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# Opening of a phase Ib/II study to investigate the safety and efficacy of Afatinib in patients with Fanconi anemia and unresectable locally advanced or metastatic head and neck squamous cell carcinoma

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## Abstract

**Background** Individuals diagnosed with Fanconi anemia (FA) present an incidence of 500- to 700-fold higher to develop head and neck squamous carcinomas (HNSCCs) compared to the general population. Effective anticancer treatments for FA-HNSCCs are missing. Several studies demonstrated that FA-HNSCCs overexpress the epithelial growth factor receptor (EGFR) and their viability is highly dependent on this pathway, as FA-HNSCCs cells are highly sensitive to EGFR inhibitors such as afatinib in preclinical models, which led to an orphan drug designation by EMA in 2018.

**Methods** The AFAN trial is a phase Ib/II, single arm, non-randomized, open-label, multicenter study to determine whether afatinib is effective and safe in patients with FA and advanced / metastatic HNSCC. Patients could be treatment-naïve or progressed to a previous systemic treatment with immunotherapy, chemotherapy or cetuximab. Afatinib will be administered orally at a starting dose of 20 mg /day (weeks 1–2), escalating to 30 mg / day at weeks 3–4 and to 40 mg / day thereafter provided no adverse events occur. Treatment will be maintained until disease progression / secondary primary tumor, loss to follow-up, unacceptable toxicity, patient withdrawal or death. Dose reductions and delays will be allowed. All patients will undergo periodic tumor assessments by CT or MRI scan every 12 weeks (3 months) from the start of the study treatment until progression / SPT or patient withdrawal. The primary endpoint is objective response rate (ORR) after 9 months of study treatment initiation according to RECIST V1.1. Secondary endpoints include disease control rate, duration of response, disease-free survival, overall survival, safety, patient reported outcomes and ancillary studies. The expected sample size is 25 patients calculated using a Simon II stage design ($\alpha = 0.1$; $\beta = 80\%$), taking as null hypothesis a 9-month ORR of 20% and an alternative ORR of 40%.

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**Discussion** The AFAN trial will investigate if afatinib is an effective treatment in FA patients with unresectable and / or metastatic locoregionally advanced squamous cell carcinoma of the oral cavity, oropharynx or hypopharynx or larynx.

**Trial registration** EU CT 20,245,114,772,900 / www.clinicaltrials.gov NCT06648096 (August, 1st 2024).

**Keywords** Fanconi anemia, Head and neck squamous cell carcinoma, EGFR inhibitors, Afatinib

## Background

Fanconi anemia (FA) is a germline DNA repair disorder characterized by genome instability. Individuals diagnosed with FA are genetically predisposed to leukemias and solid tumors, with a cumulative cancer incidence of 86% by age 50. Head and neck squamous cell carcinomas (HNSCC) is the type of cancer most commonly associated with FA, with an increased probability of 500- to 700 folds, reaching an incidence of 14% by the age of 40 years [1–4].

The clinical presentation of HNSCC in patients with FA is very aggressive and commonly it is diagnosed at advanced stages. The cellular mechanisms driving the development of FA-HNSCC arise from key disease hallmarks, including chromosomal fragility and hypersensitivity to interstrand crosslink (ICL)-inducing drugs [5]. The FA/BRCA pathway is activated during replication, and the proteins involved participate in the mechanisms of interstrand crosslink (ICL) repair. Defects in ICL repair increase susceptibility to DNA breakage and rearrangement, promoting an increase in oncogene alteration and loss of tumor suppressor genes [6]. Defects in FA proteins lead to dysregulation of pathways related to the cell cycle, resulting in increased p53/p21 activation, cell death, cell reprogramming defects and inflammation [6–8]. Therefore, patients with FA usually show limited efficacy with standard therapies to treat HNSCC, such as chemo- and radiotherapy, since their non-cancerous cells have an increased sensitivity to these two mainstay treatments [9]. Often they require reduced doses, which affects the efficacy of treatments [10]. Moreover, due to the rarity of this disease, data is limited on use of chemotherapy either at standard doses or reduced doses in patients with FA-HNSCC [11, 12]. Consequently, surgical resection is often the first-line therapeutic approach. However, surgery is not always a viable option.

The tyrosine kinase inhibitors (TKIs) are an effective treatment for achieving a complete response and improved survival rates in patients with HNSCC [13]. Among novel TKIs, stands afatinib, a potent and selective ErbB family blocker. Afatinib covalently binds to and irreversibly blocks signaling from all homo- and heterodimers formed by the ErbB family members EGFR (ErbB1), HER2 (ErbB2), ErbB3 and ErbB4. The clinical proof of concept for afatinib in recurrent or metastatic HNSCC has been established in a phase II study with afatinib versus cetuximab and a phase III study versus methotrexate, in which the anti-tumoral activity of afatinib was at least comparable to the activity of standard of care [14, 15]. FA-HNSCC cells overexpress EGFR and are highly dependent on this pathway for survival [16]. In line with these findings, preclinical studies in FA-HNSCC cell lines and xenografted and transgenic mouse models, showed that afatinib treatment inhibited the growth of subcutaneous tumors derived from FA-patient cell lines known to co-express erbB receptors [16]. This preclinical data led to the orphan drug designation of afatinib for FA-HNSCC by the European Medicines Agency (EMA) in 2018. More recently, whole genome sequencing and RNAseq data from a relevant number of tumors was reported to show that EGFR is one of the most overexpressed and amplified cancer driver gene in FA HNSCC compared to sporadic HNSCC [9].

The aim of the AFAN trial is to provide a new and effective treatment therapy for patients with FA diagnosed with HNSCC by targeting EGFR overexpressed in their malignant cells using the irreversible TKI afatinib.

## Methods/design

### Design

The AFAN trial (EU-CT: 2024-511477-29-00/NCT: NCT06648096) is a phase Ib/II, single-arm, open-label, multicenter study designed to evaluate the efficacy and safety of afatinib in patients with FA diagnosed with locoregionally unresectable and/or metastatic HNSCC. This study will follow a Simon II stage design: in the first stage 12 patients will be recruited and at least 2 confirmed responses according to RECIST v1.1 [17] will be required to follow to the second stage. In the second stage, the trial will include 13 additional patients. A total of 7 responses out of the 25 patients will be required to declare the study positive in terms of efficacy.

The primary endpoint for efficacy in the AFAN trial will be objective response rate (ORR), after 9 months of study treatment initiation (Fig. 1). As secondary efficacy endpoints, disease control rate (DCR), duration of response (DoR), disease free survival (DFS) and overall survival (OS) will be evaluated. Safety and tolerability of afatinib in patients with FA-HNSCC will be assessed by the frequency and severity of adverse events (AEs) and treatment-related adverse events (TRAEs) assessed by National Cancer Institute (NCI) common terminology criteria for adverse events (CTCAE) v5.0. In addition, AFAN trial will assess the correlation between

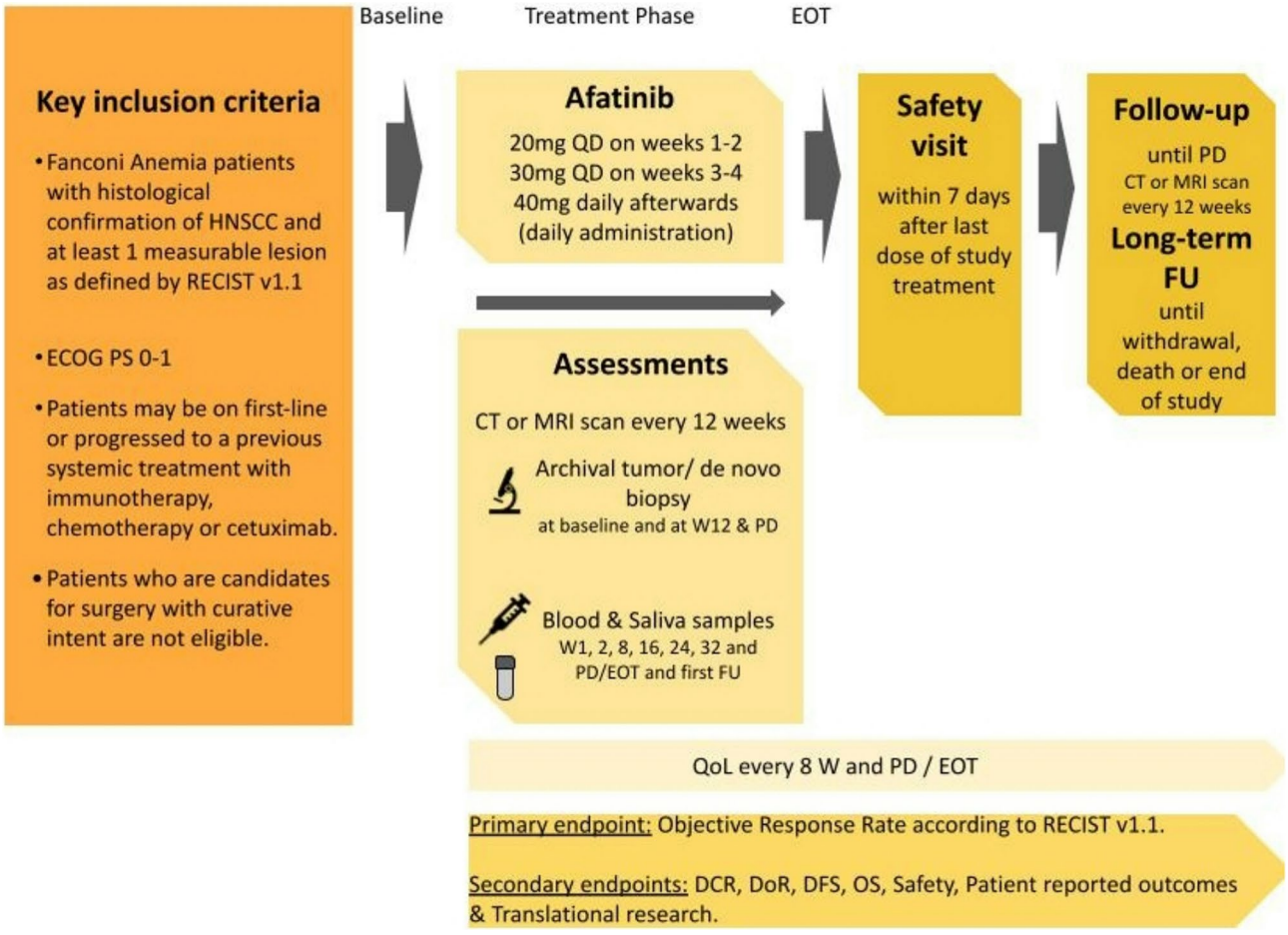


**Fig. 1** The AFAN study protocol design

pathology-specific genetic alterations (i.e. EGFR genetic alterations) at baseline and during treatment and the efficacy of afatinib for HNSCC.

The trial will be conducted in two sites, one in Spain (IR Sant Pau, Hospital de Sant Pau, Barcelona) and the other in Germany (Hannover University Hospital, Hannover). The first site was already opened in Nov2024, and the recruitment time is estimated to last approximately 30 months. The end of study is planned for 1Q2029.

### Target population and allocation

AFAN trial will recruit patients, male or female, ≥ 18 years, with Eastern Cooperative Oncology Group (ECOG) performance status (PS) 0–1, having a histological confirmation of HNSCC and at least 1 measurable lesion as defined by RECIST v1.1. Patients may be on first-line or progressed to a previous systemic treatment with immunotherapy, chemotherapy or cetuximab (Fig. 1).

### Inclusion criteria

1. Written informed consent according to local guidelines, must be signed and dated by the participant and investigator prior to performing any protocol procedure.
2. Patients ≥ 18 years of age.
3. Confirmed diagnosis of Fanconi anemia.
4. Histologically or cytologically confirmed unresectable or locoregionally advanced squamous cell carcinoma of the oral cavity, oropharynx, hypopharynx, larynx, nasopharynx, paranasal sinuses or salivary glands. Patients with distal metastasis (M1, AJCC 8th ed.) are also eligible.
5. Tumor not a candidate for resection prior to afatinib due to technical inability to resect (tumor fixation/invasion in the skull base, cervical vertebrae, nasopharynx or fixed lymph nodes) and/or low surgical cure [T3-T4, N2-N3; AJCC 8th ed.]).
6. Patients must not be candidates for other curative standard treatment options including radiotherapy, chemotherapy or immunotherapy.

7. Patients must have at least 1 measurable lesion by computed tomography (CT) scan or magnetic resonance imaging (MRI) as defined by RECIST v1.1 [17].
8. Previous anticancer treatment is allowed if it ends 6 weeks or 5 half-lives, whichever is shorter, before the expected date of start of the study treatment.
9. Previous locoregional treatments such as radiotherapy are allowed.
10. ECOG PS < 2 at inclusion.
11. Adequate organ and bone marrow functions, as defined below:

a) Neutrophils > 1000 cells/microliter.
b) Platelets > 50,000 cells/microliter.
c) Hemoglobin > 8 g/dL.
d) Creatinine < 1.5 x upper limit normal (ULN) with clearance > 50 mL/min.
e) Total bilirubin < 1.5 x ULN. Note: patients with Gilbert's may be included with bilirubin < 2 x ULN.
f) Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) < 2.5 x ULN or < 5 ULN if liver metastases are present.
g) International normalized ratio (INR) and prothrombin time (PT) < 1.5 x ULN.

12. Female patients must either:

a) Be of nonchildbearing potential:

i. Postmenopausal *(defined as at least 1 year without any menses) prior to screening, or.
ii. Documented surgically sterile (e.g., hysterectomy, bilateral salpingectomy, bilateral oophorectomy, or bilateral tubal occlusion).

**Those who are amenorrheic due to an alternative medical cause are not considered postmenopausal and must follow the criteria for childbearing potential subjects.*
*OR.*

b) If of childbearing potential:

i. Agree not to try to become pregnant during the study and for at least 1 months after the final study drug administration,
a And have a negative urine or serum pregnancy test within 7 days prior to Day 1 (females with false positive results and documented verification of negative pregnancy status are eligible for participation),
b And if heterosexually active, agree to abstinence (if in line with the usual preferred lifestyle of the patient) or consistently use a condom plus 1 form of highly effective birth control per locally accepted standards starting at screening and throughout the study period and for at least 1 month after the final study drug administration.

13. Female patients must agree not to breastfeed or donate ovules starting at screening and throughout the study period, and for at least 1 month after the final study drug administration.
14. Male patients must not donate sperm starting at screening and throughout the study period, and for at least 1 month after the final study drug administration.
15. Male patients with a partner with childbearing potential, or who is pregnant or breastfeeding must agree to abstinence or use a condom plus 1 form of highly effective birth control throughout the study period and for at least 1 month after the final study drug administration.
16. Patient agrees not to participate in another interventional study while on treatment in the present study.

**Exclusion criteria**

1. Patients who are candidates for surgery with curative intent are not eligible.
2. Less than two weeks from surgical resection or other major surgical procedure at start of treatment. Planned surgery for other diseases.
3. Previous treatment with EGFR small molecule inhibitors, EGFR inhibitory antibodies and/or any investigational agents for the treatment of HNSCC within 4 weeks prior to the selection was not allowed.

**Note** *Previous treatment with chemotherapy and/or radiotherapy is allowed.*

4. Patient must have recovered from any previous treatment toxicity to Grade ≤ 2.
5. Existence of any other intercurrent malignant disease is not allowed within the previous 2 years to inclusion.

**Note** *Patients with non-melanoma skin cancer, curatively treated localized prostate cancer, or carcinoma in situ of any type (if complete resection was performed) are allowed.*

6. Active severe infectious disease in the 4 weeks prior to the initiation of study treatment, including human immunodeficiency virus (HIV) infection or chronic Hepatitis B or C.

7. Patient has documented history of a cerebral vascular event (stroke or transient ischemic attack), or the following criteria for cardiac disease:
    a. Myocardial infarction or unstable angina pectoris within 6 months of enrollment.
    b. History of serious ventricular arrhythmia (i.e., ventricular tachycardia or ventricular fibrillation), high-grade atrioventricular block, or other cardiac arrhythmias requiring antiarrhythmic medications (except for atrial fibrillation that is well controlled with antiarrhythmic medication); history of QT interval prolongation.
    c. New York Heart Association (NYHA) class III or greater congestive heart failure or left ventricular ejection fraction of < 40%.
8. Participants with QTcF interval (corrected) > 470 msec at screening.
9. History of interstitial lung disease requiring corticosteroids or pneumonitis.
10. Gastrointestinal disorders that may interfere with the absorption of the study drug or chronic diarrhea.
11. Patient has known hypersensitivity to afatinib or to any excipient contained in the drug formulation.
12. Female patients who are or intend to be pregnant or breastfeeding during their participation in the study or 1 month after the final study drug administration.
13. Patients unable to comply with the protocol as determined by the investigator.
14. The patient is currently participating in another clinical trial that would interfere with the radiological imaging schedule or any other determinations required in this protocol.
15. Patient has other underlying medical conditions that, in the opinion of the investigator, would impair the ability of the patient to receive or tolerate the planned treatment and follow-up.
16. Patients with psychiatric disorders that may interfere with monitoring.

### Study treatment

All the patients included in the AFAN trial will receive continuous daily treatment with afatinib by oral route of administration in line with the scheduled escalation of doses (Fig. 2). First, all patients will start at 20 mg/day (weeks 1–2), then 30 mg/day (weeks 3–4) to finally reach a steady-state of 40 mg/day after 1 month (week 5 and thereafter). The dose escalation will occur if no hematologic or other relevant toxicities are observed (following CTCAE V5.0 < grade 2). Those patients that could experience AEs during the first month that limit dose escalation to 40 mg/day, may require dose interruptions and proper management of the AEs but may reach later to the 40 mg/day dose. Afatinib 40 mg daily will be the highest dose allowed (Fig. 2). Dose reductions up to 20 mg/day and delays will be allowed to manage emerging toxicities. No dose reduction below 20 mg is allowed.

Treatment with afatinib will be maintained until disease progression (PD)/secondary primary tumor (SPT), loss to follow-up, unacceptable toxicity, patient withdrawal or death.

### Objectives and endpoints

The main objective of the AFAN trial is to investigate the efficacy of afatinib when administered as therapy in patients with FA-HNSCC of the oral cavity, oropharynx or hypopharynx or larynx. The primary endpoint for efficacy is established as the objective response rate (ORR) according to RECIST V1.1 [17] during the first 9 months after study treatment initiation.

Secondary objectives will be focused on determining the safety and clinical efficacy outcomes by evaluation of the following:

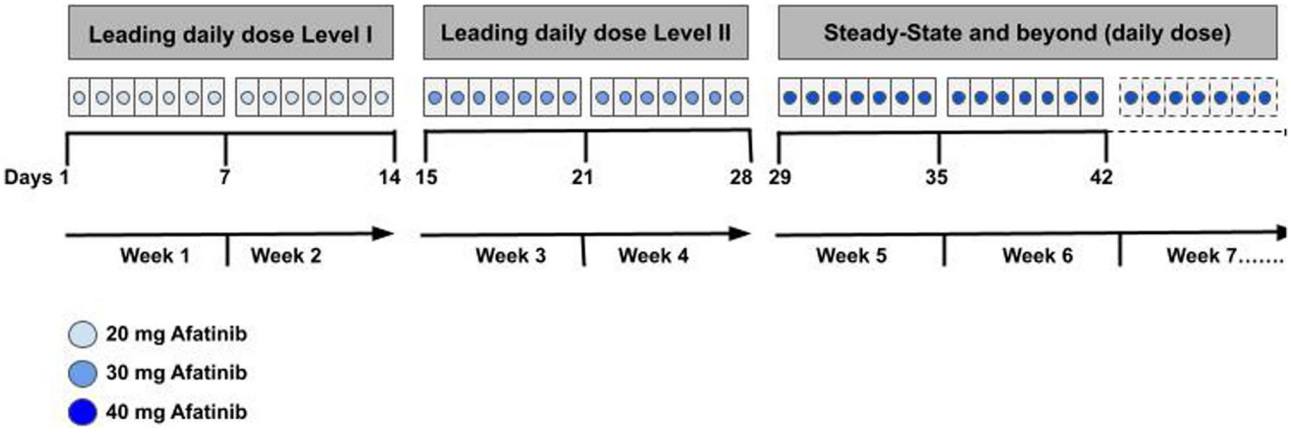


**Fig. 2** Treatment Schedule

- DCR, defined as the percentage of patients with complete response (CR) or partial response (PR), according to ORR definition for the trial, or maintained stable disease (SD) as their overall best response, assessed by imaging follow-up (CT scan/MRI) and RECIST V1.1 criteria. Stable disease should be maintained for at least 4 months to be considered as a DCR event.
- DoR, defined as the time from first confirmed response (complete or partial response), according to ORR definition for the trial, to the date of the documented PD as determined using RECIST V1.1 criteria or death due to any cause, whichever occurs first.
- DFS, defined as the time from first dosing date to the date of PD/SPT according to RECIST V1.1. The following definitions to each event to be considered apply:
  - Progression events include:
    - Local recurrence is defined as recurrence and/or PD of the tumor in the primary anatomic region (T-site) and/or adjacent to it in the image.
    - Regional recurrence is defined as the appearance of the tumor in the neck nodes (N site) on imaging. For a lymph node to be considered pathologic (i.e., new lesion) the following criteria apply:
      - Positive biopsy (biopsy prevails over negative CT MRI.
      - Measurable lymph node (i.e., at least 15 mm on the minor axis) unless the biopsy is negative and/or the CT/MRI is negative.
      - CT MRI-positive lymph node of any size, unless biopsy is negative.
    - Distant recurrence is defined as a spread of disease beyond the area of the primary tumor and/or neck nodules on imaging. Solitary distant metastases should be biopsied to discard SPT.
  - Secondary primary tumor (SPT) events (defined by *Hong et al., 1990*) include:
    - Upper aerodigestive tract cancers diagnosed more than three years after the primary cancer.
    - Any new tumor of different histological subtype.
    - Isolated lesions in distant locations and suspicion of new tumors in other anatomical regions should be biopsied.
- OS, defined as the time elapsed from the first dose of study treatment until death from any cause. Patients alive and free of events at the date of the analysis will be censored at their last known contact. Survival will be assessed by recording patient status at each visit.
- Safety and tolerability of afatinib in patients with FA-HNSCC will be assessed by the frequency and severity of AEs and TRAEs assessed by NCI CTCAE v5.0.
- Patient reported health-related quality of life (HRQoL), assessed through the European Organization for Research and Treatment of Cancer Quality of Life Questionnaire C30 (EORTC QLQ-C30), version 3, EORTC head and neck cancer specific complementary module QLQ-HN43 and EQ-5D Health Status Questionnaire.

Exploratory objectives and endpoints aim:

- To assess genetic alterations present in tumor tissue, saliva and plasma at baseline and saliva and plasma samples during treatment to evaluate their correlation with the efficacy of afatinib.
- To evaluate the levels of tumor DNA in plasma and saliva and evaluate their correlation with the efficacy of afatinib.

These exploratory endpoints will be correlated with efficacy endpoints such as ORR, DFS and OS to find potential prognostic value.

**Study assessments**

All patients must have the informed consent signed prior the start of the screening period (28 days). In the screening phase several determinations must be carried out prior to the start of treatment. In order to evaluate eligibility, during the screening period all data related to medical history (medical and oncology specific), demography, smoking and alcohol use, review of the inclusion and exclusion criteria. Baseline tumor assessments are required to be performed within 4 weeks prior to the start of study treatment, by MRI or CT scan according to RECIST v1.1 including head, neck, chest and all suspected regions with cancer lesions. The same radiological method must be used throughout the study. Screening clinical visits will also include the following assessments: ECOG PS, a complete physical exam, including examination of head and neck and skin, 12-leads electrocardiogram (ECG), left ventricular ejection fraction (LVEF) assessment and recording of any prior AE and

concomitant medications, laboratory determinations (hematology, serum chemistry, coagulation, urinalysis and pregnancy test [if applicable]).

During the treatment phase, clinical visits will be performed at weeks 1, 2, and 4 during the first month, followed by monthly visits thereafter. These visits will encompass the recording of vital signs and body weight, complete physical examination, ECOG PS, and blood laboratory analysis, including hematology, and serum biochemistry. Tumor imaging assessments will be performed every 12 weeks (± 1 week) until disease recurrence or SPT, loss to follow-up or unacceptable toxicity. The same radiological method employed at baseline must be consistently used throughout the study and the imaging schedule should remain unaltered regardless of any treatment modification such as dose delays or interruptions. The safety and tolerability of afatinib, as well as the use of other concomitant medications will be continuously monitored. The end of treatment visit will include the same assessments. Pregnancy tests, if applicable, will be performed monthly and at the end of treatment. Additional unscheduled visits may be performed according to the investigator criteria when necessary.

A 12-lead ECG will be performed every two months, at PD and at the end of treatment to monitor any changes in cardiac function. Additionally, LFEV will be assessed at the end of treatment visits.

QoL will be assessed at baseline and every 8 weeks using the EORTC QLQ-C30, EORTC QLQ-H&N35 and EQ-5D Health Status questionnaires.

After the end of treatment for other reasons than PD, patients will continue having tumor assessments every 12 weeks (± 1 week) until PD, death, loss of follow-up, total patient consent withdrawal (refusing any trial procedure), or end of study. Long term follow-up to determine status (alive, death, loss of follow up, etc.) will be carried out for those patients that progressed. These follow-ups may be performed by phone if patients are not coming to the clinic due to other reasons. The end of study is defined as the Last Patient Last Visit (LPLV) and will be estimated at 42 months after the first patient first visit (FPFV). Please see Table 1 for the specific schedule of assessments.

### Translational research

The AFAN trial includes an ambitious translational project that requires the collection of an archival tumor tissue at baseline, paraffin or frozen sample of the primary tumor from the initial diagnosis (minimum 10–15 slides) or a more recent biopsy before first dose of study treatment, for retrospective central evaluation of somatic molecular alterations or tumor biomarkers expression levels. No central pathological review will be needed to include the patient in the trial. Additionally, optional *de novo* tumor biopsy could be collected in week 12 and at PD if the patient accepts the participation in the sub-study and the intervention is considered safe and feasible.

The study also includes the collection of blood and saliva samples at baseline and during the treatment (at weeks: 1, 2, 8, 16, 24, 32, including at PD, end of treatment and first follow-up) to identify and characterize DNA, RNA, or protein markers known or suspected to be of relevance to the mechanisms of action, or the development of resistance to afatinib, which may include but are not limited to EGFR-related biomarkers or ctDNA. This sub-study may aid in the identification of those patients who might preferentially benefit from treatment with afatinib.

### Statistical planning

The total sample size of this trial (25 patients) was calculated using a Simon II stage design (*Simon, 1989; Jung et al., 2004;* http://cancer.unc.edu/biostatistics/program/ivanova/SimonsTwoStageDesign.aspx*)*, taking as null hypothesis an estimated ORR (after 9 months) with standard current therapies including chemoradiotherapy, cetuximab combinations or immunotherapies of 20% ($H_0$)(*Vermorken et al., 2008; Burtness et al., 2005; Rubió-Casadevall et al., 2023; Ferris et al., 2016; Cohen et al., 2019*) and an alternative ORR of 40% ($H_1$) to consider the efficacy of afatinib clinically relevant. With an alpha error of 0.1 and power of 80%, the total number of evaluable patients to be recruited will be 25.

The trial will include 12 patients on the first stage and will require at least 2 confirmed responses according to RECIST v1.1 out of the first 12 patients. If the futility analysis is passed, the trial will include 13 additional patients in the second stage. A total of 7 responses out of the 25 patients will be required to declare the study positive in terms of efficacy.

Sample size also takes into consideration the feasibility of the accrual given the rare nature of the disease under study. The expected number of patients with FA-HNSCC diagnosed in the two countries involved in the trial (Spain and Germany), is 5–10 patients/year.

The data will be analyzed in the following populations:

1. *Full analysis set (FAS)*: All patients who have been enrolled in the trial.
2. *Evaluable population per protocol (PP)*: All patients fulfilling all eligibility criteria without any protocol deviation that makes the patient invalid for the primary endpoint evaluation and having at least a radiological assessment after the first dose of study treatment.
3. *Safety population*: All patients receiving at least one dose of treatment with afatinib.

**Table 1** Study determinations in the AFAN study

| **Study period** | **Selection** | **Treatment period** | | | | | | | | | | | | **EoT** | **FU** | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Visit** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13–23** | **EoT** | **FU V1** | **FU V2 - *n*** |
| **Weeks after visit** | **−4** | **0** | **1** | **2** | **4** | **8** | **12** | **16** | **20** | **24** | **28** | **32** | **36-*n*** | | | * |
| **Day**[1] | **−28 -to −1** | **1** | **8 (+/−2)** | **15 (+/−2)** | **29 (+/−2)** | **57 (+/−2)** | **85 (+/−2)** | **113 (+/−2)** | **141 (+/−2)** | **169 (+/−2)** | **197 (+/−2)** | **225 (+/−2)** | **Repeat visits 9–12** | **0–7 after last dose** | **28 (+/−7) After EoT** | * (+/−7) |
| Informed consent | *Prior to any study specific determination* | | | | | | | | | | | | | | | |
| Demographics, height, smoking, alcohol use | X | | | | | | | | | | | | | | | |
| Medical record | X | | | | | | | | | | | | | | | |
| Vital signs and body weight | | X | X | X | X | X | X | X | X | X | X | X | X | X | X | |
| Physical Exam including skin examination | | X | | X | X | X | X | X | X | X | X | X | X | X | X | |
| Head and neck examination [2] | | X | | X | | X | | X | | X | | X | | X | X | X[3] |
| Tumor Imaging [3] | X | | | | | | X | | | X | | | X[3] | X | X[4] | X |
| ECOG Performance status | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X[4] |
| Tobacco habits | X | X | | X | | X | | X | | X | | X | | X | X | X[5] |
| 12-lead ECG | X | | | X | | | | X | | | | X | | X[5] | | |
| LVEF [6] | X | | | | | | | | | | | X | | X | | |
| Health-related quality of life [7] | | X | | | | X | | X | | X | | X | X[7] | X | X[7] | X[7] |
| Inclusion/exclusion criteria review | X | X[8] | | | | | | | | | | | | | | |
| Dispensing of study medication | | X | | | X | X | X | X | X | X | X | X | X | | | |
| Study drug treatment [9] | | Oral, continuous, once a day | | | | | | | | | | | | | | |
| Compliance verification [10] | | | X | | X | X | X | X | X | X | X | X | X | X | | |
| Adverse events | | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X[11] |
| Concomitant treatment | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X[12] | X[12] |
| Safety laboratory tests [13] | X | X | | X | X | X | | X | | X | | X | | X | X | |
| Pregnancy test [14] | X | X | | | X | X | X | X | X | X | X | X | X | X | | |
| Tumor tissue preservation [15] | | X | | | | | X[15] | | | | | | | X[15] | | |
| Blood and saliva samples for R + D [16] | X | X | | X | | X | | X | | X | | X | | X | X | |

**Table 1** (continued)

| Study period | Selection | Treatment period | | | | | | | | | | | | EoT | FU | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Visit** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13–23** | **EoT** | **FU V1** | **FU V2 - *n*** |
| **Weeks after visit** | **−4** | **0** | **1** | **2** | **4** | **8** | **12** | **16** | **20** | **24** | **28** | **32** | **36-*n*** | | | * |
| **Day[1]** | **−28 -to −1** | **1** | **8 (+/−2)** | **15 (+/−2)** | **29 (+/−2)** | **57 (+/−2)** | **85 (+/−2)** | **113 (+/−2)** | **141 (+/−2)** | **169 (+/−2)** | **197 (+/−2)** | **225 (+/−2)** | **Repeat visits 9–12** | **0–7 after last dose** | **28 (+/−7) After EoT** | * (+/−7) |
| Pharmacogenetics [17] | | X | | | | | | | | | | | | | | |
| Vital status | | | | | | | | | | | | | | | X | X |

EoT: The End of Treatment visit is performed within 7 days of the last administration of study medication. If a patient permanently discontinued study medication at a scheduled visit, then the EoT visit will be conducted instead of the scheduled visit

FU V1 The first follow-up visit is performed 28 days (±7 days) after the EoT

* FU Before Progression/SPT:

Patients ending treatment without progression should remain in the study, and should be followed up according to RECIST 1.1 every 12 weeks (approximately 3 months) until progression/SPT, initiation of new subsequent anticancer therapy, death, loss of follow-up, total patient consent withdrawal (refusing any trial procedure), or end of study (whichever occurs first). Further CT/MRI scans could be performed upon suspicion of disease progression according to standard clinical practice and physician criteria

* FU After Progression/SPT:

The following follow-up visits are conducted every 16 weeks until week 112 from Visit 2 and every 24 weeks thereafter until the end of the study. Once tumor recurrence has been radiologically confirmed, only the following data, vital status, drug-related adverse events and cancer therapy, will be obtained. At this point, scheduled visits can be replaced by telephone contacts

[1] Recommended day for the visit. In the event that the visit is delayed beyond the planned schedule, it is recommended that the patient have sufficient medication during this period

[2] In case of suspected tumor recurrence, it should be confirmed by imaging within 2 weeks, preferably followed by biopsy. If recurrence is not radiologically confirmed, radiological imaging should continue according to the original schedule

[3] Imaging should include CT, MRI or PET-CT of the head and neck, chest and, if clinically indicated, any other site of suspected or known disease. **The same radiological technique should be used throughout the study**, observing the imaging guidelines. Imaging will be performed until tumor recurrence/SPT at the following times:

● Every 12 weeks (approx. 3 months) (±1 week) until disease recurrence/SPT, loss to follow-up or unacceptable toxicity

● In the event of early discontinuation or discontinuation/delay of medication the imaging schedule should NOT be changed; patients should continue imaging according to the predefined schedule until tumor recurrence or study completion

[4] Carry out until tumor recurrence/SPT

[5] If not performed within the previous 8 days per standard facility procedures

[6] LVEF is assessed by echocardiography/MUGA. The same procedure should be used throughout the study

[7] HRQoL: EORTC QLQ-C30, EORTC QLQ-H&N-C35, and EQ-5D. Carry out every 8 weeks until tumor recurrence

[8] Certain eligibility criteria are assessed at Visit 2, before study treatment administration

[9] Continuous, daily treatment with Afatinib starting at Visit 2

[10] After one week of treatment, compliance with treatment should be reviewed with the patient to ensure that the medication is being taken correctly. Starting at Visit 5, treatment compliance is calculated and entered into the eCRF

[11] After the first follow-up visit (FU V1), only drug-related adverse events are recorded

[12] Information on possible concomitant treatments is collected up to FU V1. After FU V1, only information on cancer therapy is collected, if applicable

[13] This includes hematology, serum biochemistry and urinalysis. Urinalysis is only performed at screening and at the EoT

[14] Pregnancy testing for women of childbearing potential is mandatory prior to treatment, every 4 weeks during treatment and at EoT. If pregnancy test during selection is performed within 72 h of the first dose of study treatment, it does not need to be repeated on Visit 2

[15] Biomarker analysis on preserved tissue samples is compulsory at baseline and optional at visit on week 12 and at PD. At baseline, it is required a paraffin or frozen sample of the primary tumor from the initial diagnosis (minimum 10–15 slides) and a biopsy if feasible. Samples are sent to the central laboratory for exploratory biomarker analysis only if the patient has given written informed consent to do so

[16] Frequency may be modified to every 4 weeks to record changes in relevant pathological time points such as disease progression or response

[17] Pharmacogenetic analysis is optional. Sample collection is only performed if the patient has given written informed consent to do so. The sample can be obtained at Visit 2 or later

The ORR during the first 9 months, which will serve as the primary endpoint, is assessed locally by the investigator through imaging (CT scan/MRI) following RECIST 1.1. ORR is considered the percentage/proportion of patients with confirmed CR or PR as their best overall response during the first 9 months after study treatment initiation. The protocol requires confirmation of the response. The primary analysis will be based on the FAS. The analysis will be replicated in the PP population as sensitivity analysis. The ORR will be estimated by binomial proportion, dividing the number of patients with confirmed CR or PR by the total in the studied population. The corresponding exact 2-sided 90% confidence intervals (CIs) will be provided. Changes in tumor size from baseline will be calculated and displayed graphically by waterfall plot.

Regarding the analysis of the secondary efficacy endpoints:

The DCR will include the percentage/proportion of patients with CR, PR, or SD (maintained > 4 months) as their best overall response throughout the study period. The DCR will be estimated by binomial proportion, dividing the number of patients with CR, PR or SD by the total number of patients. The corresponding exact 2-sided 95% CIs will be provided.

Time-to-event endpoints such as DoR, DFS and OS will be summarized using Kaplan-Meier method and displayed graphically. The mean, median, and rates at several relevant timepoints with their corresponding 95% CIs will be provided. Patients alive and free of events at the date of the analysis will be censored at their last known tumor assessment. Patients who start a new treatment line without event will be censored on the date of first dose of the subsequent anticancer treatment.

QoL will be summarized by mean and standard error (SE), median, range and 95% CI of absolute scores will be reported for each of the subscales of the questionnaires. The mean change of absolute scores from baseline (and 95% CI) will also be assessed. Line charts depicting the means and mean changes of items and subscales over time will be provided. Additional exploratory analyses may be performed, such as repeated measures, mixed effects modeling and analyses of patients subgroups according to efficacy.

The primary analysis of secondary efficacy endpoints will be performed in the FAS. Efficacy variables, including primary and secondary endpoints, will be reported for subgroups to find potential correlation between patient characteristics (i.e., age, gender, ECOG, AJCC stage, differentiation, or previous treatments) and the clinical outcomes to afatinib.

Regarding the analysis of the secondary safety endpoints:

AEs, treatment-related AEs (TRAEs) and AEs leading to temporary or permanent treatment discontinuation or dose reductions will be provided as counts or patients affected and frequencies in the safety population. Events will be reported by grade according to NCI CTCAE V5.0.

Regarding the analysis of the secondary exploratory endpoints:

- -The genetic alterations present in tumor tissue, saliva and plasma at baseline and saliva and plasma samples during treatment will be assessed and reported as minor allele frequency and percentage of patients presenting specific alterations. Genetic alterations may be used to stratify the population and find potential associations with prognosis.
- -For the ctDNA in blood and saliva samples, the summary statistics [mean and SE, median, range and 95% CI] of absolute value of tumor DNA levels will be reported at each timepoint. The mean change of tumor DNA presence from baseline (and 95% CI) will also be assessed. Line charts depicting the means and mean changes over time will be provided.

Exploratory endpoints will be correlated with efficacy endpoints such as ORR, DFS and OS to find potential prognostic value.

Analysis will be based on observed data, and missing data for drop-outs are not replaced by methods like LOCF (last option carried forward).

## Discussion

In this manuscript, we outline the design of an international and challenging phase Ib/II clinical trial aimed at identifying potential new treatment options for patients with FA-HNSCC. This scenario is complex, primarily due to the low incidence of FA-HNSCC. In addition, there is a lack of previous experience in FA-HNSCC trials given the fact that AFAN is the first clinical trial for this indication in the history of medicine. Previous research has involved small retrospective observational cohorts, such as the French cohort, which included eight patients with FA and solid tumors during childhood [18], or the United States NCI initiative for FA, which is conducting a study to improve early cancer detection. As of 2024, 55 patients with FA and various cancer types have been included [19]. The largest dataset comprises a retrospective meta-analysis of 119 patients with FA-HNSCC treated with standard therapies, suggesting prioritization of surgical resection and a limited role for platinum-based chemotherapy in this context [10]. Anticipating the challenge in achieving the expected sample size, the study has been promoted and communicated in scientific congresses and disseminated through patient advocacy groups and medical associations dedicated to patients with FA and cancer

care. The opening of additional sites and countries might be considered to boost accrual. Moreover, the study was designed using a Simon II stage design that will optimize the accrual and allow early detection of efficacy signals in a smaller initial subset of patients. The design also uses a single-arm strategy to minimize sample size, which in this case has limited impact on later data interpretation as no standard treatment option are defined and placebos in such an advanced setting would be not ethically acceptable.

Our study is pioneering, as it represents the first interventionist clinical trial specifically aiming to treat FA-HNSCC. To date, no clinical trial has been dedicated to FA-HNSCC, with existing evidence derived from retrospective cohorts and the use of approved therapies for HNSCC, whose etiology differs significantly from that of patients with FA. FA is majorly caused by mutations in FANC genes leading to defects in the FA/BRCA pathway, which is involved in cell cycle regulation and DNA repair [6, 20, 21]. Consequently, patients with FA exhibit a higher degree of genome instability, promoting the accumulation of DNA damage and cellular stress [7, 22–25]. Consequently, tumor cells in FA-HNSCC exhibit molecular and etiological differences resulting in more aggressive behavior and less histological differentiation compared to those in the general population. FA-HNSCC also has a greater dependency on EGFR due to its high amplification and overexpression when compared to sporadic HN-SCC [9, 15]. These cellular differences also influence the clinical presentation of the disease, which tends to manifest earlier in life, typically between 20 and 40 years, most commonly in the oral cavity and is usually initially diagnosed at an advanced stage [9]. Therefore, due to all these features, the FA-HNSCC is extremely challenging to treat. Surgical resection is often difficult and, in many cases unfeasible, while standard systemic treatments such as chemo- and radiotherapy, exhibit significantly higher toxicity rates due to the frailty of patients with FA and limited efficacy [3]. Additionally, most of the treatment options for FA are directed to ameliorate the progressive bone marrow failure mainly focusing on hematopoietic stem cell transplantation, such as our novel options using gene therapy to generate disease-free autologous CD34 + hematopoietic stem cells [26]. These treatments may contribute to increased survival and life expectancy, with more patients reaching adulthood with the corresponding increase in HNSCC. Therefore, there is an urgent need to seek for other effective and safe treatment options for FA-HNSCC [10].

EGFR inhibitors have shown no survival benefit when compared to methotrexate in advanced or metastatic HNSCC [27]. Accordingly, afatinib showed comparable efficacy to cetuximab and methotrexate in metastatic pretreated HNSCC [14, 28]. It is expected that patients with FA might experience greater benefit with afatinib based on their genetic alterations which generate greater tumor EGFR-dependency as evidenced in preclinical models [16]. Supporting our hypothesis, a case report of a woman with FA-HNSCC of the oral cavity treated with gefitinib reported a reduction in tumor of 80% [29]. Based on the preclinical and clinical evidence, the EMA granted an orphan drug designation for the use of afatinib in patients with FA-HNSCC. Nevertheless, the protocol has established several safety measures for the early detection of lack of efficacy. Patients will be monitored every month with complete clinical and laboratory exams, and tumor imaging will be performed every 3 months which is a similar schedule than in the real-world. Patients candidates for curative treatment options are excluded, so they are not exposed to increased risks.

In order to minimize the potential side effects commonly associated with afatinib, we designed a dose escalation schedule, starting at 20 mg/day of afatinib to ensure patient safety during the first months, which is the expected timing for the occurrence of these events. Moreover, patients will be closely monitored the first month, having clinical visits with full haematological tests at weeks 1, 2 and 4. Cardiac function will be closely monitored at baseline using LVEF and 12-lead ECG, and approximately every 3 months using 12-lead ECG during the treatment phase. The protocol already considers measures to mitigate AEs in case of occurrence, including treatment interruption and dose reductions, medical intervention, or discontinuation. Besides the previously described pathologies, we also provided recommendations and guidelines to follow in case of other less frequent events that may arise upon afatinib treatment such as: severe hepatic impairment, gastrointestinal perforations, issues on cardiac contractility (left ventricular fraction), interactions with P-glycoprotein, patients with galactose intolerance.

Translational research will delve deeper into the genetic alterations of FA-HNSCC and their effects in afatinib efficacy. These will provide highly valuable molecular data and samples in such a rare disease with very limited information and tumor samples available for research. Furthermore, the AFAN trial takes into consideration the patient reported QoL which is a strength of the design that will allow not only to evaluate the efficacy of treatment from a patient centric perspective.

In conclusion, the AFAN trial constitutes a comprehensive and complete study that will provide new insights into the therapeutic options to treat patients with FA-HNSCC, hopefully providing a potential effective treatment to halt cancer progression, expanding lifespan and preserving quality of life of the patients.

*Abbreviations: HNSCC (head and neck squamous cell carcinoma), RECIST (response evaluation criteria in solid*

*tumors), ECOG (eastern cooperative oncology group), QD (once per day), W (week), PD (progression disease), EOT (end of treatment), FU (follow-up visit), QoL (quality of life), CT (computed tomography), MRI (magnetic resonance imaging), DCR (disease control rate), DoR (duration of response), DFS (disease-free survival) and OS (overall survival).*

**Abbreviations**

| | |
|---|---|
| AEs | Adverse Event |
| AJCC | American Joint Committee on Cancer |
| ALT | Alanine Aminotransferase |
| AST | Aspartate Aminotransferase |
| CI | Confidence Interval |
| CR | Complete Response |
| CT | Computed Tomography |
| CTCAE | Common Terminology Criteria For Adverse Events |
| ctDNA | Circulating Tumor DNA |
| DCR | Disease Control Rate |
| DFS | Disease-Free Survival |
| DNA | Deoxyribonucleic Acid |
| DoR | Duration Of Response |
| ECG | Electrocardiogram |
| ECOG | Eastern Cooperative Oncology Group |
| EGFR | Epithelial Growth Factor Receptor |
| EMA | European Medicines Agency |
| EORTC | European Organization For Research And Treatment Of Cancer |
| ERBB | Erythroblastic leukemia viral oncogene homologue receptors |
| FA | Fanconi Anemia |
| FAS | Full Analysis Set |
| FDA | Food and Drug Administration |
| FPFV | First Patient First Visit |
| G-CSF | Granulocyte Colony-Stimulating Factor |
| HER2 | Human Epidermal Growth Factor Receptor 2 |
| HIV | Human Immunodeficiency Virus |
| HNSCC | Head And Neck Squamous Cell Carcinoma |
| HSCT | Hematopoietic Stem Cell Transplantation |
| ICL | Interstrand Crosslinks |
| IDL | Interstitial Lung Disease |
| INR | International Normalized Ratio |
| LOCF | Last Option Carried Forward |
| LPLV | Last Patient Last Visit |
| LVEF | Left Ventricular Ejection Fraction |
| MDS | Myelodysplastic Syndrome |
| MRI | Magnetic Resonance Imaging |
| MRI | Magnetic Resonance Imaging |
| NSCLC | Non-Small Cell Lung Cancer |
| NYHA | New York Heart Association |
| ODD | Orphan Drug Designation |
| ORR | Objective Response Rate |
| OS | Overall Survival |
| PD | Progression Of The Disease |
| PP | Per Protocol |
| PR | Partial Response |
| PT | Prothrombin Time |
| QLQ | Quality Of Life Questionnaire |
| QoL | Quality Of Life |
| QTcF | Corrected Qt Interval Calculated With Fridericia Formula |
| RECIST | Response Evaluation Criteria In Solid Tumors |
| RNA | Ribonucleic Acid |
| SD | Stable disease |
| SE | Standard Error |
| SPT | Secondary Primary tumor |
| TKIs | Tyrosine Kinase Inhibitors |
| TRAEs | Treatment-Related Adverse Event |
| ULN | Upper Limit Of Normal |

## Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12885-025-14619-6.

Supplementary Material 1.

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**Authors' contributions**

This study was designed by all co-authors. The author(s) meet criteria for authorship as recommended by the International Committee of Medical Journal Editors (ICMJE).

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**Data availability**

The study is reported in accordance with the CONSORT guidelines. The full protocol is attached to this manuscript.

## Declarations

**Ethics approval and consent to participate**

The study will be conducted in accordance with the principles of the Helsinki Declaration Adopted by the 18th World Medical Assembly, Helsinki, Finland, June 1964 updated to its latest version Fortaleza, Brazil, October 2013 (Appendix 17). With the Good Clinical Practice (GCP) standards issued by the Working Party on Medicinal Product Efficacy of the European Economic Community (1990) (CPMP/ICH/135/95) and the laws and regulations in force in Europe.

**Consent for publication**

Not applicable.

**Competing interests**

MEMM has received honoraria as consultant from Boehringer Ingelheim España SA. JM and JS have filed a patent of the afatinib use to FA patients with HNSCC. J.S. has signed agreements in the last 3 years (advisory boards, service provision, research collaboration, or material transfer) with Boehringer Ingelheim, Glaxo Smith Kline, Moderna Therapeutics, Roche, Rocket Pharmaceuticals and Pfizer. All remaining authors declare that they have no competing interests.

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## Publisher's Note