these clinical trials, is still necessary.

2.2.2. Cereblon (CRBN)- targeting agents

The Cullin 4-RING ligase (CRL4) complexes are known to exert a significant role in tumorigenesis through their roles in the control of DNA damage response and DNA repair. CRBN is a substrate recognition subunit of CRL4 that represents a great therapeutic potential, especially in hematological cancers. IMiD agents, that encompass the antiemetic agent thalidomide and its derivative lenalidomide and pomalidomide, are being clinically evaluated and/or approved for the treatment of different hematological malignancies in the relapse setting. CRBN was identified in 2004 as primary target of thalidomide and as the mediator of the reported teratogenic activity of the drug [99]. However, this discovery also shed light onto the mechanism of action of IMiDs in MM patients, in which the proteasome-mediated degradation of the transcription factors Ikaros and Aiolos with a crucial role in the determining of B-cell fate, has been shown to depend on CRBN E3 ligase activity [100–102]. IMiD binding at a conserved pocket near the C-terminus of CRL4^{CRBN}-mediated CRBN polyubiquitination triggers and proteasome-dependent degradation of its substrates, in a process termed "molecular glue" [102]. In MM, IMiD-induced degradation of Ikaros and Aiolos leads to interferon regulatory factor 4 (IRF4) downregulation and interleukin-2 (IL-2) upregulation, followed by apoptotic cell death [101, 103]. In both preclinical and clinical settings, IMiDs are being used successfully in combination with proteasome inhibitors, steroids, and monoclonal antibodies as anti-myeloma therapy, and as single agents in other hematological malignancies, including MDS with deletion of chromosome 5q, CLL, and mantle cell lymphoma (MCL) [104]. A novel set of thalidomide analogs has been developed recently by Celgene Corp. which includes the cereblon E3 ligase modulators (CELMoDs) CC-122 (avadomide), CC-220 (iberdomide), and CC-885, all being evaluated in active clinical trials. Thanks to the presence of a conserved glutarimide required for CRBN binding in its structure, CC-122 contains exerts pleiotropic immunomodulatory and antitumor activities [105,106]. Mechanistically, CC-122 binds CRL4^{CRBN} E3 ligase to induce the degradation of Ikaros and Aiolos in MM cells, de-repression of interferon (IFN)-regulated genes, and co-stimulation of T cells followed by apoptotic cell death in in vitro and in vivo models of diffuse large B-cell lymphoma (DLBCL) [106,107]. Following these results, CC-122 recently entered into clinical trials with patients suffering from B-NHL, MM, and CLL (Table 1). In a dose-escalation phase 1 clinical trial (NCT01421524) aimed at evaluating the safety, tolerability, pharmacokinetics and preliminary efficacy of CC-122 in patients with relapsed cancers, including MM, B-NHL and advanced solid tumors, CC-122 monotherapy showed an acceptable safety profile with a maximum tolerated dose (MTD) of 3.0 mg and with manageable AEs reported after a median duration of treatment of 58 days (44% fatigue, 29% neutropenia, 15% diarrhea) [46]. Authors reported one complete response (CR) and two partial response (PR) in B-NHL patients, being the compound able to evoke the degradation of Aiolos in circulating B and T cells and to reduce the number of B cells in the peripheral blood of the patients [104].

CC-220 is another thalidomide derivative developed, among others, for the treatment of relapsed/refractory MM, and with enhanced capacity to trigger CRBN-mediated degradation of Aiolos [108]. In a dose-escalation phase 1 study (NCT01733875), no severe AEs were

attributed to CC-220 when administrated daily to healthy volunteers. The compound was generally well tolerated and was able to evoke the degradation of both Ikaros and Aiolos in peripheral blood mononuclear cells (PBMCs) [49]. Following preclinical reports of CC-220 synergistic interaction with the proteasome inhibitor bortezomib with or without the glucocorticoid dexamethasone regarding Ikaros and Aiolos degradation and anti-myeloma activity, and of efficacy of CC-220/anti-CD38 (daratumumab) combination in vitro [109], a subsequent dose-escalation phase 1b/2a study was launched with relapsed or refractory (R/R) MM patients to evaluate the efficacy and safety of the CELMoD when administered either as single agent or in combination with dexamethasone, with or without daratumumab or bortezomib therapies (NCT02773030). Preliminary results showed an overall response rate (ORR) of 29% in these heavily pretreated patients, with no MTD reached. Main AEs in patients receiving CC-220 and dexamethasone combination were manageable and included grade 3-4 neutropenia (29%), infections (25%), and thrombocytopenia (12%) [110] (Table 1).

2.2.3. MDM2 antagonists

In hematologic malignancies in which p53 is predominantly expressed in its wt form, such as AML, the pharmacological targeting of MDM2 has become particularly attractive.

The Nutlin derivative RG7112 [111] was the first MDM2-P53 disruptor to enter into the clinic, where it efficiently led to p53 stabilization and to the upregulation of some apoptosis and cell cycle regulators (BAX, PUMA, FAS, TNFRSF10B, CDKN1A) in cells from R/R AML and CLL patients [111]. In AML patients, this agent was active either as single agent or in combination with cytarabine, allowing some cases to reach a CR [111,112]. However, given the frequency of hematological AEs (neutropenia, thrombocytopenia and hemorrhage) and gastrointestinal toxicity following RG7112 administration, a more potent and selective Nutlins derivative, i.e. the pyrrolidine RG7388 (RO5503781, idasanutlin), was developed [52]. In preclinical models, this second-generation MDM2 inhibitor harbored similar activity as its precursor, but at doses that were significantly lower [52]. In clinical settings, this compound was well tolerated and showed significant activity with manageable toxicity in R/R AML patients [113]. Different RG7388-based combinations are being evaluated clinically. Among these, a phase 1b/2 trial (NCT02670044) is currently investigating the safety, tolerability, and preliminary efficacy of idasanutlin in combination with the BCL-2 antagonist venetoclax in patients with R/R AML who are not eligible for chemotherapy. Another ongoing phase 1b/2 trial (NCT03850535) is evaluating the safety, efficacy, and pharmacological properties of RG7388 when given alongside cytarabine and daunorubicin to newly diagnosed AML patients.

CGM097 has been evaluated in monotherapy in a first-in-human dose-escalation phase 1 study (NCT01760525) in patients with advanced solid cancers and with wt *TP53* gene (Table 1). Results were disappointing with relatively limited activity and notable AEs including grade 3–4 thrombocytopenia and/or neutropenia [114].

The DS3032b compound is being tested clinically since 2013 and is now under evaluation in patients with advanced solid tumors or lymphomas, or with other hematological cancers like AML, MDS, MM, acute lymphoblastic leukemia (ALL) and chronic myelogenous leukemia (CML) (NCT02319369, NCT01877382, NCT02579824, Table 1). First reports from these trials highlighted a general lack of efficacy of DS3032b although 77% of patients had stable disease, with main hematological AEs being thrombocytopenia and neutropenia [115]. In the subgroup of patients with R/R AML or high-risk MDS, a decrease in bone marrow infiltration by tumoral blasts was detected in 15/38 cases and a CR was observed in two AML patients, although a TP53-mutated clone appeared alongside the treatment in these two cases [116].

In two phase 1 studies (NCT02319369 and NCT01877382), milademetan showed promising activity in patients with R/R AML, high-risk MDS, or with solid tumors or lymphomas, with 3 patients presenting a CR. Although almost all the cases presented grade 3–4 AEs including

Table 2

MDM2 ligands reported in literature grouped by scaffold (*) PDB identification code is used for those ligands without any specific name.

Chemical scaffold	Compound	PDBID	PubChem CID	References
pyrrolidine	RO5313109	4JRG	45139212	[52]
I J	RO5316533	4JSC	59249286	[52]
imidazoline	Nutlin-2	1RV1	5288631	[51]
	IMY*	1TTV	49867154	[123]
	WK-298	3LBJ	24969086	[124]
	WK-23 DI E*	3LBK	44825260	[124]
	BG-7112	4DIJ 4IPF	57406853	[125]
	Nutlin-3a	4HG7	11433190	[127]
	Nutlin-3a	4J3E	11433190	[128]
	RO0503918	4J74	71816470	[129]
	RO5045331	4J7D	58894234	[129]
	RO5524529	4J7E	71763144	[129]
	NUT*	5Z02	11433190	NA
imidazolo	NU1^ 2V8 *	52AF 4003	50006417	NA [120]
IIIIuazoie	2V8 6GG*	40Q3 517F	126963310	[130]
	6GG*	5J7G	126963310	[131]
	HOW*	6129	71155423	NA
	HTZ*	6Q9L	138320068	[132]
	HU8 *	6Q9O	67032088	[132]
3-pyrroline-2-one	4NJ*	4ZFI	122177104	[133]
furanone	4NX*	4ZGK	122177105	[133]
nydantoin	RO-2443	3VBG	130083437	[134]
	13Q 0R2 *	4ERE	56591324	[136]
	AM-8553	4ERF	56965957	[136]
	0Y7 *	4HBM	56591282	[135]
	2SW*	40AS	68000593	[137]
piperidinone	2U0 *	40DE	73386675	[138]
	2U5 *	40GN	73386677	[138]
	35 S*	4Q04	58573999	[139]
	351* AM 7200	4QOC	67999919 77109199	[140]
morpholinone	1MN*	4W12 4IV7	71305070	[141]
morphonnone	1MO*	4JV9	71305071	[142]
	1MQ*	4JVE	137347970	[142]
	1MY*	4JWR	71305074	[142]
	2TW*	40BA	71544420	[143]
	2TZ*	40CC	73386674	[138]
	201 *	40DF	73386676	[138]
	206 ^	40G1 40GV	73386670	[138]
dihydroisoquinolinone	NVP-	400 V 47.YF	53240420	[130]
uniyuroisoqumonnone	CGM097	1211	332 10 120	[01]
	NVP-	4ZYI	58437867	[61]
	CGM097			
	4SS*	4ZYC	91820721	[144]
isoindolinone	TUZ*	7BIR	135282535	[145]
	TV5 *	7BIT	135282593	[145]
	IUW^ TVH*	7BIV 7BIO	135282862	[145]
	TVK*	7BJ6	155883011	[145]
	U3Z*	7BMG	135281739	[145]
indolinone	RO5027344	4LWT	117071888	[146]
imidazolopyrrolidinone	6ZT*	5LN2	71565460	[147]
	Siremadlin	5OC8	71678098	[148]
	EYH*	6GGN	71678945	[149]
dihudroimidozothiozolo	HRE*	6Q96 2W7V	89601515	[132]
amyaroimiaazotniazoie	VZV" IT7*	3VZV 3W60	58007243	[150]
benzodiazepinedione	DIZ*	1T4F	656933	[152]
Spiro-oxindole	RO5353	4LWV	56649312	[153]
·	RO8994	4LWU	53236857	[146]
	MI-63-	3LBL	49867311	[124]
	analog			
	1MT*	4JVR	71305073	[142]
	6SK*	5LAV	122198471	[154]
	05J" 6SS*	5LAW	110439041	[154]
	BI-0252	5J.AZ	118439587	[154]
	SAR405838	5TRF	53476877	[155]
	H28 *	6I3S	137349477	[156]



nausea, diarrhea, vomiting, fatigue, anemia, thrombocytopenia, neutropenia, hypotension, hypokalemia, and hypomagnesemia, the safety profile was considered acceptable [116–118]. Importantly, among 79 patients evaluated with liposarcoma, solid tumors, and lymphomas, 47 of them (60%) presented a stable disease [117]. Following these encouraging trials, milademetan remains currently under clinical evaluation in different cancer subtypes.

First results of a phase 1 clinical trial testing APG-115 in patients with solid tumors (NCT02935907) showed that dose-limiting toxicities (DTLs), including essentially thrombocytopenia and fatigue, but also decreased neutrophil and white blood cell counts among others, were detected [119]. No information about drug efficacy is available so far.

2.3. Clinical activity of the DUB modulator VLX1570

VLX1570 is a competitive inhibitor of proteasome DUB activity, and an analogue of the previously described compound b-AP15, that shows selective inhibitory activity for USP14 with an $\rm IC_{50}$ in the low

micromolar range of concentrations [120]. Its enhanced activity against DUB activity in MM cells over other tumor types prompted its evaluation in patients with R/R MM. In a phase 1 study aimed at characterizing its safety, tolerability, and pharmacokinetic profile (NCT02372240), a total of 15 patients having relapsed after proteasome inhibitor and IMiD therapies received either a low dose (0.05–0.60 mg/kg) or a high dose (1.2–2.0 mg/kg) of the compound. In the low-dose groups, although only minimal AEs were observed, including grade 1–2 fatigue, rash, nausea, and anemia, and DLTs were not reported, half of the patients were withdrawn from the study due to progressive disease. In the high-dose group, patients developed fatal pulmonary toxicity within days of receiving two doses of the compound. These disappointing results led to premature study termination [121].

3. Inhibitors in the pipeline

As stated previously, four proteins belonging to the UPS, i.e. HOIP, MDM2, USP7, and USP14, have been identified as promising targets for

cancer therapy and they could attract a widespread interest in the near future. Having information related to the structure and the binding mechanism for these proteins is crucial to make possible the design of new inhibitors by applying structure-based drug design techniques. The Protein Data Bank (PDB, https://www.rcsb.org/) is the main worldwide repository of 3D structures of macromolecules including proteins, nucleic acids, and their complexes. Over the past few years, the number of crystal structures involving the aforementioned proteins, including complexes with small molecules, has grown dramatically. Having available structural information allowed the application of structurebased methods for designing new inhibitors [122].

3.1. Structure-based design of E3 ubiquitin-protein ligase inhibitors

3.1.1. Identification of new potent MDM2-interacting drugs

The huge number of entries in the PDB (more than 110 3D structures are available) demonstrates the interest of the scientific community in the MDM2 protein. Interestingly, from the chemical point of view, small molecules complexed to MDM2 involve a high variety of chemical scaffolds, being most of them heterocyclic compounds (Table 2). The molecular name reported in the original reference has been used for those molecules without a standard name.

The binding site of MDM2/HDM2 is mostly hydrophobic [157], and most of the MDM2 inhibitors have been shown to mimic the three key p53 residues Phe19, Trp23, and Leu26, which are part of an α -helix of that protein. Different chemical families have been assessed for the inhibition of the MDM2-p53 by targeting the aforementioned interactions.

The molecular design has played a pivotal role during the development of these candidates. Structure-based drug design (SBDD) techniques, mainly represented by docking and molecular dynamics simulations, have been widely applied not only for gaining insights into MDM2 structural features [158] but also to guide the hit to lead process. The benzodiazepinedione (BDP) family was described for the first time by Grasberger et al. as antagonists of HDM2-p53 interaction [152]. The crystal structure of the BDP-HDM2 complex (PDBID: 1T4F, 1T4E) revealed that this family of compounds is able to interact with the p53 binding site, by reproducing the interaction pattern of this latter (recognizing the Phe19, Trp23, and Leu26 residues) and by mimicking its α -helix structure. Thanks to the analysis of the binding site available in the PDB, authors were able to enhance the pharmacokinetic properties of compound 1, leading to the more potent and orally bioavailable compound 19 [159]. More recently, SBDD was used in the discovery of AM-8553, using molecular docking to predict the binding affinities of piperidinone derivatives [136].

Structure-activity relationship (SAR) analysis has been applied on known MDM2 inhibitors to design more potent derivatives with better drug-like properties. In the case of Nutlins, the application of scaffold hopping approach led to the identification of dihydroimidazothiazole derivatives as potent MDM2 inhibitors [150]. AMG-232, which is currently in clinical phase 2, was identified as a result of a SAR analysis applied on the AM-8553 inhibitor previously described [137].

The identification of the spiro-oxindole SAR405838 was based on the optimization of a previously studied derivatives, designed using a structure-based approach to target the three key residues involved in p53 interaction [160]. This small molecule has been advanced to phase 1 clinical trial and has a K_i of 0.88 nM. The co-crystal structure of the SAR405838-MDM2 complex showed not only the direct interaction of the three key amino acid residues of p53 but also additional interactions not observed on the p53-MDM2 complex, e.g. Val14 and Thr16, which induce the refolding of the short MDM2 N-terminal region. Altogether, these characteristics give to SAR405838 a high affinity and specificity character for the inhibition of MDM2. Durable tumor regression or complete tumor growth inhibitor is observed when using SAR405838 in RS4, acute leukemia, mouse xenograft models of osteosarcoma, colon cancer, and prostate cancer [155].

Inspired by Wang's work, Gollner et al. designed spiro[3H-indole-

Table 3

List of crystallized HOIP structures complexed with a small molecule available in the Protein Data Bank grouped according to their chemical scaffold.

Core	Ligand	PDBID	PubChem CID	References
NH_O	compound $5_{\mathbf{a}}$	6GZY	137349349	[164]
∧ .NH ∠O	Ligand 3	6SC7	145946065	[165]
	Ligand 4	6SC8	145946064	
	Ligand 2	6SC5	145946063	
	HOIPIN-8	6KC6	146035892	[166]
		6SC9	146018040	[165]
ОН	HOIPIN-1	6KC5	146035891	[166]

Table 4

List of crystallized USP7 structures complexed with a small molecule available in the Protein Data Bank grouped according to their chemical scaffold.

Chemical scaffold	Ligand	PDBID	PubChem CID	References
	compound 7	6VN5	146026046	[167]
N= O_N	compound 2	5WHC	135567363	[168]
۰ آ	L55	6M1K	155804189	[169]
Ĭ	FT827	5NGF	137348762	[170]
	FT671	5NGE	121457027	[170]
NH NH	compound 5	5N9T	131750088	[171]
NHo	GNE6776	5UQX	122531750	[172]
1 2	GNE6640	5UQV	122531786	[172]
N				
0	compound 46	6F5H	132990905	[173]
	compound 1_b	6VN4	146026045	[167]
NH	compound 16	5N9R	131750081	[171]
∕∕_S	compound 14	6VN6	146026047	[167]
	compound 23	6VN3	138610287	
L N	compound 18	6VN2	146672986	
0	XL188	5VS6	131953451	[174]
	9HS*	5VSK	131953452	
NH	compound 1_a	5VSB	71278663	

3,2'-pyrrolidin] – 2(1H)-one derivatives and applied structure-based leading to BI-0252 inhibitor. Molecular docking was applied to predict and rank the binding affinity of candidates [154].

The isoindolinone scaffold was identified as MDM2 inhibitors and explored using SAR studies, showing a characteristic binding mode in which isonindolinone scaffold is placed abutting Phe19 and interacts with Val93 and Tyr67 [161]. To optimize this kind of structures, authors made use of quantum mechanics and ligand-based drug design to finally identify compound 14 with an IC50 = 4 nM [145] (Fig. 2).

3.1.2. Design of novel HOIP antagonists

HOIP sequence is composed of three main domains: zing finger (ZF) domains, NPI4-type zinc finger 1 (NZF1), and NPI4-type zinc finger 2 (NFZ2). NZF1 domain acts as a ubiquitin-binding site able to interact

Table 5

List of crystallized USP14 structures complexed with a small molecule available in the Protein Data Bank.

Chemical Scaffold	Ligand	PDBID	PubChem CID	Reference
NH	IU1 IU1–248 A8L IU1–47	6IIK 6IIN 6IIM 6IIL	675434 135393505 34134404 675477	[175]

with NEMO and Lys63 ubiquitin chains. These interactions are necessary for the recruitment of LUBAC in different signaling pathways.

HOIP RBR E3 ligase located at the C-terminal of HOIP is formed by a RING1, IBR, and RING2 core. RING1 is the one in charge of recruiting the E2-Ub complex. IBR domain contains an allosteric ubiquitin-binding site that can promote the binding of the Ub-E2 complex (allosteric activation). The RING2 domain contains the catalytic cysteine (Cys885), responsible for the covalent linkage between LUBAC and the donor ubiquitin. Close to this catalytic cysteine, His887 (also called catalytic histidine) plays a vital role in the final aminolysis reaction [162–164].

The elongated conformation of the donor ubiquitin is accomplished by hydrophobic interactions of RING2 (HOIP) with the Ile36 patch and the C-terminus (Ub).

Interactions between RING2-LDD and the acceptor ubiquitin brings the nucleophilic amine from the Met1 residue closer to the catalytic cysteine (at distance 3.5 Å) [162] linked to the donor ubiquitin by its Gly76 residue via a thioester bond, allowing the aminolysis reaction.

Interestingly, all ligands reported in the PDB (Table 3) are nonreversible inhibitors that target Cys885. Most of them are located in the same region, establishing a secondary interaction with His889 via Hbond, and with Phe888 in most of the cases in presence of arene-arene interactions. Only molecule L68 (HOIPIN-8, the ligand included in the PDBID 6SC9) is located in a different region.

The crystal structure of the RING2-LDD domain of HOIP in complex with a covalent inhibitor (compound 5_a) was firstly described by Johansson et al. in 2019 [164]. The cyclopentyl pyridone scaffold interacts through H-bond with the His889 backbone. The ester chain of 5_a lay within an orthogonal pocket to the ledge and interacted with the hydroxyl side chain of Ser899, this interaction led to a conformation of the small molecule where the Michael acceptor of 5_a approached the catalytic cysteine Cys885 and promoted enough residence time to form a covalent bond. One year after, the same group described the interaction mechanism of tetrahydroquinolinone derivatives [164]. These compounds interact with HOIP protein, establishing H-bonds with His889 and His887 [165], and aromatic interactions with Phe888. Their studies led to the design of HOIPIN-1 and - 8 ligands [166]. Interestingly, HOIPIN-8 filled a pocket on the opposite surface of Cys885. It had different interactions with HOIP, such as a salt bridge between the ligand carboxylate and Arg935 and hydrogen bonds between the ligand pyrazole group and Asp936 [165] (Fig. 3).

3.2. Uncovering new DUB modulators

3.2.1. Discovery of USP7-blocking agents

USP7 is structured in seven domains, the N-terminal TRAF-like (TNF receptor-associated factor) domain, the catalytic core domain, and the five C-terminal ubiquitin-like domains, UBL1–5. Residues 208–882 of USP7 contain the catalytic domain (CD) and the first three Ubl domains. USP7 catalytic domain comprises residues 208–560, located between the TRAF-like and UBL domains. The catalytic site is formed by a triad of Cys223, His464, and Asp481 residues. These residues are located in a cleft between two regions known as Thumb and Palm. The Finger region is the one in charge of binding to ubiquitin. The main role of His464, is to deprotonate the thiol group of the Cys223 and initiate the nucleophilic



Fig. 4. Binding cavity described for co-crystal structures available in the PDB (green).



HOIPIN-1



HOIPIN-8



compound 5_a

Fig. 3. Molecular structure of compounds described as HOIP inhibitors in the PDB.



Fig. 5. Molecular representation of USP7 inhibitors reported in the PDB.

attack of the final isopeptide bond. The role of the Asp481 residue is the restriction of the side-chain rotation of the His464. The active site conformation of the isolated USP7 catalytic domain is different from the ones observed in the available structures of other USPs. To an isolated USP7, the USP7 catalytic triad is found in an unproductive conformation. Ubiquitination of USP7 leads to the activation of the enzyme [176].

Most of the described USP7 ligands in the PDB correspond to reversible inhibitors except for FT827, which acts as an irreversible inhibitor targeting the catalytic Cys223 (Table 4). According to the information available in the PDB, most of them are placed in the same region, establishing interactions with Gln297, Phe409, and Val296. Molecules included in 5UQX, 5WHC, and 5UQV are located in a different region (Fig. 4).

After the three-dimensional structures for USP7 became available, SBDD approaches were rapidly applied to address the design of new USP7 inhibitors using molecular docking, molecular dynamics simulations [177], or 3D pharmacophore models [178]. Covalent docking was applied for predicting the binding mechanism of irreversible inhibitors, e.g. for the most specific USP7 inhibitors available P22077 and P50429 [179].

Several covalent and non-covalent inhibitors of USP7 have been designed and synthesized in the early last few years. More in detail, the first two crystal structure USP7-small molecule found in the literature are from year 2017. Covalent inhibitor FT827 and a non-covalent inhibitor FT671 were identified in an ubiquitin-rhodamine screening assay of approximately 500.000 compounds available at FORMA

Therapeutics. Their pyrazolo[3,4-*d*] pyrimidine-4-one-piperidine scaffold allows them to interact with the USP7 thumb-palm domain, which guides the ubiquitin C terminus into the active site of USP7, specifically with Asp295, Val296, and Gln297 of the thumb subdomain and Phe409 and Tyr465 of the palm subdomain. In the case of FT671, the parafluorophenyl group is extended towards the fingers subdomain and the 3-fluoropyrazole group points towards the catalytic center abutting the catalytic Cys223. For its part, FT827 covalently binds to catalytic Cys223 through its vinylsulfonamide moiety. Both compounds selectively inhibit USP7 in a panel of 38 DUBs from diverse families [170].

Selective USP7 inhibitors GNE-6640 and GNE-6776 were reported in the same year. Crystallographic data show that these compounds show a non-covalently interaction with USP7 at a distance of 12 Å from the catalytic site. Their inhibition mode of action would be explained by the interaction with USP7 acidic residues involved in recognizing the ubiquitin Lys48 side chain. They have been shown to be selective to USP7 in a panel of 36 other DUBs. The co-crystal structures demonstrate that phenol rings of phenol-aminopyridine moieties fit the USP7 pocket, while the hydroxyl groups are able to stabilize the interaction via Hbond with His403 and the 2-aminopyridine interacts with Asp349. The NH of the pyridine carboxamide moiety of GNE-6776 engaged an interaction with Asp305 while the NH of the indazole moiety of GNE-6640 makes van der Waals interaction in the shallow between α 5 and α 6 [172].

Consecutively in time, several crystal structures were reported involving different chemical families. The oxadiazole scaffold was involved in a face-to-face π -stacking interaction with Tyr348 [168], e.g. compound 2-USP7 complex revealed several interactions where the phenol ring locates into the hydrophobic pocket establishing an edge-to-face interaction with Phe324 and the hydroxyl group of the ring interact via H-bond with His403. Quinazolone scaffold can interact with the catalytic site. The co-crystal structure of the potent USP7 inhibitor compound 1_a, led to the development of XL188, a highly potent and selective inhibitor of USP7. The selectivity of XL188 was assessed against a panel of 41 purified DUBs. The analysis of the co-crystal structure XL188-USP7 catalytic domain showed that the compound interacted with the S4-S5 pocket of USP7, between the Palm and Thumb area, about 5 Å away of the catalytic triad. The quinazolinone ketone scaffold interacted with USP7 forming H-bonds with peptide back-bone nitrogen atoms of Arg408 and Phe409. The quinazolinone cyclic nitrogen formed an H-bond with the amide side chain of Gln297. The tertiary hydroxy group not only interacted via H-bond with the carboxylic group of Asp295 but also with the peptide backbone nitrogen of Val296. Moreover, the phenyl moiety of compounds 1_a and XL188, fitted on the S4 pocket and interacted by the aromatic rings of Tyr514, His456, Phe409, and aliphatic chains of Lys420 and Arg408 [174]. Rational and structure-guided design has also been useful in the design of USP7 inhibitors. Particularly, SAR analysis was of utmost importance in the design of pyrazolopyrimidine inhibitors and in identifying compound 2a. Although most of the reported inhibitors target the catalytic domain, the co-crystal structure of compound 2a complexed in human DUB revealed an allosteric binding site of USP7. The phenyl ring of compound 2_a is fitted with the hydrophobic cavity generated due to a conformational change of Phe 409, not seen in the apo form or in the ubiquitin-conjugated USP7 structures. This ligand was sited in an exosite of about 5.5 Å away from the catalytic Cys223. The rational analysis of compounds 2_a interactions allow the design of compounds 4 and 5, which include a benzylic amine group onto the heterocyclic core, where the nitrogen atom of the heterocyclic core interacts with Gln351 and the nitrogen of the pyrazole moiety interacts with Phe409, both via H-bond [171].

Taking compound 2_a as a template, scaffold-hopping leads to compound 46, which showed excellent selectivity in a panel of 21 USP at a fixed concentration of 10 μ M [173].

After a gap of one year, the co-crystal of a pyrazolopyrimidone derivative (L55) and USP7 was reported. The complex shows that the



Fig. 6. USP14 structure with ubiquitin bound (adapted from [181]).

pyrazolopyrimidone group interacts with Gln297, Arg408, and Phe409, whereas the (4-aminomethyl)phenyl group of L55 establishes an H-bond with Gln351. The pyrazole ring of the ligand also interacted with Phe409 via π - π stacking. Moreover, the hydroxyl group of the inhibitor also made hydrogen bond interactions with Asp295 and Val296. Compound L55 did not inhibit several DUBs, such as UCH-L1, UCH-L5, USP2, USP25, USP28, and USP11 at 20 μ M, indicating its excellent inhibitory selectivity for USP7 [169].

Pyridylbenzofuran derivatives have been the latest scaffold described as USP7 ligands in the PDB. Compound 41, a highly potent, selective, and orally bioavailable USP7 inhibitor was obtained through SAR studies. Compound 41 shows tumor growth inhibition in both p53 mutant and p53 wild type cancer cell lines. Thus, USP7 inhibitors can suppress tumor growth through several different pathways. Nevertheless, in order to design 41, interaction studies of different compounds in X-ray co-crystal structures were necessary. In that case, compounds 1_b and 7 showed to occupy the same allosteric pocket of the palm region although their main scaffold was different, 4-hydroxy piperidine and benzofuran, respectively. Both compounds interact via H-bonds with Val296, Asp295, and the phenol moiety of Tyr465. Interestingly, the cocrystallization of compound 14 confirmed two more interactions with USP7, interacting in a reversible way with Arg408 and Gln297. After compound 14 and some more, they designed compound 18 with a succinimide moiety introduced to the structure, this moiety was able to interact with Phe409 and Gln297 [167] (Fig. 5).

3.2.2. Design of USP14 inhibitors

The crystal structures of the 45-kDa catalytic domain of USP14 in isolation and in a complex with ubiquitin aldehyde reveal precise structural features. In the absence of ubiquitin binding, the catalytic cleft leading to the active site of USP14 is blocked by two surface loops. When USP14 is bonded by ubiquitin, it induces a significant conformational change that translocates the two surface loops, as a result, it allows the access of the ubiquitin C-terminus to the active site. These structural observations, in conjunction with biochemical characterization, identify important regulatory mechanisms for USP14 [180].

The full-length human USP14 contains 494 amino acids, with a 9-kDa Ubl domain at its N-terminus followed by a 45-kDa catalytic domain. The catalytic domain of USP14 comprises three main domains: Fingers, Palm, and Thumb (Fig. 6). The three-domain association creates a pronounced binding surface between the Fingers and the Palm–Thumb scaffold, which is predicted to bind to ubiquitin. The active site of free USP14 is already well formed before substrate binding [180].

The N_d1 atom in the imidazole ring of the candidate catalytic histidine (His435) is approximately 3.3 A° away from the S_Y atom in the side chain of the catalytic cysteine (Cys 114), in accordance with a hydrogen bond distance. A third residue, Asp451, stabilizes His435 by accepting a hydrogen bond from its N_E1 atom. Thus, Cys114, His435, and Asp451 form a catalytic chord in the active site of free USP14 [180].

IU1 is a well-known small molecule for being the first one showing



Fig. 7. Molecular structure of IU1 and IU1–248 USP14 inhibitors.

inhibition and excellent selectivity for USP14 over the other DUBs (Table 5). All ligands described by Wang and co-workers are reversible inhibitors, and all are placed in the same region, establishing interactions mainly with Ser431, Gln197, and Asp199. Wang and co-workers reported a co-crystal structure of the catalytic domain of USP14 interacting with IU1 and three derivatives. Authors showed that all the inhibitors likely interacted with a previously unknown steric binding site in USP14, so that they could block the access of the C-terminus of ubiquitin to the active site of USP14, thus inhibiting USP14 activity [175].

Authors then characterized IU1-USP14, His426, Tyr436, and Tyr476 were seen to have an important role due to their contacts formed with the benzene ring of IU1, via both hydrophobic and π - π stacking interactions. The orientation of the benzene ring of IU1 was blocked by the 2 methyl groups of the pyrrole ring, so that, the existence of these methyl groups was essential for retention of the activity of IU1 derivatives, thus the π - π stacking interactions were essential (Fig. 7).

IU1–248 has been shown to be the best compound designed by Wang and co-workers, exhibiting a 10-fold higher potency than IU1. Nevertheless, additional efforts will be required to achieve the characterization of an ideal inhibitor for USP14 [175].

4. Conclusions and perspectives

Ubiquitin-regulating enzymes are a rich resource of attractive targets for developing small molecules cancer therapeutics due to their essential role in a myriad of signaling pathways controlling cell fate. Studies during the last two decades have shown that deregulated E3 ubiquitin ligases play a critical role in the development, progression, and response of human cancers to conventional chemotherapy, and their targeting by the means of specific modulators has greatly improved the prognosis of a subset of cancer patients, especially in hematological cancers. In the case of DUBs, although significant progress has been made in our understanding of their biology and mechanisms of action, there is considerable work to be done in order to move the use of these molecules into the clinical practice. Well-controlled studies must be performed in order to fully understand the mechanisms of regulation and the exact roles of the various endogenous DUBs in health and disease The first paths to better understand their roles and to fairly evaluate their function(s) in a specific cancer model would be to develop libraries of potential, structurebased DUB antagonists based on the crystal structures that have been recently resolved, and to screen them for their selectivity, specificity and efficacy in vitro using elaborated co-culture models. Of note, these models should be able to recreate the specific tumor microenvironment of each cancer subtype, as this player is a crucial determinant of drug efficacy involved in most of the failures experimented during the switch from preclinical to clinical drug development. This approach would also allow identifying the best targets for future DUB-based drug development.

Importantly, regulating the E1-E2-E3 cascade or a selected set of DUBs may represent some limitations due to the important and complex roles exerted by each of these players in cellular processes that are conserved in normal tissues. In addition, the use of these molecules may apply only to those enzymes with well-defined active sites and catalytic functions, limiting their application as a whole. In this context, targeted

protein degradation (TPD) exemplified by the proteolysis targeting chimeras (PROTACs) technology has recently emerged as a promising approach besides general targeting of E3 and DUBs activity, since it allows a more fine-tuned control of pathological targets in cancer, among other diseases. Indeed, in contrast to conventional enzyme inhibitors, PROTACs can elicit selective intracellular proteolysis thanks to their specific heterobifunctional structure associating a protein-binding moiety capable of engaging an E3 ubiquitin ligase, with another part that can selectively bind to the target protein, being these two moieties covalently associated by a linker. Main benefits of the TPD approach include the possibility to deplete the target proteins very fast (generally in minutes), the elimination of protein variants, and its reversibility, that globally offer more fine-tuned control. This new approach, already under clinical evaluation in different cancer subtypes, will undoubtedly improve the field of precision medicine in oncology.

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Conflicts of interest

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References

- R. Yau, K. Doerner, E. Castellanos, D. Haakonsen, A. Werner, N. Wang, X. Yang, N. Martinez-Martin, M. Matsumoto, V. Dixit, M. Rape, Assembly and function of heterotypic ubiquitin chains in cell-cycle and protein quality control, 918-933. e20, Cell 171 (2017), https://doi.org/10.1016/J.CELL.2017.09.040.
 K. Heger, K. Wickliffe, A. Ndoja, J. Zhang, A. Murthy, D. Dugger, A. Maltzman,
- [2] K. Heger, K. Wickliffe, A. Ndoja, J. Zhang, A. Murthy, D. Dugger, A. Maltzman, F. de Sousa, E. Melo, J. Hung, Y. Zeng, E. Verschueren, D. Kirkpatrick, D. Vucic, W. Lee, M. Roose-Girma, R. Newman, S. Warming, Y. Hsiao, L. Kömüves, J. Webster, N. K, V. Dixit, OTULIN limits cell death and inflammation by deubiquitinating LUBAC, Nature 559 (2018) 120–124, https://doi.org/10.1038/ S41586-018-0256-2.
- [3] Q. Liang, T. Dexheimer, P. Zhang, A. Rosenthal, M. Villamil, C. You, Q. Zhang, J. Chen, C. Ott, H. Sun, D. Luci, B. Yuan, A. Simeonov, A. Jadhav, H. Xiao, Y. Wang, D. Maloney, Z. Zhuang, A selective USP1-UAF1 inhibitor links deubiquitination to DNA damage responses, Nat. Chem. Biol. 10 (2014) 298–304, https://doi.org/10.1038/NCHEMBIO.1455.
- [4] J. Wang, M. Maldonado, The ubiquitin-proteasome system and its role in inflammatory and autoimmune diseases, Cell. Mol. Immunol. 3 (2006) 255–261.
- [5] M.J. Clague, C. Heride, S. Urbé, The demographics of the ubiquitin system, Trends Cell Biol. 25 (2015) 417–426, https://doi.org/10.1016/j. tcb.2015.03.002.
- [6] A. Hershko, A. Ciechanover, The ubiquitin system, Annu. Rev. Biochem. 67 (2003) 425–479, https://doi.org/10.1146/annurev.biochem.67.1.425.
- [7] X. Zhang, S. Linder, M. Bazzaro, Drug development targeting the ubiquitin–proteasome system (UPS) for the treatment of human cancers, Cancers 12 (2020), https://doi.org/10.3390/CANCERS12040902.
- [8] A.J. George, Y.C. Hoffiz, A.J. Charles, Y. Zhu, A.M. Mabb, A comprehensive atlas of E3 ubiquitin ligase mutations in neurological disorders, Front. Genet. 9 (2018) 1–17, https://doi.org/10.3389/fgene.2018.00029.

- [9] L. Bedford, J. Lowe, L. Dick, R. Mayer, J. Brownell, Ubiquitin-like protein conjugation and the ubiquitin-proteasome system as drug targets, Nat. Rev. Drug Discov. 10 (2011) 29–46, https://doi.org/10.1038/NRD3321.
- [10] M. Scheffner, S. Kumar, Mammalian HECT ubiquitin-protein ligases: Biological and pathophysiological aspects, Biochim. Biophys. Acta - Mol. Cell Res. 1843 (2014) 61–74, https://doi.org/10.1016/j.bbamcr.2013.03.024.
- [11] Q. Fan, Q. Wang, R. Cai, H. Yuan, M. Xu, The ubiquitin system: orchestrating cellular signals in non-small-cell lung cancer, 2020 251, Cell. Mol. Biol. Lett. 25 (2020) 1–19, https://doi.org/10.1186/S11658-019-0193-6.
- [12] A. Amerik, M. Hochstrasser, Mechanism and function of deubiquitinating enzymes, Biochim. Biophys. Acta 2004 (1695) 189–207, https://doi.org/ 10.1016/J.BBAMCR.2004.10.003.
- [13] J. Kim, K. Park, S. Chung, O. Bang, C. Chung, Deubiquitinating enzymes as cellular regulators, J. Biochem. 134 (2003) 9–18, https://doi.org/10.1093/JB/ MVG107.
- [14] G. LaPlante, W. Zhang, Targeting the ubiquitin-proteasome system for cancer therapeutics by small-molecule inhibitors, Cancers 13 (2021), https://doi.org/ 10.3390/CANCERS13123079.
- [15] P. D'Arcy, S. Linder, Molecular pathways: translational potential of deubiquitinases as drug targets, Clin. Cancer Res. 20 (2014) 3908–3914, https:// doi.org/10.1158/1078-0432.CCR-14-0568.
- [16] D. Rousseau, D. Cannella, J. Boulaire, P. Fitzgerald, A. Fotedar, R. Fotedar, Growth inhibition by CDK-cyclin and PCNA binding domains of p21 occurs by distinct mechanisms and is regulated by ubiquitin-proteasome pathway, Oncogene 18 (1999) 4313–4325, https://doi.org/10.1038/SJ.ONC.1202686.
- [17] P. Hydbring, A. Castell, L. Larsson, MYC modulation around the CDK2/p27/SKP2 Axis, Genes (Basel) 8 (2017), https://doi.org/10.3390/GENES8070174.
- [18] K. Kojima, M. Konopleva, I.J. Samudio, M. Shikami, M. Cabreira-Hansen, T. McQueen, V. Ruvolo, T. Tsao, Z. Zeng, L.T. Vassilev, M. Andreeff, MDM2 antagonists induce p53-dependent apoptosis in AML: Implications for leukemia therapy, Blood 106 (2005) 3150–3159, https://doi.org/10.1182/blood-2005-02-0553.
- [19] C.-H. Yeh, M. Bellon, C. Nicot, FBXW7: a critical tumor suppressor of human cancers, 2018 171, Mol. Cancer 17 (2018) 1–19, https://doi.org/10.1186/ S12943-018-0857-2.
- [20] K. Crusio, B. King, L. Reavie, I. Aifantis, The ubiquitous nature of cancer: the role of the SCF(Fbw7) complex in development and transformation, Oncogene 29 (2010) 4865–4873, https://doi.org/10.1038/ONC.2010.222.
- [21] X. Sun, X. He, L. Yin, M. Komada, R. Sears, M. Dai, The nucleolar ubiquitinspecific protease USP36 deubiquitinates and stabilizes c-Myc, Proc. Natl. Acad. Sci. U. S. A. 112 (2015) 3734–3739, https://doi.org/10.1073/ PNAS.1411713112.
- [22] K. Kriegsmann, M. Baertsch, M. Awwad, M. Merz, D. Hose, A. Seckinger, A. Jauch, N. Becker, A. Benner, M. Raab, J. Hillengass, U. Bertsch, J. Dürig, H. Salwender, M. Hänel, M. Fenk, M. Munder, K. Weisel, C. Müller-Tidow, H. Goldschmidt, M. Hundemer, Cereblon-binding proteins expression levels correlate with hyperdiploidy in newly diagnosed multiple myeloma patients, Blood Cancer J. 9 (2019), https://doi.org/10.1038/S41408-019-0174-Z.
- [23] Z. Wang, Y. Sun, Targeting p53 for novel anticancer therapy, Transl. Oncol. 3 (2010) 1–12, https://doi.org/10.1593/tlo.09250.
- [24] A.C. Joerger, A.R. Fersht, The p53 pathway: origins, inactivation in cancer, and emerging therapeutic approaches, Annu. Rev. Biochem. 85 (2016) 375–404, https://doi.org/10.1146/annurev-biochem-060815-014710.
- [25] A. Feki, I. Irminger-Finger, Mutational spectrum of p53 mutations in primary breast and ovarian tumors, Crit. Rev. Oncol. Hematol. 52 (2004) 103–116, https://doi.org/10.1016/j.critrevonc.2004.07.002.
- [26] W. Hu, Z. Feng, A.J. Levine, The regulation of multiple p53 stress responses is mediated through MDM2, Genes Cancer 3 (2012) 199–208, https://doi.org/ 10.1177/1947601912454734.
- [27] M. Oren, A. Damalas, T. Gottlieb, D. Michael, J. Taplick, J. Leal, R. Maya, M. Moas, R. Seger, Y. Taya, A. Ben-Ze'Ev, Regulation of p53: intricate loops and delicate balances, Ann. N. Y. Acad. Sci. 973 (2002) 374–383, https://doi.org/ 10.1111/J.1749-6632.2002.TB04669.X.
- [28] J. Yuan, K. Luo, L. Zhang, J.C. Cheville, Z. Lou, USP10 regulates p53 localization and stability by deubiquitinating p53, Cell 140 (2010) 384–396, https://doi.org/ 10.1016/J.CELL.2009.12.032.
- [29] J. Yuan, K. Luo, L. Zhang, J. Cheville, Z. Lou, USP10 regulates p53 localization and stability by deubiquitinating p53, Cell 140 (2010) 384–396, https://doi.org/ 10.1016/J.CELL.2009.12.032.
- [30] K. Allton, A. Jain, H. Herz, W. Tsai, S. Jung, J. Qin, A. Bergmann, R. Johnson, M. Barton, Trim24 targets endogenous p53 for degradation, Proc. Natl. Acad. Sci. U. S. A. 106 (2009) 11612–11616, https://doi.org/10.1073/PNAS.0813177106.
- [32] M. Magiera, S. Mora, B. Mojsa, I. Robbins, I. Lassot, S. Desagher, Trim17mediated ubiquitination and degradation of Mcl-1 initiate apoptosis in neurons, Cell Death Differ. 20 (2013) 281–292, https://doi.org/10.1038/CDD.2012.124.
- [33] M. Sancho, D. Leiva, E. Lucendo, M. Orzáez, Understanding MCL1: from cellular function and regulation to pharmacological inhibition, FEBS J. (2021), https:// doi.org/10.1111/FEBS.16136.
- [34] J. Wang, S. Katz, E. Morgan, D. Yang, X. Pan, M. Xu, Proapoptotic protein BIM as a novel prognostic marker in mantle cell lymphoma, Hum. Pathol. 93 (2019) 54–64, https://doi.org/10.1016/J.HUMPATH.2019.08.008.

- [35] S. Soh, S. Ong, A novel BIM deletion polymorphism: implications and lessons for cancer targeted therapies, Rinsho. Ketsueki. 54 (2013) 1714–1719, https://doi. org/10.11406/rinketsu.54.1714.
- [36] R. Ley, K. Balmanno, K. Hadfield, C. Weston, S. Cook, Activation of the ERK1/2 signaling pathway promotes phosphorylation and proteasome-dependent degradation of the BH3-only protein, Bim, J. Biol. Chem. 278 (2003) 18811–18816, https://doi.org/10.1074/JBC.M301010200.
- [37] W.L. Yang, G. Jin, C.F. Li, Y.S. Jeong, A. Moten, D. Xu, Z. Feng, W. Chen, Z. Cai, B. Darnay, W. Gu, H.K. Lin, Cycles of ubiquitination and deubiquitination critically regulate growth factor-mediated activation of Akt signaling, Sci. Signal. 6 (2013), https://doi.org/10.1126/SCISIGNAL.2003197/SUPPL_FILE/6_RA3_SM. PDF.
- [38] S. Kim, H. Kim, H. Byeon, D. Kim, C. Kim, FOXO3 induces ubiquitylation of AKT through MUL1 regulation, Oncotarget 8 (2017) 110474–110489, https://doi.org/ 10.18632/ONCOTARGET.22793.
- [39] W. Yang, G. Jin, C. Li, Y. Jeong, A. Moten, D. Xu, Z. Feng, W. Chen, Z. Cai, B. Darnay, W. Gu, H. Lin, Cycles of ubiquitination and deubiquitination critically regulate growth factor-mediated activation of Akt signaling, Sci. Signal. 6 (2013), https://doi.org/10.1126/SCISIGNAL.2003197.
- [40] R. Ali, M. Alabdullah, I. Miligy, M. Normatova, R. Babaei-Jadidi, A. Nateri, E. Rakha, S. Madhusudan, ATM regulated PTEN degradation Is XIAP E3 ubiquitin ligase mediated in p85α deficient cancer cells and influence platinum sensitivity, Cells 8 (2019), https://doi.org/10.3390/CELLS8101271.
- [41] G. Carrà, C. Panuzzo, D. Torti, G. Parvis, S. Crivellaro, U. Familiari, M. Volante, D. Morena, M.F. Lingua, M. Brancaccio, A. Guerrasio, P.P. Pandolfi, G. Saglio, R. Taulli, A. Morotti, G. Carrà, C. Panuzzo, D. Torti, G. Parvis, S. Crivellaro, U. Familiari, M. Volante, D. Morena, M.F. Lingua, M. Brancaccio, A. Guerrasio, P. P. Pandolfi, G. Saglio, R. Taulli, A. Morotti, Therapeutic inhibition of USP7-PTEN network in chronic lymphocytic leukemia: a strategy to overcome TP53 mutated/ deleted clones, Oncotarget 8 (2017) 35508–35522, https://doi.org/10.18632/ ONCOTARGET.16348.
- [42] M.S. Song, L. Salmena, A. Carracedo, A. Egia, F. Lo-Coco, J. Salmena, P. P. Pandolfi, The deubiquitinylation and localization of PTEN are regulated by a HAUSP-PML network, Nature 455 (2008) 813–817, https://doi.org/10.1038/ nature07290.
- [43] G. Maubach, A.-C. Schmädicke, M. Naumann, NEMO links nuclear factor-kB to human diseases, Trends Mol. Med. 23 (2017) 1138–1155, https://doi.org/ 10.1016/J.MOLMED.2017.10.004.
- [44] J. Niu, Y. Shi, K. Iwai, Z. Wu, LUBAC regulates NF-κB activation upon genotoxic stress by promoting linear ubiquitination of NEMO, EMBO J. 30 (2011) 3741–3753, https://doi.org/10.1038/EMBOJ.2011.264.
- [45] P. Gough, I.A. Myles, Tumor necrosis factor receptors: pleiotropic signaling complexes and their differential effects, Front. Immunol. 11 (2020), https://doi. org/10.3389/FIMMU.2020.585880.
- [46] P.E. Cockram, M. Kist, S. Prakash, S.-H. Chen, I.E. Wertz, D. Vucic, Ubiquitination in the regulation of inflammatory cell death and cancer, Cell Death Differ. 28 (2021) 591, https://doi.org/10.1038/S41418-020-00708-5.
- [47] M. O'Donnell, D. Legarda-Addison, P. Skountzos, W. Yeh, A. Ting, Ubiquitination of RIP1 regulates an NF-kappaB-independent cell-death switch in TNF signaling, Curr. Biol. 17 (2007) 418–424, https://doi.org/10.1016/J.CUB.2007.01.027.
- [48] L. Dalla Via, C. Nardon, D. Fregona, Targeting the ubiquitin-proteasome pathway with inorganic compounds to fight cancer: a challenge for the future, Future Med. Chem. 4 (2012) 525–543, https://doi.org/10.4155/FMC.11.187.
- [49] M. Gonzalez-Santamarta, G. Quinet, D. Reyes-Garau, B. Sola, G. Roué, M. Rodriguez, Resistance to the proteasome inhibitors: lessons from multiple myeloma and mantle cell lymphoma, Adv. Exp. Med. Biol. 1233 (2020) 153–174, https://doi.org/10.1007/978-3-030-38266-7_6.
- [50] A. Ventura, D.G. Kirsch, M.E. McLaughlin, D.A. Tuveson, J. Grimm, L. Lintault, J. Newman, E.E. Reczek, R. Weissleder, T. Jacks, Restoration of p53 function leads to tumour regression in vivo, Nature 445 (2007) 661–665, https://doi.org/ 10.1038/nature05541.
- [51] L.T. Vassilev, B.T. Vu, B. Graves, D. Carvajal, F. Podlaski, Z. Filipovic, N. Kong, U. Kammlott, C. Lukacs, C. Klein, N. Fotouhi, E.A. Liu, In vivo activation of the p53 pathway by small-molecule antagonists of MDM2, Science 303 (2004) 844–848, https://doi.org/10.1126/SCIENCE.1092472.
- [52] Q. Ding, Z. Zhang, J.-J. Liu, N. Jiang, J. Zhang, T.M. Ross, X.-J. Chu, D. Bartkovitz, F. Podlaski, C. Janson, C. Tovar, Z.M. Filipovic, B. Higgins, K. Glenn, K. Packman, L.T. Vassilev, B. Graves, Discovery of RG7388, a potent and selective p53–MDM2 inhibitor in clinical development, J. Med. Chem. 56 (2013) 5979–5983, https://doi.org/10.1021/JM400487C.
- [53] L.T. Vassilev, MDM2 inhibitors for cancer therapy, Trends Mol. Med. 13 (2007) 23–31, https://doi.org/10.1016/j.molmed.2006.11.002.
- [54] M.B. Kastan, Wild-type p53: tumors can't stand it, Cell 128 (2007) 837–840, https://doi.org/10.1016/j.cell.2007.02.022.
- [55] L.M.S. Lau, J.K. Nugent, X. Zhao, M.S. Irwin, HDM2 antagonist Nutlin-3 disrupts p73-HDM2 binding and enhances p73 function, Oncogene 27 (2008) 997–1003, https://doi.org/10.1038/sj.onc.1210707.
- [56] G. Ambrosini, E.B. Sambol, D. Carvajal, L.T. Vassilev, S. Singer, G.K. Schwartz, Mouse double minute antagonist Nutlin-3a enhances chemotherapy-induced apoptosis in cancer cells with mutant p53 by activating E2F1, Oncogene 26 (2007) 3473–3481, https://doi.org/10.1038/sj.onc.1210136.
- [57] G.A. LaRusch, M.W. Jackson, J.D. Dunbar, R.S. Warren, D.B. Donner, L.D. Mayo, Nutlin3 blocks vascular endothelial growth factor induction by preventing the interaction between hypoxia inducible factor 1α and Hdm2, Cancer Res. 67 (2007) 450–454, https://doi.org/10.1158/0008-5472.CAN-06-2710.

- [58] G. Sarek, S. Kurki, J. Enbäck, G. Iotzova, J. Haas, P. Laakkonen, M. Laiho, P. M. Ojala, Reactivation of the p53 pathway as a treatment modality for KSHVinduced lymphomas, J. Clin. Invest. 117 (2007) 1019–1028, https://doi.org/ 10.1172/JCI30945.
- [59] M. Zheng, J. Yang, X. Xu, J.T. Sebolt, S. Wang, Y. Sun, Efficacy of MDM2 inhibitor MI-219 against lung cancer cells alone or in combination with MDM2 knockdown, a XIAP inhibitor or etoposide, Anticancer Res. 30 (2010) 3321–3331.
- [60] S. Shangary, D. Qin, D. McEachern, M. Liu, R.S. Miller, S. Qiu, Z. Nikolovska-Coleska, K. Ding, G. Wang, J. Chen, D. Bernard, J. Zhang, Y. Lu, Q. Gu, R.B. Shah, K.J. Pienta, X. Ling, S. Kang, M. Guo, Y. Sun, D. Yang, S. Wang, Temporal activation of p53 by a specific MDM2 inhibitor is selectively toxic to tumors and leads to complete tumor growth inhibition, Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 3933–3938. https://doi.org/10.1073/pnas.0708917105.
- [61] P. Holzer, K. Masuya, P. Furet, J. Kallen, T. Valat-Stachyra, S. Ferretti, J. Berghausen, M. Bouisset-Leonard, N. Buschmann, C. Pissot-Soldermann, C. Rynn, S. Ruetz, S. Stutz, P. Chène, S. Jeay, F. Gessier, Discovery of a dihydroisoquinolinone derivative (NVP-CGM097): a highly potent and selective MDM2 inhibitor undergoing phase 1 clinical trials in p53wt tumors, J. Med. Chem. 58 (2015) 6348–6358, https://doi.org/10.1021/ACS. JMEDCHEM.5B00810.
- [62] E. Weisberg, E. Halilovic, V.G. Cooke, A. Nonami, T. Ren, T. Sanda, I. Simkin, J. Yuan, B. Antonakos, L. Barys, M. Ito, R. Stone, I. Galinsky, K. Cowens, E. Nelson, M. Sattler, S. Jeay, J.U. Wuerthner, S.M. McDonough, M. Wiesmann, J. D. Griffin, Inhibition of wild-type p53-expressing aml by the novel small molecule HDM2 inhibitor CGM097, Mol. Cancer Ther. 14 (2015) 2249–2259, https://doi.org/10.1158/1535-7163.MCT-15-0429.
- [63] E.C. Townsend, M.A. Murakami, A. Christodoulou, A.L. Christie, J. Köster, T. A. DeSouza, E.A. Morgan, S.P. Kallgren, H. Liu, S.-C. Wu, O. Plana, J. Montero, K. E. Stevenson, P. Rao, R. Vadhi, M. Andreeff, P. Armand, K.K. Ballen, P. Barzaghi-Rinaudo, S. Cahill, R.A. Clark, V.G. Cooke, M.S. Davids, D.J. DeAngelo, D. M. Dorfman, H. Eaton, B.L. Ebert, J. Etchin, B. Firestone, D.C. Fisher, A. S. Freedman, I.A. Galinsky, H. Gao, J.S. Garcia, F. Garnache-Ottou, T.A. Graubert, A. Gutierrez, E. Halilovic, M.H. Harris, Z.T. Herbert, S.M. Horwitz, G. Inghirami, A.M. Intlekofer, M. Ito, S. Izraeli, E.D. Jacobsen, C.A. Jacobson, S. Jeay, I. Jeremias, M.A. Kelliher, R. Koch, M. Konopleva, N. Kopp, S.M. Kornblau, A. L. Kung, T.S. Kupper, N.R. LeBoeuf, A.S. LaCasce, E. Lees, L.S. Li, A.T. Look M. Murakami, M. Muschen, D. Neuberg, S.Y. Ng, O.O. Odejide, S.H. Orkin, R. R. Paquette, A.E. Place, J.E. Roderick, J.A. Ryan, S.E. Sallan, B. Shoji, L. B. Silverman, R.J. Soiffer, D.P. Steensma, K. Stegmaier, R.M. Stone, J. Tamburini, A.R. Thorner, P. van Hummelen, M. Wadleigh, M. Wiesmann, A.P. Weng, J. U. Wuerthner, D.A. Williams, B.M. Wollison, A.A. Lane, A. Letai, M. M. Bertagnolli, J. Ritz, M. Brown, H. Long, J.C. Aster, M.A. Shipp, J.D. Griffin, D. M. Weinstock, The public repository of xenografts enables discovery and randomized phase II-like trials in mice, Cancer Cell 29 (2016) 574-586, https:// doi.org/10.1016/J.CCELL.2016.03.008.
- [64] V. Arnhold, K. Schmelz, J. Proba, A. Winkler, J. Wünschel, J. Toedling, H. E. Deubzer, A. Künkele, A. Eggert, J.H. Schulte, P. Hundsdoerfer, V. Arnhold, K. Schmelz, J. Proba, A. Winkler, J. Wünschel, J. Toedling, H.E. Deubzer, A. Künkele, A. Eggert, J.H. Schulte, P. Hundsdoerfer, Reactivating TP53 signaling by the novel MDM2 inhibitor DS-3032b as a therapeutic option for high-risk neuroblastoma, Oncotarget 9 (2017) 2304–2319, https://doi.org/10.18632/ ONCOTARGET.23409.
- [65] J. Ishizawa, K. Nakamaru, T. Seki, K. Tazaki, K. Kojima, D. Chachad, R. Zhao, L. Heese, W. Ma, M.C.J. Ma, C. DiNardo, S. Pierce, K.P. Patel, A. Tse, R.E. Davis, A. Rao, M. Andreeff, Predictive gene signatures determine tumor sensitivity to MDM2 inhibition, Cancer Res 78 (2018) 2721–2731, https://doi.org/10.1158/ 0008-5472.CAN-17-0949.
- [66] A. Aguilar, J. Lu, L. Liu, D. Du, D. Bernard, D. McEachern, S. Przybranowski, X. Li, R. Luo, B. Wen, D. Sun, H. Wang, J. Wen, G. Wang, Y. Zhai, M. Guo, D. Yang, S. Wang, Discovery of 4-((3'R,4'S,5'R)-6"-Chloro-4'-(3-chloro-2-fluorophenyl)-1'ethyl-2"-oxodispiro[cyclohexane-1,2'-pyrrolidine-3',3"-indoline]-5'carboxamido)bicyclo[2.2.2]octane-1-carboxylic Acid (AA-115/APG-115): a potent and orally active murine double minute 2 (MDM2) inhibitor in clinical development, J. Med. Chem. 60 (2017) 2819–2839, https://doi.org/10.1021/ ACS_JMEDCHEM.6B01665.
- [67] C.H. Chan, J.K. Morrow, C.F. Li, Y. Gao, G. Jin, A. Moten, L.J. Stagg, J.E. Ladbury, Z. Cai, D. Xu, C.J. Logothetis, M.C. Hung, S. Zhang, H.K. Lin, Pharmacological inactivation of Skp2 SCF ubiquitin ligase restricts cancer stem cell traits and cancer progression, Cell 154 (2013) 556–568, https://doi.org/10.1016/j. cell.2013.06.048.
- [68] X. Liu, J. Xu, J. Zhou, Q. Shen, Oridonin and its derivatives for cancer treatment and overcoming therapeutic resistance, Genes Dis. 8 (2021) 448–462, https://doi. org/10.1016/j.gendis.2020.06.010.
- [69] H.L. Huang, H.Y. Weng, L.Q. Wang, C.H. Yu, Q.J. Huang, P.P. Zhao, J.Z. Wen, H. Zhou, L.H. Qu, Triggering Fbw7-mediated proteasomal degradation of c-Myc by oridonin induces cell growth inhibition and apoptosis, Mol. Cancer Ther. 11 (2012) 1155–1165, https://doi.org/10.1158/1535-7163.MCT-12-0066.
- [70] K. Kubaichuk, T. Kietzmann, Involvement of E3 ligases and deubiquitinases in the control of HIF-α subunit abundance, Cells 8 (2019) 598, https://doi.org/ 10.3390/cells8060598.
- [71] X. Zhang, T. Meng, S. Cui, L. Feng, D. Liu, Q. Pang, P. Wang, Ubiquitination of nonhistone proteins in cancer development and treatment, Front. Oncol. 10 (2021) 3418, https://doi.org/10.3389/fonc.2020.621294.
- [72] F. Colland, E. Formstecher, X. Jacq, C. Reverdy, C. Planquette, S. Conrath, V. Trouplin, J. Bianchi, V.N. Aushev, J. Camonis, A. Calabrese, C. Borg-Capra, W. Sippl, V. Collura, G. Boissy, J.C. Rain, P. Guedat, R. Delansorne, L. Daviet,

Small-molecule inhibitor of USP7/HAUSP ubiquitin protease stabilizes and activates p53 in cells, Mol. Cancer Ther. 8 (2009) 2286–2295, https://doi.org/10.1158/1535-7163.MCT-09-0097.

- [73] D. Chauhan, Z. Tian, B. Nicholson, K.G.S. Kumar, B. Zhou, R. Carrasco, J. L. Mcdermott, C.A. Leach, M. Fulcinniti, M.P. Kodrasov, J. Weinstock, W. D. Kingsbury, T. Hideshima, P.K. Shah, S. Minvielle, M. Altun, B.M. Kessler, P. Richardson, N. Munshi, K.C. Anderson, A. Small, Molecule Inhibitor of Ubiquitin-Specific protease-7 induces apoptosis in multiple myeloma cells and overcomes bortezomib resistance, Cancer Cell 22 (2013) 345–358, https://doi.org/10.1016/i.ccr.2012.08.007.A.
- [74] X.X. Sun, K.B. Challagundla, M.S. Dai, Positive regulation of p53 stability and activity by the deubiquitinating enzyme Otubain 1, EMBO J. 31 (2012) 576–592, https://doi.org/10.1038/EMBOJ.2011.434.
- [75] L. Liu, Y. Hua, D. Wang, L. Shan, Y. Zhang, J. Zhu, H. Jin, H. Li, Z. Hu, W. Zhang, A sesquiterpene lactone from a medicinal herb inhibits proinflammatory activity of TNF-α by inhibiting ubiquitin-conjugating enzyme UbcH5, Chem. Biol. 21 (2014) 1341–1350, https://doi.org/10.1016/J.CHEMBIOL.2014.07.021.
- [76] Z. Ly, K.M. Williams, L. Yuan, J.H. Atkison, S.K. Olsen, Crystal structure of a human ubiquitin E1–ubiquitin complex reveals conserved functional elements essential for activity, J. Biol. Chem. 293 (2018) 18337–18352, https://doi.org/ 10.1074/JBC.RA118.003975.
- [77] M.L. Hyer, M.A. Milhollen, J. Ciavarri, P. Fleming, T. Traore, D. Sappal, J. Huck, J. Shi, J. Gavin, J. Brownell, Y. Yang, B. Stringer, R. Griffin, F. Bruzzese, T. Soucy, J. Duffy, C. Rabino, J. Riceberg, K. Hoar, A. Lublinsky, S. Menon, M. Sintchak, N. Bump, S.M. Pulukuri, S. Langston, S. Tirrell, M. Kuranda, P. Veiby, J. Newcomb, P. Li, J.T. Wu, J. Powe, L.R. Dick, P. Greenspan, K. Galvin, M. Manfredi, C. Claiborne, B.S. Amidon, N.F. Bence, A small-molecule inhibitor of the ubiquitin activating enzyme for cancer treatment, 2018 242, Nat. Med. 24 (2018) 186–193, https://doi.org/10.1038/nm.4474.
- [78] S.H. Barghout, A.D. Schimmer, E1 enzymes as therapeutic targets in cancer, Pharmacol. Rev. 73 (2021) 1–58, https://doi.org/10.1124/ PHARMREV.120.000053.
- [79] T.A. Soucy, P.G. Smith, M.A. Milhollen, A.J. Berger, J.M. Gavin, S. Adhikari, J. E. Brownell, K.E. Burke, D.P. Cardin, S. Critchley, C.A. Cullis, A. Doucette, J. J. Garnsey, J.L. Gaulin, R.E. Gershman, A.R. Lublinsky, A. McDonald, H. Mizutani, U. Narayanan, E.J. Olhava, S. Peluso, M. Rezaei, M.D. Sintchak, T. Talreja, M.P. Thomas, T. Traore, S. Vyskocil, G.S. Weatherhead, J. Yu, J. Zhang, L.R. Dick, C.F. Claiborne, M. Rolfe, J.B. Bolen, S.P. Langston, An inhibitor of NEDD8-activating enzyme as a new approach to treat cancer, Nature 458 (2009) 732–736, https://doi.org/10.1038/nature07884.
- [80] J. Zhuang, F. Shirazi, R. Singh, I. Kuiatse, H. Wang, H. Lee, Z. Berkova, A. Berger, M. Hyer, N. Chattopadhyay, S. Syed, J. Shi, J. Yu, V. Shinde, S. Tirrell, R. Jones, Z. Wang, R. Davis, R. Orlowski, Ubiquitin-activating enzyme inhibition induces an unfolded protein response and overcomes drug resistance in myeloma, Blood 133 (2019) 1572–1584, https://doi.org/10.1182/BLOOD-2018-06-859686.
- [81] S. Best, T. Hashiguchi, A. Kittai, N. Bruss, C. Paiva, C. Okada, T. Liu, A. Berger, A. V. Danilov, Targeting ubiquitin-activating enzyme induces ER stress-mediated apoptosis in B-cell lymphoma cells, Blood Adv. 3 (2019) 51–62, https://doi.org/10.1182/BLOODADVANCES.2018026880.
- [82] S.H. Barghout, P.S. Patel, X. Wang, G.W. Xu, S. Kavanagh, O. Halgas, S.F. Zarabi, M. Gronda, R. Hurren, D.V. Jeyaraju, N. MacLean, S. Brennan, M.L. Hyer, A. Berger, T. Traore, M. Milhollen, A.C. Smith, M.D. Minden, E.F. Pai, R. Hakem, A.D. Schimmer, Preclinical evaluation of the selective small-molecule UBA1 inhibitor, TAK-243, in acute myeloid leukemia, Leukemia 33 (2018) 37–51, https://doi.org/10.1038/s41375-018-0167-0.
- [83] Y. Liu, S. Awadia, A. Delaney, M. Sitto, C. Engelke, H. Patel, A. Calcaterra, S. Zelenka-Wang, H. Lee, J. Contessa, N. Neamati, M. Ljungman, T.S. Lawrence, M.A. Morgan, A. Rehemtulla, UAE1 inhibition mediates the unfolded protein response, DNA damage and caspase-dependent cell death in pancreatic cancer, Transl. Oncol. 13 (2020), 100834, https://doi.org/10.1016/J. TRANON.2020.100834.
- [84] G. Liu, J. Yu, R. Wu, L. Shi, X. Zhang, W. Zhang, X. Zhong, Y. Wang, H. Li, Y. Shen, C. Wu, R. Yu, M. Niu, X. Liu, GRP78 determines glioblastoma sensitivity to UBA1 inhibition-induced UPR signaling and cell death, 2021 128. 12, Cell Death Dis. (2021) 1–13, https://doi.org/10.1038/s41419-021-04023-w.
- [85] S. Majeed, M.K. Aparnathi, L. Song, A.D. Schimmer, M.S. Tsao, G. Liu, B.H. Lok, Abstract 2699: targeting an ubiquitin-activating enzyme in small-cell lung cancer (SCLC), Cancer Res. 79 (2019), https://doi.org/10.1158/1538-7445.AM2019-2699.
- [86] A. McHugh, K. Fernandes, A.P. South, J.E. Mellerio, J.C. Salas-Alanís, C.M. Proby, I.M. Leigh, M.K. Saville, A. McHugh, K. Fernandes, A.P. South, J.E. Mellerio, J. C. Salas-Alanís, C.M. Proby, I.M. Leigh, M.K. Saville, Preclinical comparison of proteasome and ubiquitin E1 enzyme inhibitors in cutaneous squamous cell carcinoma: the identification of mechanisms of differential sensitivity, Oncotarget 9 (2018) 20265–20281, https://doi.org/10.18632/ONCOTARGET.24750.
- [87] S. Best, T. Liu, N. Bruss, A. Kittai, A. Berger, A.V. Danilov, Pharmacologic inhibition of the ubiquitin-activating enzyme induces ER stress and apoptosis in chronic lymphocytic leukemia and ibrutinib-resistant mantle cell lymphoma cells, Https://Doi.Org/10.1080/10428194.2019.1616190 60 (2019) 2946–2950, https://doi.org/10.1080/10428194.2019.1616190.
- [88] M.L. Hyer, M.A. Milhollen, J. Ciavarri, P. Fleming, T. Traore, D. Sappal, J. Huck, J. Shi, J. Gavin, J. Brownell, Y. Yang, B. Stringer, R. Griffin, F. Bruzzese, T. Soucy, J. Duffy, C. Rabino, J. Riceberg, K. Hoar, A. Lublinsky, S. Menon, M. Sintchak, N. Bump, S.M. Pulukuri, S. Langston, S. Tirrell, M. Kuranda, P. Veiby, J. Newcomb, P. Li, J.T. Wu, J. Powe, L.R. DIck, P. Greenspan, K. Galvin,
 - M. Manfredi, C. Claiborne, B.S. Amidon, N.F. Bence, A small-molecule inhibitor of

A.M. Montagut et al.

the ubiquitin activating enzyme for cancer treatment, Nat. Med. 24 (2018) 186–193, https://doi.org/10.1038/nm.4474.

- [89] M. Milhollen, D. Sappal, J. Duffy, K. Hoar, J. Huck, P. Sha, E. Koenig, M. Hyer, J. Ciavarri, N. Bence, 577 characterization of the cellular mechanism of action of the first in class investigational inhibitor of the ubiquitin activating enzyme, MLN7243, Eur. J. Cancer 50 (2014) 186, https://doi.org/10.1016/s0959-8049 (14)70703-8.
- [90] D.W. Rasco, K.P. Papadopoulos, M. Pourdehnad, A.K. Gandhi, P.R. Hagner, Y. Li, X. Wei, R. Chopra, K. Hege, J. DiMartino, K. Shih, A first-in-human study of novel cereblon modulator avadomide (CC-122) in advanced malignancies, Clin. Cancer Res. 25 (2019) 90–98, https://doi.org/10.1158/1078-0432.CCR-18-1203.
- [91] P. Holzer, K. Masuya, P. Furet, J. Kallen, T. Valat-Stachyra, S. Ferretti, J. Berghausen, M. Bouisset-Leonard, N. Buschmann, C. Pissot-Soldermann, C. Rynn, S. Ruetz, S. Stutz, P. Chène, S. Jeay, F. Gessier, Discovery of a dihydroisoquinolinone derivative (NVP-CGM097): a highly potent and selective MDM2 inhibitor undergoing phase 1 clinical trials in p53wt tumors, J. Med. Chem. 58 (2015) 6348–6358, https://doi.org/10.1021/acs.jmedchem.5b00810.
- [92] C.C. Bjorklund, J. Kang, M. Amatangelo, A. Polonskaia, M. Katz, H. Chiu, S. Couto, M. Wang, Y. Ren, M. Ortiz, F. Towfic, J.E. Flynt, W. Pierceall, A. Thakurta, Iberdomide (CC-220) is a potent cereblon E3 ligase modulator with antitumor and immunostimulatory activities in lenalidomide- and pomalidomideresistant multiple myeloma cells with dysregulated CRBN, Leukemia 34 (2020) 1197–1201, https://doi.org/10.1038/s41375-019-0620-8.
- [93] Y.Z. Drew, W. Rasco, Nehal J. Lakhani, Yufeng Li, Lichuang Men, Hengbang Wang, Jiao Ji, Yuefen Tang, Zhiyan Liang, Alex Amaya, Kathy Estkowski, Joan Sun, Yingjie Huang, Dajun Yang, Yifan ZhaiDrew, W. Rasco, Nehal J. Lakhani, Yufeng Li, Lichuang Men, Hengbang Wan, A phase I study of a novel MDM2 antagonist APG-115 in patients with advanced solid tumors, J. Clin. Oncol. 37 (2019) 3126, https://doi.org/10.1200/ JCO.2019.37.15_suppl.3126.
- [94] T. Han, M. Goralski, N. Gaskill, E. Capota, J. Kim, T.C. Ting, Y. Xie, N.S. Williams, D. Nijhawan, Anticancer sulfonamides target splicing by inducing RBM39 degradation via recruitment to DCAF15, Science 356 (2017), https://doi.org/ 10.1126/science.aal3755.
- [95] N.Rita Assi, Hagop Kantarjian, Tapan M. Kadia, Naveen Pemmaraju, Elias Jabbour, J.E.C. Jain, Naval Daver, Zeev Estrov, Taisuke Uehara, Takashi Owa, G. Borthakur, Final results of a phase 2, open-label study of indisulam, idarubicin and cytarabine in patients with relapsed or refractory acute myeloid leukemia and high-risk myelodysplastic syndrome, Cancer 124 (2018) 2758–2765, https://doi.org/10.1002/cncr.31398.Final.
 [96] H. Gao, E.F. Yamasaki, K.K. Chan, L.L. Shen, R.M. Snapka, Chloroquinoxaline
- [96] H. Gao, E.F. Yamasaki, K.K. Chan, L.L. Shen, R.M. Snapka, Chloroquinoxaline sulfonamide (NSC 339004) is a topoisomerase $II\alpha/\beta$ poison, Cancer Res. 60 (2000) 5937–5940.
- [97] Z.H. Jia, X.G. Wang, H. Zhang, Overcome cancer drug resistance by targeting epigenetic modifications of centrosome, Cancer Drug Resist. 2 (2019) 210–224, https://doi.org/10.20517/cdr.2018.010.
- [98] H.Q. Wu, D. Baker, H. Ovaa, Small molecules that target the ubiquitin system, Biochem. Soc. Trans. 48 (2020) 479–497, https://doi.org/10.1042/ BST20190535.
- [99] Q. Shi, L. Chen, Cereblon: a protein crucial to the multiple functions of immunomodulatory drugs as well as cell metabolism and disease generation, J. Immunol. Res. 2017 (2017), https://doi.org/10.1155/2017/9130608.
- [100] G. Lu, R.E. Middleton, H. Sun, M. Naniong, C.J. Ott, C.S. Mitsiades, K.-K. Wong, J. E. Bradner, J. William, G. Kaelin, The myeloma drug lenalidomide promotes the cereblon-dependent destruction of ikaros proteins, Sci. (80-.) 343 (2014) 305–309, https://doi.org/10.1126/SCIENCE.1244917.
- [101] J. Krönke, N.D. Udeshi, A. Narla, P. Grauman, S.N. Hurst, M. McConkey, T. Svinkina, D. Heckl, E. Comer, X. Li, C. Ciarlo, E. Hartman, N. Munshi, M. Schenone, S.L. Schreiber, S.A. Carr, B.L. Ebert, Lenalidomide causes selective degradation of IKZF1 and IKZF3 in multiple myeloma cells, Science 343 (2014) 301–305, https://doi.org/10.1126/SCIENCE.1244851.
- [102] E.S. Fischer, K. Böhm, J.R. Lydeard, H. Yang, M.B. Stadler, S. Cavadini, J. Nagel, F. Serluca, V. Acker, G.M. Lingaraju, R.B. Tichkule, M. Schebesta, W.C. Forrester, M. Schirle, U. Hassiepen, J. Ottl, M. Hild, R.E.J. Beckwith, J.W. Harper, J. L. Jenkins, N.H. Thomä, Structure of the DDB1–CRBN E3 ubiquitin ligase in complex with thalidomide, Nature 512 (2014) 49–53, https://doi.org/10.1038/ nature13527.
- [103] A. Lopez-Girona, D. Heintel, L.-H. Zhang, D. Mendy, S. Gaidarova, H. Brady, J. B. Bartlett, P.H. Schafer, M. Schreder, A. Bolomsky, B. Hilgarth, N. Zojer, H. Gisslinger, H. Ludwig, T. Daniel, U. Jäger, R. Chopra, Lenalidomide downregulates the cell survival factor, interferon regulatory factor-4, providing a potential mechanistic link for predicting response, Br. J. Haematol. 154 (2011) 325–336, https://doi.org/10.1111/J.1365-2141.2011.08689.X.
- [104] D.W. Rasco, K.P. Papadopoulos, M. Pourdehnad, A.K. Gandhi, P.R. Hagner, Y. Li, X. Wei, R. Chopra, K. Hege, J. DiMartino, K. Shih, A. First-in-Human, Study of novel cereblon modulator avadomide (CC-122) in advanced malignancies, Clin. Cancer Res. 25 (2019) 90–98, https://doi.org/10.1158/1078-0432.CCR-18-1203.
- [105] C. Cubillos-Zapata, R. Cordoba, J. Avendaño-Ortiz, C. Arribas-Jiménez, E. Hernández-Jiménez, V. Toledano, T. Villaescusa, V. Moreno, E. López-Collazo, CC-122 immunomodulatory effects in refractory patients with diffuse large B-cell lymphoma, Oncoimmunology 5 (2016), e1231290, https://doi.org/10.1080/ 2162402X.2016.1231290.
- [106] P.R. Hagner, H.-W. Man, C. Fontanillo, M. Wang, S. Couto, M. Breider, C. Bjorklund, C.G. Havens, G. Lu, E. Rychak, H. Raymon, R.K. Narla, L. Barnes, G. Khambatta, H. Chiu, J. Kosek, J. Kang, M.D. Amantangelo, M. Waldman, A. Lopez-Girona, T. Cai, M. Pourdehnad, M. Trotter, T.O. Daniel, P.H. Schafer,

A. Klippel, A. Thakurta, R. Chopra, A.K. Gandhi, CC-122, a pleiotropic pathway modifier, mimics an interferon response and has antitumor activity in DLBCL, Blood 126 (2015) 779–789, https://doi.org/10.1182/BLOOD-2015-02-628669.

- [107] J. Krönke, E.C. Fink, P.W. Hollenbach, K.J. MacBeth, S.N. Hurst, N.D. Udeshi, P. P. Chamberlain, D.R. Mani, H.W. Man, A.K. Gandhi, T. Svinkina, R.K. Schneider, M. McConkey, M. Järås, E. Griffiths, M. Wetzler, L. Bullinger, B.E. Cathers, S. A. Carr, R. Chopra, B.L. Ebert, Lenalidomide induces ubiquitination and degradation of CK1α in del(5q) MDS, Nature 523 (2015) 183–188, https://doi.org/10.1038/nature14610.
- [108] M. Matyskiela, W. Zhang, H. Man, G. Muller, G. Khambatta, F. Baculi, M. Hickman, L. LeBrun, B. Pagarigan, G. Carmel, C. Lu, G. Lu, M. Riley, Y. Satoh, P. Schafer, T. Daniel, J. Carmichael, B. Cathers, P. Chamberlain, A. Cereblon, Modulator (CC-220) with improved degradation of ikaros and aiolos, J. Med. Chem. 61 (2018) 535–542, https://doi.org/10.1021/ACS.JMEDCHEM.6B01921.
- [109] M. Amatangelo, C.C. Bjorklund, J. Kang, A. Polonskaia, S. Viswanatha, A. Thakurta, Iberdomide (CC-220) has synergistic anti-tumor and immunostimulatory activity against multiple myeloma in combination with both bortezomib and dexamethasone, or in combination with daratumumab in vitro, Blood 132 (2018) 1935, https://doi.org/10.1182/blood-2018-99-113383.
- [110] S. Lonial, M. Amatangelo, R. Popat, M.C. Minnema, J.A. Zonder, J. Larsen, A. Oriol Rocafiguera, E.L. Campagnaro, P. Rodriguez Otero, A.Z. Badros, D. S. Siegel, S. Jagannath, S. Bringhen, M. Gironella, M.F. Kaiser, G. Cook, B. Gamberi, P. Sonneveld, T.V. Nguyen, M.S. Chen, T. Homan, C.C. Bjorklund, M. Wang, W.E. Pierceall, A. Bensmaine, A. Thakurta, T. Peluso, N.W.C.J. Van De Donk, Translational and clinical evidence of a differentiated profile for the novel CELMOD, iberdomide (CC-220), Blood 134 (2019) 3119, https://doi.org/ 10.1182/blood-2019-124298.
- [111] M. Andreeff, K.R. Kelly, K. Yee, S. Assouline, R. Strair, L. Popplewell, D. Bowen, G. Martinelli, M.W. Drummond, P. Vyas, M. Kirschbaum, S.P. Iyer, V. Ruvolo, G. M.N. Gonzalez, X. Huang, G. Chen, B. Graves, S. Blotner, P. Bridge, L. Jukofsky, S. Middleton, M. Reckner, R. Rueger, J. Zhi, G. Nichols, K. Kojima, Results of the phase I trial of RG7112, a small-molecule MDM2 antagonist in leukemia, Clin. Cancer Res. 22 (2016) 868–876, https://doi.org/10.1158/1078-0432.CCR-15-0481.
- [112] K. Yee, G. Martinelli, S. Assouline, M. Kasner, N. Vey, K.R. Kelly, M. W. Drummond, M. Dennis, K. Seiter, S. Blotner, L. Jukofsky, S. Middleton, J. Zhi, G. Chen, H. Zhong, G. Nichols, Phase 1b study of the MDM2 antagonist RG7112 in combination with 2 doses/schedules of cytarabine, Blood 122 (2013) 498, https://doi.org/10.1182/blood.V122.21.498.498.
- [113] B. Reis, L. Jukofsky, G. Chen, G. Martinelli, H. Zhong, W.V. So, M.J. Dickinson, M. Drummond, S. Assouline, M. Hashemyan, M. Theron, S. Blotner, J.-H. Lee, M. Kasner, S.-S. Yoon, R. Rueger, K. Seiter, S.A. Middleton, K.R. Kelly, N. Vey, K. Yee, G. Nichols, L.-C. Chen, W.E. Pierceall, Acute myeloid leukemia patients' clinical response to idasanutlin (RG7388) is associated with pre-treatment MDM2 protein expression in leukemic blasts, Haematologica 101 (2016) e185–e188, https://doi.org/10.3324/HAEMATOL.2015.139717.
- [114] S. Bauer, G. Demetri, S. Jeay, R. Dummer, N. Guerreiro, D.S. Tan, A. Kumar, C. Meille, L. Van Bree, E. Halilovic, J.U. Wuerthner, P. Cassier, A phase I, openlabel, multi-center, dose escalation study of oral NVP-CGM097, a p53/HDM2protein-protein interaction inhibitor, in adult patients with selected advanced solid tumors, Ann. Oncol. 27 (2016) vi116, https://doi.org/10.1093/ANNONC/ MDW368.09.
- [115] M.M. Gounder, T.M. Bauer, G.K. Schwartz, T. Masters, R.D. Carvajal, S. Song, P. Kumar, R. Gajee, O. Zernovak, M.M. Rosen, J.P. Kochan, S. Chen, D.M. Hyman, S. Gokmen, F. Meric-Bernstam, P. LoRusso, N. Somaiah, A.M. Weise, D.S. Hong, A phase 1 study of the MDM2 inhibitor DS-3032b in patients (pts) with advanced solid tumors and lymphomas, 2581–2581, J. Clin. Oncol. 34 (2016), https://doi. org/10.1200/JCO.2016.34.15 SUPPL.2581.
- [116] C.D. DiNardo, J. Rosenthal, M. Andreeff, O. Zernovak, P. Kumar, R. Gajee, S. Chen, M. Rosen, S. Song, J. Kochan, T. Limsakun, R. Olin, Phase 1 dose escalation study of MDM2 inhibitor DS-3032b in patients with hematological malignancies - preliminary results, Blood 128 (2016) 593, https://doi.org/ 10.1182/BLOOD.V128.22.593.593.
- [117] T.M. Bauer, M.M. Gounder, A.M. Weise, G.K. Schwartz, R.D. Carvajal, P. Kumar, O. Zernovak, A. Beck, J. Doyle, J. Mendell-Harary, J.P. Kochan, S. Chen, P. LoRusso, W.D. Tap, N. Somaiah, D.M. Hyman, F. Meric-Bernstam, D.S. Hong, A phase 1 study of MDM2 inhibitor DS-3032b in patients with well/dedifferentiated liposarcoma (WD/DD LPS), solid tumors (ST) and lymphomas (L), J. Clin. Oncol. 36 (2018) 11514, https://doi.org/10.1200/JCO.2018.36.15_ suppl.11514.
- [118] M.M. Gounder, T.M. Bauer, G.K. Schwartz, T. Masters, R.D. Carvajal, S. Song, P. Kumar, R. Gajee, O. Zernovak, M.M. Rosen, J.P. Kochan, S. Chen, D.M. Hyman, S. Gokmen, F. Meric-Bernstam, P. LoRusso, N. Somaiah, A.M. Weise, D.S. Hong, A phase 1 study of the MDM2 inhibitor DS-3032b in patients (pts) with advanced solid tumors and lymphomas, J. Clin. Oncol. 34 (2016), https://doi.org/10.1200/ JCO.2016.34.15_suppl.2581, 2581-2581.
- [119] D.W. Rasco, N.J. Lakhani, Y. Li, L. Men, H. Wang, J. Ji, Y. Tang, Z. Liang, A. Amaya, K. Estkowski, J. Sun, Y. Huang, D. Yang, Y. Zhai, A phase I study of a novel MDM2 antagonist APG-115 in patients with advanced solid tumors, J. Clin. Oncol. 37 (2019), https://doi.org/10.1200/JCO.2019.37.15_suppl.3126, 3126-3126.
- [120] X. Wang, P. D'Arcy, T.R. Caulfield, A. Paulus, K. Chitta, C. Mohanty, J. Gullbo, A. Chanan-Khan, S. Linder, Synthesis and evaluation of derivatives of the proteasome deubiquitinase inhibitor b-AP15, Chem. Biol. Drug Des. 86 (2015) 1036–1048, https://doi.org/10.1111/CBDD.12571.

- [121] E.K. Rowinsky, A. Paner, J.G. Berdeja, C. Paba-Prada, P. Venugopal, K. Porkka, J. Gullbo, S. Linder, A. Loskog, P.G. Richardson, O. Landgren, Phase 1 study of the protein deubiquitinase inhibitor VLX1570 in patients with relapsed and/or refractory multiple myeloma, 2020 385, Investig. N. Drugs 38 (2020) 1448–1453, https://doi.org/10.1007/\$10637-020-00915-4.
- [122] N. Estrada-Ortiz, C.G. Neochoritis, A. Dömling, How to design a successful p53-MDM2/X interaction inhibitor: a thorough overview based on crystal structures, ChemMedChem 11 (2016) 757–772, https://doi.org/10.1002/cmdc.201500487.
- [123] D.C. Fry, S.D. Emerson, S. Palme, B.T. Vu, C.M. Liu, F. Podlaski, NMR structure of a complex between MDM2 and a small molecule inhibitor, J. Biomol. Nmr. 30 (2004) 163–173, https://doi.org/10.1023/B:JNMR.0000048856.84603.9B.
- [124] G.M. Popowicz, A. Czarna, S. Wolf, K. Wang, W. Wang, A. Dömling, T.A. Holak, Structures of low molecular weight inhibitors bound to MDMX and MDM2 reveal new approaches for p53-MDMX/MDM2 antagonist drug discovery, Cell Cycle 9 (2010) 1104–1111, https://doi.org/10.4161/cc.9.6.10956.
- [125] P. Furet, P. Chène, A. De Pover, T.S. Valat, J.H. Lisztwan, J. Kallen, K. Masuya, The central value concept provides an entry in a new class of non peptide inhibitors of the p53-MDM2 interaction, Bioorg. Med. Chem. Lett. 22 (2012) 3498–3502, https://doi.org/10.1016/J.BMCL.2012.03.083.
- [126] C. Tovar, B. Graves, K. Packman, Z. Filipovic, B.H.M. Xia, C. Tardell, R. Garrido, E. Lee, K. Kolinsky, K.H. To, M. Linn, F. Podlaski, P. Wovkulich, B. Vu, L. T. Vassilev, MDM2 small-molecule antagonist RG7112 activates p53 signaling and regresses human tumors in preclinical cancer models, Cancer Res. 73 (2013) 2587–2597, https://doi.org/10.1158/0008-5472.CAN-12-2807.
- [127] B. Anil, C. Riedinger, J.A. Endicott, M.E.M. Noble, The structure of an MDM2-Nutlin-3a complex solved by the use of a validated MDM2 surface-entropy reduction mutant, Acta Crystallogr. D. Biol. Crystallogr. 69 (2013) 1358–1366, https://doi.org/10.1107/S0907444913004459.
- [128] B. Vu, P. Wovkulich, G. Pizzolato, A. Lovey, Q. Ding, N. Jiang, J.J. Liu, C. Zhao, K. Glenn, Y. Wen, C. Tovar, K. Packman, L. Vassilev, B. Graves, Discovery of RG7112: a small-molecule MDM2 inhibitor in clinical development, ACS Med. Chem. Lett. 4 (2013) 466–469, https://doi.org/10.1021/ML4000657/SUPPL_ FILE/ML4000657_SI_001.PDF.
- [129] D.C. Fry, C. Wartchow, B. Graves, C. Janson, C. Lukacs, U. Kammlott, C. Belunis, S. Palme, C. Klein, B. Vu, Deconstruction of a nutlin: dissecting the binding determinants of a potent protein-protein interaction inhibitor, ACS Med. Chem. Lett. 4 (2013) 660–665, https://doi.org/10.1021/ML400062C/SUPPL_FILE/ ML400062C SI 001.PDF.
- [130] A. Vaupel, G. Bold, A. De Pover, T. Stachyra-Valat, J. Hergovich-Lisztwan, J. Kallen, K. Masuya, P. Furet, Tetra-substituted imidazoles as a new class of inhibitors of the p53–MDM2 interaction, Bioorg. Med. Chem. Lett. 24 (2014) 2110–2114, https://doi.org/10.1016/J.BMCL.2014.03.039.
- [131] A. Twarda-Clapa, S. Krzanik, K. Kubica, K. Guzik, B. Labuzek, C.G. Neochoritis, K. Khoury, K. Kowalska, M. Czub, G. Dubin, A. Dömling, L. Skalniak, T.A. Holak, 1,4,5-trisubstituted imidazole-based p53-MDM2/MDMX antagonists with aliphatic linkers for conjugation with biological carriers, J. Med. Chem. 60 (2017) 4234–4244, https://doi.org/10.1021/ACS.JMEDCHEM.7B00104/SUPPL_FILE/ JM7B00104 SI 001.CSV.
- [132] J. Kallen, A. Izaac, S. Chau, E. Wirth, J. Schoepfer, R. Mah, A. Schlapbach, S. Stutz, A. Vaupel, V. Guagnano, K. Masuya, T.M. Stachyra, B. Salem, P. Chene, F. Gessier, P. Holzer, P. Furet, Structural states of Hdm2 and HdmX: X-ray elucidation of adaptations and binding interactions for different chemical compound classes, ChemMedChem 14 (2019) 1305–1314, https://doi.org/ 10.1002/CMDC.201900201.
- [133] E. Surmiak, A. Twarda-Clapa, K.M. Zak, B. Musielak, M.D. Tomala, K. Kubica, P. Grudnik, M. Madej, M. Jablonski, J. Potempa, J. Kalinowska-Tluscik, A. Dömling, G. Dubin, T.A. Holak, A unique Mdm2-binding mode of the 3-pyrrolin-2-one- and 2-furanone-based antagonists of the p53-Mdm2 interaction, ACS Chem. Biol. 11 (2016) 3310–3318, https://doi.org/10.1021/ ACSCHEMBIO.6800596/SUPPL.FUE/CB6B00596 S1 001.PDF.
- [134] B. Graves, T. Thompson, M. Xia, C. Janson, C. Lukacs, D. Deo, P. Di Lello, D. Fry, C. Garvie, K. Sen Huang, L. Gao, C. Tovar, A. Lovey, J. Wanner, L.T. Vassilev, Activation of the p53 pathway by small-molecule-induced MDM2 and MDMX dimerization, Proc. Natl. Acad. Sci. U. S. A. 109 (2012) 11788–11793, https:// doi.org/10.1073/PNAS.1203789109/~/DCSUPPLEMENTAL.
- [135] K. Michelsen, J.B. Jordan, J. Lewis, A.M. Long, E. Yang, Y. Rew, J. Zhou, P. Yakowec, P.D. Schnier, X. Huang, L. Poppe, Ordering of the N-terminus of human MDM2 by small molecule inhibitors, J. Am. Chem. Soc. 134 (2012) 17059–17067, https://doi.org/10.1021/JA305839B/SUPPL_FILE/JA305839B_ SI_001.PDF.
- [136] Y. Rew, D. Sun, F. Gonzalez-Lopez De Turiso, M.D. Bartberger, H.P. Beck, J. Canon, A. Chen, D. Chow, J. Deignan, B.M. Fox, D. Gustin, X. Huang, M. Jiang, X. Jiao, L. Jin, F. Kayser, D.J. Kopecky, Y. Li, M.C. Lo, A.M. Long, K. Michelsen, J. D. Oliner, T. Osgood, M. Ragains, A.Y. Saiki, S. Schneider, M. Toteva, P. Yakowec, X. Yan, Q. Ye, D. Yu, X. Zhao, J. Zhou, J.C. Medina, S.H. Olson, Structure-based design of novel inhibitors of the MDM2-p53 interaction, J. Med. Chem. 55 (2012) 4936–4954, https://doi.org/10.1021/jm300354j.
- [137] D. Sun, Z. Li, Y. Rew, M. Gribble, M.D. Bartberger, H.P. Beck, J. Canon, A. Chen, X. Chen, D. Chow, J. Deignan, J. Duquette, J. Eksterowicz, B. Fisher, B.M. Fox, J. Fu, A.Z. Gonzalez, F. Gonzalez-Lopez De Turiso, J.B. Houze, X. Huang, M. Jiang, L. Jin, F. Kayser, J. Liu, M.C. Lo, A.M. Long, B. Lucas, L.R. McGee, J. McIntosh, J. Mihalic, J.D. Oliner, T. Osgood, M.L. Peterson, P. Roveto, A. Y. Saiki, P. Shaffer, M. Toteva, Y. Wang, Y.C. Wang, S. Wortman, P. Yakowec, X. Yan, Q. Ye, D. Yu, M. Yu, X. Zhao, J. Zhou, J. Zhu, S.H. Olson, J.C. Medina, Discovery of AMG 232, a potent, selective, and orally bioavailable MDM2-p53

inhibitor in clinical development, J. Med. Chem. 57 (2014) 1454–1472, https://doi.org/10.1021/jm401753e.

- [138] A.Z. Gonzalez, Z. Li, H.P. Beck, J. Canon, A. Chen, D. Chow, J. Duquette, J. Eksterowicz, B.M. Fox, J. Fu, X. Huang, J. Houze, L. Jin, Y. Li, Y. Ling, M.C. Lo, A.M. Long, L.R. McGee, J. McIntosh, J.D. Oliner, T. Osgood, Y. Rew, A.Y. Saiki, P. Shaffer, S. Wortman, P. Yakowec, X. Yan, Q. Ye, D. Yu, X. Zhao, J. Zhou, S. H. Olson, D. Sun, J.C. Medina, Novel inhibitors of the MDM2-p53 interaction featuring hydrogen bond acceptors as carboxylic acid isosteres, J. Med. Chem. 57 (2014) 2963–2988, https://doi.org/10.1021/JM401911V/SUPPL_FILE/ JM401911V SI 001.PDF.
- [139] M. Yu, Y. Wang, J. Zhu, M.D. Bartberger, J. Canon, A. Chen, D. Chow, J. Eksterowicz, B. Fox, J. Fu, M. Gribble, X. Huang, Z. Li, J. Liu, M.C. Lo, D. McMinn, J.D. Oliner, T. Osgood, Y. Rew, A.Y. Saiki, P. Shaffer, X. Yan, Q. Ye, D. Yu, X. Zhao, J. Zhou, S.H. Olson, J.C. Medina, D. Sun, Discovery of potent and simplified piperidinone-based inhibitors of the MDM2-p53 interaction, ACS Med. Chem. Lett. 5 (2014) 894–899, https://doi.org/10.1021/ML500142B/SUPPL_ FILE/ML500142B_SI_001.PDF.
- [140] Y. Wang, J. Zhu, J. Liu, X. Chen, J. Mihalic, J. Deignan, M. Yu, D. Sun, F. Kayser, L.R. McGee, M.C. Lo, A. Chen, J. Zhou, Q. Ye, X. Huang, A.M. Long, P. Yakowec, J.D. Oliner, S.H. Olson, J.C. Medina, Optimization beyond AMG 232: Discovery and SAR of sulfonamides on a piperidinone scaffold as potent inhibitors of the MDM2-p53 protein–protein interaction, Bioorg. Med. Chem. Lett. 24 (2014) 3782–3785, https://doi.org/10.1016/J.BMCL.2014.06.073.
- [141] Y. Rew, D. Sun, X. Yan, H.P. Beck, J. Canon, A. Chen, J. Duquette, J. Eksterowicz, B.M. Fox, J. Fu, A.Z. Gonzalez, J. Houze, X. Huang, M. Jiang, L. Jin, Y. Li, Z. Li, Y. Ling, M.C. Lo, A.M. Long, L.R. McGee, J. McIntosh, J.D. Oliner, T. Osgood, A. Y. Saiki, P. Shaffer, Y.C. Wang, S. Wortman, P. Yakowec, Q. Ye, D. Yu, X. Zhao, J. Zhou, J.C. Medina, S.H. Olson, Discovery of AM-7209, a potent and selective 4amidobenzoic acid inhibitor of the MDM2-p53 interaction, J. Med. Chem. 57 (2014) 10499–10511, https://doi.org/10.1021/JM501550P/SUPPL_FILE/ JM501550P_SI_001.PDF.
- [142] F.G.L. De Turiso, D. Sun, Y. Rew, M.D. Bartberger, H.P. Beck, J. Canon, A. Chen, D. Chow, T.L. Correll, X. Huang, L.D. Julian, F. Kayser, M.C. Lo, A.M. Long, D. McMinn, J.D. Oliner, T. Osgood, J.P. Powers, A.Y. Saiki, S. Schneider, P. Shaffer, S.H. Xiao, P. Yakowec, X. Yan, Q. Ye, D. Yu, X. Zhao, J. Zhou, J. C. Medina, S.H. Olson, Rational design and binding mode duality of MDM2-p53 inhibitors, J. Med. Chem. 56 (2013) 4053–4070, https://doi.org/10.1021/ JM400293Z/SUPPL_FILE/JM400293Z_SL_001.PDF.
- [143] A.Z. Gonzalez, J. Eksterowicz, M.D. Bartberger, H.P. Beck, J. Canon, A. Chen, D. Chow, J. Duquette, B.M. Fox, J. Fu, X. Huang, J.B. Houze, L. Jin, Y. Li, Z. Li, Y. Ling, M.C. Lo, A.M. Long, L.R. McGee, J. McIntosh, D.L. McMinn, J.D. Oliner, T. Osgood, Y. Rew, A.Y. Saiki, P. Shaffer, S. Wortman, P. Yakowec, X. Yan, Q. Ye, D. Yu, X. Zhao, J. Zhou, S.H. Olson, J.C. Medina, D. Sun, Selective and potent morpholinone inhibitors of the MDM2-p53 protein-protein interaction, J. Med. Chem. 57 (2014) 2472–2488, https://doi.org/10.1021/JM401767K/SUPPL_ FILE/JM401767K SI_001.PDF.
- [144] F. Gessier, J. Kallen, E. Jacoby, P. Chène, T. Stachyra-Valat, S. Ruetz, S. Jeay, P. Holzer, K. Masuya, P. Furet, Discovery of dihydroisoquinolinone derivatives as novel inhibitors of the p53–MDM2 interaction with a distinct binding mode, Bioorg. Med. Chem. Lett. 25 (2015) 3621–3625, https://doi.org/10.1016/J. BMCL.2015.06.058.
- [145] G. Chessari, I.R. Hardcastle, J.S. Ahn, B. Anil, E. Anscombe, R.H. Bawn, L. D. Bevan, T.J. Blackburn, I. Buck, C. Cano, B. Carbain, J. Castro, B. Cons, S. J. Cully, J.A. Endicott, L. Fazal, B.T. Golding, R.J. Griffin, K. Hagerty, S. J. Harnor, K. Hearn, S. Hobson, R.S. Holvey, S. Howard, C.E. Jennings, C. N. Johnson, J. Lunec, D.C. Miller, D.R. Newell, M.E.M. Noble, J. Reeks, C. H. Revill, C. Riedinger, J.D. Denis St., E. Tamanini, H. Thomas, N.T. Thompson, M. Vinković, S.R. Wedge, P.A. Williams, N.E. Wilsher, B. Zhang, Y. Zhao, Structure-based design of potent and orally active isoindolinone inhibitors of MDM2-p53 protein-protein interaction, J. Med. Chem. 64 (2021) 4071–4088, https://doi.org/10.1021/acs.jmedchem.0c02188.
- [146] Z. Zhang, Q. Ding, J.J. Liu, J. Zhang, N. Jiang, X.J. Chu, D. Bartkovitz, K.C. Luk, C. Janson, C. Tovar, Z.M. Filipovic, B. Higgins, K. Glenn, K. Packman, L. T. Vassilev, B. Graves, Discovery of potent and selective spiroindolinone MDM2 inhibitor, RO8994, for cancer therapy, Bioorg. Med. Chem. 22 (2014) 4001–4009, https://doi.org/10.1016/J.BMC.2014.05.072.
- [147] P. Furet, K. Masuya, J. Kallen, T. Stachyra-Valat, S. Ruetz, V. Guagnano, P. Holzer, R. Mah, S. Stutz, A. Vaupel, P. Chène, S. Jeay, A. Schlapbach, Discovery of a novel class of highly potent inhibitors of the p53–MDM2 interaction by structure-based design starting from a conformational argument, Bioorg. Med. Chem. Lett. 26 (2016) 4837–4841, https://doi.org/10.1016/J. BMCL-2016.08.010.
- [148] S. Jeay, S. Ferretti, P. Holzer, J. Fuchs, E.A. Chapeau, M. Wartmann, D. Sterker, V. Romanet, M. Murakami, G. Kerr, E.Y. Durand, S. Gaulis, M. Cortes-Cros, S. Ruetz, T.M. Stachyra, J. Kallen, P. Furet, J. Würthner, N. Guerreiro, E. Halilovic, A. Jullion, A. Kauffmann, E. Kuriakose, M. Wiesmann, M.R. Jensen, F. Hofmann, W.R. Sellers, Dose and schedule determine distinct molecular mechanisms underlying the efficacy of the p53-MDM2 inhibitor HDM201, Cancer Res. 78 (2018) 6257–6267, https://doi.org/10.1158/0008-5472.CAN-18-0338.
- [149] A. Vaupel, P. Holzer, S. Ferretti, V. Guagnano, J. Kallen, R. Mah, K. Masuya, S. Ruetz, C. Rynn, A. Schlapbach, T. Stachyra, S. Stutz, M. Todorov, S. Jeay, P. Furet, In vitro and in vivo characterization of a novel, highly potent p53-MDM2 inhibitor, Bioorg. Med. Chem. Lett. 28 (2018) 3404–3408, https://doi.org/ 10.1016/J.BMCL.2018.08.027.
- [150] M. Miyazaki, H. Naito, Y. Sugimoto, H. Kawato, T. Okayama, H. Shimizu, M. Miyazaki, M. Kitagawa, T. Seki, S. Fukutake, M. Aonuma, T. Soga, Lead

optimization of novel p53-MDM2 interaction inhibitors possessing dihydroimidazothiazole scaffold, Bioorg. Med. Chem. Lett. 23 (2013) 728–732, https://doi.org/10.1016/j.bmcl.2012.11.091.

- [151] M. Miyazaki, H. Naito, Y. Sugimoto, K. Yoshida, H. Kawato, T. Okayama, H. Shimizu, M. Miyazaki, M. Kitagawa, T. Seki, S. Fukutake, Y. Shiose, M. Aonuma, T. Soga, Synthesis and evaluation of novel orally active p53–MDM2 interaction inhibitors, Bioorg. Med. Chem. 21 (2013) 4319–4331, https://doi. org/10.1016/J.BMC.2013.04.056.
- [152] B.L. Grasberger, T. Lu, C. Schubert, D.J. Parks, T.E. Carver, H.K. Koblish, M. D. Cummings, L.V. LaFrance, K.L. Milkiewicz, R.R. Calvo, D. Maguire, J. Lattanze, C.F. Franks, S. Zhao, K. Ramachandren, G.R. Bylebyl, M. Zhang, C.L. Manthey, E. C. Petrella, M.W. Pantoliano, I.C. Deckman, J.C. Spurlino, A.C. Maroney, B. E. Tomczuk, C.J. Molloy, R.F. Bone, Discovery and cocrystal structure of benzodiazepinedione HDM2 antagonists that activate p53 in cells, J. Med. Chem. 48 (2005) 909–912, https://doi.org/10.1021/jm049137g.
- [153] Z. Zhang, X.J. Chu, J.J. Liu, Q. Ding, J. Zhang, D. Bartkovitz, N. Jiang, P. Karnachi, S.S. So, C. Tovar, Z.M. Filipovic, B. Higgins, K. Glenn, K. Packman, L. Vassilev, B. Graves, Discovery of potent and orally active p53-MDM2 inhibitors RO53553 and RO2468 for potential clinical development, ACS Med. Chem. Lett. 5 (2014) 124–127, https://doi.org/10.1021/ML400359Z/SUPPL_FILE/ ML400359Z SI 001.PDF.
- [154] A. Gollner, D. Rudolph, H. Arnhof, M. Bauer, S.M. Blake, G. Boehmelt, X. L. Cockroft, G. Dahmann, P. Ettmayer, T. Gerstberger, J. Karolyi-Oezguer, D. Kessler, C. Kofink, J. Ramharter, J. Rinnenthal, A. Savchenko, R. Schnitzer, H. Weinstabl, U. Weyer-Czernilofsky, T. Wunberg, D.B. McConnell, Discovery of Novel Spiro[3H-indole-3,2'-pyrrolidin]-2(1H)-one compounds as chemically stable and orally active inhibitors of the MDM2-p53 interaction, J. Med. Chem. 59 (2016) 10147–10162, https://doi.org/10.1021/acs.jmedchem.6b00900.
- [155] S. Wang, W. Sun, Y. Zhao, D. McEachern, I. Meaux, C. Barrière, J.A. Stuckey, J. L. Meagher, L. Bai, L. Liu, C.G. Hoffman-Luca, J. Lu, S. Shangary, S. Yu, D. Bernard, A. Aguilar, O. Dos-Santos, L. Besret, S. Guerif, P. Pannier, D. Gorge-Bernat, L. Debussche, SAR405838: an optimized inhibitor of MDM2-p53 interaction that induces complete and durable tumor regression, Cancer Res. 74 (2014) 5855–5865, https://doi.org/10.1158/0008-5472, CAN-14-0799.
- [156] A. Gollner, H. Weinstabl, J.E. Fuchs, D. Rudolph, G. Garavel, K.S. Hofbauer, J. Karolyi-Oezguer, G. Gmaschitz, W. Hela, N. Kerres, E. Grondal, P. Werni, J. Ramharter, J. Broeker, D.B. McConnell, Targeted synthesis of complex spiro [3H-indole-3,2'-pyrrolidin]-2(1H)-ones by intramolecular cyclization of azomethine ylides: highly potent MDM2-p53 inhibitors, ChemMedChem 14 (2019) 88–93, https://doi.org/10.1002/CMDC.201800617.
- [157] P.H. Kussie, S. Gorina, V. Marechal, B. Elenbaas, J. Moreau, A.J. Levine, N. P. Pavletich, Structure of the MDM2 oncoprotein bound to the p53 tumor suppressor transactivation domain, Science 274 (1996) 948–953, https://doi.org/ 10.1126/science.274.5289.948.
- [158] J. Nicholson, T.R. Hupp, The molecular dynamics of MDM2, Cell Cycle 9 (2010) 1878–1881, https://doi.org/10.4161/cc.9.10.11597.
- [159] D.J. Parks, L.V. LaFrance, R.R. Calvo, K.L. Milkiewicz, J. José Marugán, P. Raboisson, C. Schubert, H.K. Koblish, S. Zhao, C.F. Franks, J. Lattanze, T. E. Carver, M.D. Cummings, D. Maguire, B.L. Grasberger, A.C. Maroney, T. Lu, Enhanced pharmacokinetic properties of 1,4-benzodiazepine-2,5-dione antagonists of the HDM2-p53 protein-protein interaction through structure-based drug design, Bioorg. Med. Chem. Lett. 16 (2006) 3310–3314, https://doi.org/ 10.1016/j.bmcl.2006.03.055.
- [160] K. Ding, Y. Lu, Z. Nikolovska-Coleska, S. Qiu, Y. Ding, W. Gao, J. Stuckey, K. Krajewski, P.P. Roller, Y. Tomita, D.A. Parrish, J.R. Deschamps, S. Wang, Structure-based design of potent non-peptide MDM2 inhibitors, J. Am. Chem. Soc. 127 (2005) 10130–10131, https://doi.org/10.1021/ja051147z.
- [161] I.R. Hardcastle, J. Liu, E. Valeur, A. Watson, U. Ahmed, T.J. Blackburn, K. Bennaceur, W. Clegg, C. Drummond, J.A. Endicott, B.T. Golding, R.J. Gri, J. Gruber, K. Haggerty, R.W. Harrington, C. Hutton, S. Kemp, X. Lu, J. M. Mcdonnell, D.R. Newell, M.E.M. Noble, S.L. Payne, C.H. Revill, C. Riedinger, Q. Xu, J. Lunec, Isoindolinone Inhibitors of the Murine Double Minute 2 (MDM2)p53 Protein–Protein Interaction: Structure–Activity Studies Leading to Improved Potency, J. Med. Chem. 2 (2011) 1233–1243, https://doi.org/10.1021/ im1011929.
- [162] P.R. Elliott, Molecular basis for specificity of the Met1-linked polyubiquitin signal, Biochem. Soc. Trans. 44 (2016) 1581–1602, https://doi.org/10.1042/ BST20160227.
- [163] T.R. Cotton, B.C. Lechtenberg, Chain reactions: molecular mechanisms of RBR ubiquitin ligases, Biochem. Soc. Trans. 48 (2020) 1737–1750, https://doi.org/ 10.1042/BST20200237.
- [164] H. Johansson, Y.C.I. Tsai, K. Fantom, C.W. Chung, S. Kümper, L. Martino, D. A. Thomas, H.C. Eberl, M. Muelbaier, D. House, K. Rittinger, Fragment-Based Covalent ligand screening enables rapid discovery of inhibitors for the RBR E3 ubiquitin ligase HOIP, J. Am. Chem. Soc. 141 (2020) 2703–2712, https://doi.org/ 10.1021/jacs.8b13193.
- [165] Y.C.I. Tsai, H. Johansson, D. Dixon, S. Martin, C. wa Chung, J. Clarkson, D. House, K. Rittinger, Single-domain antibodies as crystallization chaperones to enable structure-based inhibitor development for RBR E3 ubiquitin ligases, 83-93.e9, Cell Chem. Biol. 27 (2020), https://doi.org/10.1016/j.chembiol.2019.11.007.
- [166] D. Oikawa, Y. Sato, F. Ohtake, K. Komakura, K. Hanada, K. Sugawara, S. Terawaki, Y. Mizukami, H.T. Phuong, K. Iio, S. Obika, M. Fukushi, T. Irie, D. Tsuruta, S. Sakamoto, K. Tanaka, Y. Saeki, S. Fukai, F. Tokunaga, Molecular

bases for HOIPINs-mediated inhibition of LUBAC and innate immune responses, Commun, Biol 3 (2020), https://doi.org/10.1038/s42003-020-0882-8.

- [167] P.R. Leger, D.X. Hu, B. Biannic, M. Bui, X. Han, E. Karbarz, J. Maung, A. Okano, M. Osipov, G.M. Shibuya, K. Young, C. Higgs, B. Abraham, D. Bradford, C. Cho, C. Colas, S. Jacobson, Y.M. Ohol, D. Pookot, P. Rana, J. Sanchez, N. Shah, M. Sun, S. Wong, D.G. Brockstedt, P.D. Kassner, J.B. Schwarz, D.J. Wustrow, Discovery of potent, selective, and orally bioavailable inhibitors of USP7 with in vivo antitumor activity, J. Med. Chem. 63 (2020) 5398–5420, https://doi.org/ 10.1021/acs.jmedchem.0c00245.
- [168] P. Di Lello, R. Pastor, J.M. Murray, R.A. Blake, F. Cohen, T.D. Crawford, J. Drobnick, J. Drummond, L. Kategaya, T. Kleinheinz, T. Maurer, L. Rougé, X. Zhao, I. Wertz, C. Ndubaku, V. Tsui, Discovery of small-molecule inhibitors of ubiquitin specific protease 7 (USP7) using integrated NMR and in silico techniques, J. Med. Chem. 60 (2017) 10056–10070, https://doi.org/10.1021/ acs.jmedchem.7b01293.
- [169] M. Li, S. Liu, H. Chen, X. Zhou, J. Zhou, S. Zhou, H. Yuan, Q.L. Xu, J. Liu, K. Cheng, H. Sun, Y. Wang, C. Chen, X. Wen, N-benzylpiperidinol derivatives as novel USP7 inhibitors: structure–activity relationships and X-ray crystallographic studies, Eur. J. Med. Chem. 199 (2020), 112279, https://doi.org/10.1016/j. ejmech.2020.112279.
- [170] A.P. Turnbull, S. Ioannidis, W.W. Krajewski, A. Pinto-Fernandez, C. Heride, A.C. L. Martin, L.M. Tonkin, E.C. Townsend, S.M. Buker, D.R. Lancia, J.A. Caravella, A. V. Toms, T.M. Charlton, J. Lahdenranta, E. Wilker, B.C. Follows, N.J. Evans, L. Stead, C. Alli, V.V. Zarayskiy, A.C. Talbot, A.J. Buckmelter, M. Wang, C. L. McKinnon, F. Saab, J.F. McGouran, H. Century, M. Gersch, M.S. Pittman, C. Gary Marshall, T.M. Raynham, M. Simcox, L.M.D. Stewart, S.B. McLoughlin, J. A. Escobedo, K.W. Bair, C.J. Dinsmore, T.R. Hammonds, S. Kim, S. Urbé, M. J. Clague, B.M. Kessler, D. Komander, Molecular basis of USP7 inhibition by selective small-molecule inhibitors, Nature 550 (2017) 481–486, https://doi.org/10.1038/nature24451.
- [171] G. Gavory, C.R. O'Dowd, M.D. Helm, J. Flasz, E. Arkoudis, A. Dossang, C. Hughes, E. Cassidy, K. McClelland, E. Odrzywol, N. Page, O. Barker, H. Miel, T. Harrison, Discovery and characterization of highly potent and selective allosteric USP7 inhibitors, Nat. Chem. Biol. 14 (2018) 118–125, https://doi.org/10.1038/ nchembio.2528.
- [172] L. Kategaya, P. Di Lello, L. Rougé, R. Pastor, K.R. Clark, J. Drummond, T. Kleinheinz, E. Lin, J.P. Upton, S. Prakash, J. Heideker, M. McCleland, M. S. Ritorto, D.R. Alessi, M. Trost, T.W. Bainbridge, M.C.M. Kwok, T.P. Ma, Z. Stiffler, B. Brasher, Y. Tang, P. Jaishankar, B.R. Hearn, A.R. Renslo, M.R. Arkin, F. Cohen, K. Yu, F. Peale, F. Gnad, M.T. Chang, C. Klijn, E. Blackwood, S. E. Martin, W.F. Forrest, J.A. Ernst, C. Ndubaku, X. Wang, M.H. Beresini, V. Tsui, C. Schwerdtfeger, R.A. Blake, J. Murray, T. Maurer, I.E. Wertz, USP7 smallmolecule inhibitors interfere with ubiquitin binding, Nature 550 (2017) 534–538, https://doi.org/10.1038/nature24006.
- [173] C.R. O'Dowd, M.D. Helm, J.S.S. Rountree, J.T. Flasz, E. Arkoudis, H. Miel, P. R. Hewitt, L. Jordan, O. Barker, C. Hughes, E. Rozycka, E. Cassidy, K. McClelland, E. Odrzywol, N. Page, S. Feutren-Burton, S. Dvorkin, G. Gavory, T. Harrison, Identification and structure-guided development of pyrimidinone based USP7 inhibitors, ACS Med. Chem. Lett. 9 (2018) 238–243, https://doi.org/10.1021/ acsmedchemlett.7b00512.
- [174] I. Lamberto, X. Liu, H.S. Seo, N.J. Schauer, R.E. Iacob, W. Hu, D. Das, T. Mikhailova, E.L. Weisberg, J.R. Engen, K.C. Anderson, D. Chauhan, S. Dhe-Paganon, S.J. Buhrlage, Structure-guided development of a potent and selective non-covalent active-site inhibitor of USP7, 1490-1500.e11, Cell Chem. Biol. 24 (2017), https://doi.org/10.1016/j.chembiol.2017.09.003.
- [175] Y. Wang, Y. Jiang, S. Ding, J. Li, N. Song, Y. Ren, D. Hong, C. Wu, B. Li, F. Wang, W. He, J. Wang, Z. Mei, Small molecule inhibitors reveal allosteric regulation of USP14 via steric blockade, Cell Res. 28 (2018) 1186–1194, https://doi.org/ 10.1038/s41422-018-0091-x.
- [176] A. Pozhidaeva, I. Bezsonova, USP7: structure, substrate specificity, and inhibition, DNA Repair (Amst.) 76 (2019) 30–39, https://doi.org/10.1016/j. dnarep.2019.02.005.
- [177] Z. Wang, X. Wang, Y. Kang, H. Zhong, C. Shen, X. Yao, D. Cao, T. Hou, Binding affinity and dissociation pathway predictions for a series of USP7 inhibitors with pyrimidinone scaffold by multiple computational methods, Phys. Chem. Chem. Phys. 22 (2020) 5487–5499, https://doi.org/10.1039/d0cp00370k.
- [178] K. Misawa, S.I. Ozawa, T. Yoshida, I. Nakagome, N. Yamaotsu, S. Hirono, Identifying inhibitors of USP7-HDM2 protein-protein interaction (PPI) by the in silico fragment-mapping method, Yakugaku Zasshi 139 (2019) 827–835, https:// doi.org/10.1248/yakushi.19-00006.
- [179] A. Pozhidaeva, G. Valles, F. Wang, J. Wu, D.E. Sterner, P. Nguyen, J. Weinstock, K.G.S. Kumar, J. Kanyo, D. Wright, I. Bezsonova, USP7-specific inhibitors target and modify the enzyme's active site via distinct chemical mechanisms, 1501-1512.e5, Cell Chem. Biol. 24 (2017), https://doi.org/10.1016/j. chembiol.2017.09.004.
- [180] M. Hu, P. Li, L. Song, P.D. Jeffrey, T.A. Chenova, K.D. Wilkinson, R.E. Cohen, Y. Shi, Structure and mechanisms of the proteasome-associated deubiquitinating enzyme USP14, EMBO J. 24 (2005) 3747–3756, https://doi.org/10.1038/sj. emboj.7600832.
- [181] A. Mofers, Proteasome Inhibitors against Cancer: Determining Biology and Finding Novel Compounds, 79, Linköping University Electronic Press, 2020, https://doi.org/10.3384/diss.diva-167520.