A Phase I Dose-Escalation Study of the HIF-2 Alpha Inhibitor DFF332 in Patients with Advanced Clear-Cell Renal Cell Carcinoma

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ABSTRACT

Purpose: Mutations or silencing of the von Hippel–Lindau tumor suppressor gene accumulate hypoxia-inducible factors (HIF). HIF-2α is implicated in the oncogenesis of ~50% of patients with clear-cell renal cell carcinoma (ccRCC) but has been considered "undruggable." DFF332, an orally administered novel allosteric inhibitor of HIF-2α, showed dose-dependent antitumor efficacy in preclinical models of ccRCC.

Patients and Methods: This first-in-human study evaluated the safety, tolerability, antitumor activity, pharmacokinetics, and pharmacodynamics of DFF332 in patients with heavily pretreated advanced ccRCC. Preliminary data from the dose escalation of DFF332 monotherapy, administered orally at 50 or 100 mg weekly or 25, 50, 100, or 150 mg once daily in 28-day treatment cycles, are reported.

Results: As of January 15, 2024, 40 patients (median age, 62.5 years) received DFF332 for a median duration of 12.1 weeks. Overall, two patients (5%) achieved a partial response, and 19 (48%) achieved stable disease as the best overall response. DFF332 showed a favorable safety profile, with treatment-related adverse events occurring in 25 patients (63%). Only five patients (13%) experienced treatment-related anemia, and no hypoxia was observed. The only serious treatment-related adverse event, hypertension, was reported in one patient. The maximum tolerated dose was not reached.

Conclusions: Although clinical responses were limited in the doses evaluated, dose exploration halted prematurely, making it difficult to draw definitive conclusions about the efficacy of DFF332. Further investigation is required to establish a recommended dose regimen, assess its efficacy and safety, and evaluate its full potential as a partner in combination studies.

Introduction

The management of advanced renal cell carcinoma (RCC) has evolved significantly in recent years. For patients with advanced clear-cell RCC (ccRCC), which is the most common histologic subtype, first-line therapy involves either dual checkpoint inhibitors or an anti–PD-1 antibody in combination with a VEGF receptor agent (1). The use of VEGF-directed therapies in ccRCC, a now long-standing paradigm, is predicated on the frequent presence of alterations in the von Hippel–Lindau (*VHL*) gene. Somatic alterations in *VHL* occur in approximately 50% to 90% of patients with ccRCC, and a small proportion of patients may have germline variations (2). Disruption of the VHL protein leads to decreased ubiquitination of hypoxia-inducible factor (HIF), resulting in increased accumulation of HIF and, subsequently, increased VEGF transcription (3).

Although inhibition of VEGF and its cognate receptor remains an attractive clinical strategy, methods to inhibit upstream moieties

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Translational Relevance

Clear-cell renal cell carcinoma (ccRCC), the most common type of kidney cancer, is often driven by mutations or inactivation of the von Hippel–Lindau gene, which leads to the accumulation of hypoxia-inducible factors (HIF). HIF-2α is a key transcription factor that promotes tumor growth and angiogenesis in ccRCC. DFF332 is a novel HIF-2α inhibitor. In this first-in-human, phase I, dose-escalation study of patients with advanced ccRCC, we observed a favorable safety profile compared with similar agents in the class. DFF332 may be considered for use in combination with other therapies that have synergistic or additive effects against advanced ccRCC.

such as HIF have remained elusive until recently. Multiple small-molecule inhibitors of HIF-2α are currently under investigation as single agents or in combination with immune checkpoint inhibitors or other targeted agents. Belzutifan, an allosteric inhibitor of HIF-2α, was first approved for use in patients with germline *VHL* alterations demonstrating elements of VHL syndrome (3). A recent phase III trial, LITESPARK-005, compared belzutifan with the mammalian target of rapamycin inhibitor everolimus in patients with one to three prior lines of treatment, including prior checkpoint inhibitors and VEGF-directed therapy. The study met its primary endpoint, showing improved progression-free survival with belzutifan relative to everolimus (HR, 0.74; 95% confidence interval, 0.63–0.88; ref. 4). Although these data are compelling, of note, 34% of patients who received belzutifan had primary progressive disease (PD). Moreover, grade ≥3 anemia and hypoxia were observed in 29% and 10% of patients, respectively (4, 5). Although these on-target side effects are presumably anticipated, they can be challenging in the context of patients with advanced disease who require supportive care.

Herein, we assess DFF332, a novel small-molecule agent (such as belzutifan) that binds to the PAS-B cavity of HIF-2α and inhibits its transcriptional activity (6–8). In *in vitro* models of *VHL*-deficient ccRCC, DFF332 inhibits HIF-2α at nanomolar concentrations, translating to significant antitumor efficacy in corresponding xenograft models (7). In this study, we aimed to characterize the safety, tolerability, antitumor activity, and pharmacokinetic (PK) and pharmacodynamic activities of escalating doses of DFF332 monotherapy in patients with advanced ccRCC.

Patients and Methods

Patient eligibility

Patients aged ≥18 years with unresectable, locally advanced, or metastatic ccRCC were enrolled in the study. Patients had PD despite standard therapies, including both PD-1 checkpoint inhibitors and VEGF-targeted therapies (either as monotherapy or in combination). Patients were excluded if they had symptomatic or uncontrolled brain metastases, concomitant malignancies that were progressing or requiring active therapies, recent major surgery or radiotherapy, or significant laboratory abnormalities. Patients who had previously received treatment with an HIF-2α inhibitor were also excluded. See Supplementary Methods S1 for further details. The overall representativeness of this study population to the general ccRCC population is shown in Supplementary Table S1.

The study was conducted in accordance with the ethical principles laid down in the Declaration of Helsinki, the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use Harmonized Guidelines for Good Clinical Practice, appropriate local regulations (including European Directive 2001/20/EC and US CFR 21), and Institutional Review Board approval. All patients provided written informed consent before enrollment.

Study design and treatment

This is a first-in-human, phase I, open-label, multicenter study (CDFF332A12101, ClinicalTrials.gov ID: NCT04895748). Patients were enrolled in successive cohorts receiving increasing doses of an oral tablet formulation of DFF332. A safe starting dose of up to 25 mg daily or 120 mg weekly was determined by using body surface area–based allometric scaling to estimate the human equivalent dose and applying a safety factor of 10 on the dose causing severe toxicity in approximately 10% of rats and a safety factor of 6 on the highest nonseverely toxic doses in dogs. To ensure sufficient safety exposure margins, a starting dose of 50 mg once weekly was selected as the starting dose of DFF332. Dose escalation was based on emerging safety, PK, and biomarker data and guided by a Bayesian logistic regression model to satisfy escalation with overdose control criteria (as described in "Statistical analyses"). In all cases, the dose for an escalation cohort did not exceed a 100% increase from the previously tested safe dose.

The treatment cycle was defined as 28 days. Patients were treated until they experienced PD, as assessed based on RECIST version 1.1 criteria or unacceptable toxicity; discontinuation was also permitted at the discretion of the patient or investigator under specific circumstances.

The chemical structure and step-by-step synthesis of DFF332 can be referred from the published article (8).

Assessments

Serial blood samples for PK analyses were collected at multiple time points up to cycle 4 day 1 or cycle 5 day 1 for daily and weekly dosing cohorts, respectively. Dense PK sampling (baseline; 30 minutes; 1, 2, 3, 4, 6, 24, 72, and up to 168 hours) was performed following initial dosing with DFF332 and during the second or third cycle of therapy. DFF332 level measurements in the plasma were performed using a validated liquid chromatography–tandem mass spectrometry assay. In parallel to the PK assessments, blood was drawn for assessments of erythropoietin levels, a marker of the pharmacodynamic effect.

A newly obtained tissue biopsy was mandated at the time of study entry, and a paired biopsy was further mandated before cycle 2 day 1 of therapy. Tissue was stained for HIF-1α and HIF-2α according to previously reported methods.

Statistical analyses

The primary objective of the study was to characterize the safety and tolerability of DFF332 in patients with metastatic ccRCC. Dose escalation was guided by a Bayesian hierarchical logistic regression model along with the overdose control principle to limit the risk of dose-limiting toxicities (DLT; refs. 9, 10). Dose escalations were mutually agreed upon by investigators and the study sponsor after consideration of a dose-determining set, including patients who had received at least 75% of the planned doses of DFF332 during the 28-day DLT evaluation period. Cumulative adverse events (AE) and serious AE were tabulated using the grading established in the NCI

Common Terminology Criteria for Adverse Events, version 5.0. Tolerability was characterized by the frequency of dose interruptions, reductions, and dose intensity.

Best overall response (BOR; according to RECIST version 1.1 criteria) was a key secondary endpoint in the study. Key exploratory endpoints were HIF-1α and HIF-2α expression in tumor tissue and erythropoietin levels in the blood.

Data availability

Data are available upon reasonable request. Novartis will not provide access to patient-level data if there is a reasonable likelihood that individual patients could be reidentified. Phase I studies, by their nature, present a high risk of patient reidentification; therefore, individual patient results for phase I studies cannot be shared. In addition, clinical data, in some cases, have been collected subject to contractual or consent provisions that prohibit transfer to third parties. Such restrictions may preclude granting access under these provisions. Where codevelopment agreements or other legal restrictions prevent companies from sharing particular data, companies will work with qualified requestors to provide summary information where possible.

Results

Patient characteristics

A total of 68 patients were enrolled, of whom 40 were treated with DFF332 (**Table 1**). Patients were predominantly male (77.5%), with a median age of 62.5 years (range, 38–79 years). Among the patients, 60.0% had an Eastern Cooperative Oncology Group performance status score of 0, whereas the remaining 40.0% had a score of 1. Most patients were of International Metastatic Renal Cell Carcinoma Database Consortium intermediate risk (57.5%) category, followed by favorable risk (30.0%) and poor risk (12.5%). Patients in the study were heavily pretreated, with 50.0% of patients receiving ≥3 prior lines of therapies.

Table 1. Patient characteristics.

Baseline and clinical characteristics	All patients (*N* = 40)
Age, years [median (range)]	62.5 (38–79)
Gender, *n* (%)	
Male	31 (77.5)
Female	9 (22.5)
ECOG PS, *n* (%)	
0	24 (60.0)
1	16 (40.0)
IMDC risk at baseline, *n* (%)	
Favorable	12 (30.0)
Intermediate	23 (57.5)
Poor	5 (12.5)
Prior lines of therapies[a], *n* (%)	
1	5 (12.5)
2	15 (37.5)
3	9 (22.5)
≥4	11 (27.5)

Abbreviations: ECOG PS, Eastern Cooperative Oncology Group Performance Status; IMDC, International Metastatic Renal Cell Carcinoma Database Consortium.
[a]Prior lines of therapies here refer to regimens that can be single agents or combinations of prior antineoplastic medications.

At the time of data cutoff (January 15, 2024), of the 40 patients treated, 11 (27.5%) remained on treatment, whereas 29 (72.5%) discontinued treatment due to PD ($n = 25$), physician decision ($n = 3$), or AE not related to treatment ($n = 1$).

Safety and efficacy

The primary objective of the study was to establish the safety and tolerability of DFF332. Overall, 95.0% of patients in the study had AE, of which 62.5% had treatment-related AE (TRAE) of any grade (**Table 2**). Fatigue (37.5%) and anemia (32.5%) were the most frequently reported AE, regardless of the relationship to the treatment. However, treatment-related fatigue and anemia (as deemed by the study investigators) were observed in only 12.5% of patients each. Notably, no grade 4 TRAE were observed, and no patients experienced hypoxia. Three patients experienced grade 3 TRAE, including hypertension, lymphopenia, and weight gain (one patient each). The grade 3 hypertension was also reported as a serious AE related to the treatment and improved upon dose interruption and treatment with antihypertensives. No DLT were recorded throughout the escalation. No pattern of early or delayed toxicity was observed with either daily or weekly DFF332 dosing (Supplementary Fig. S1).

Efficacy was a secondary objective in this study. Two male patients (5.0%), ages 66 and 63 years and each on 25 mg once daily and 100 mg once daily, respectively, achieved confirmed partial responses (PR), according to investigator assessments using RECIST version 1.1 criteria. In addition, 19 patients (47.5%) had stable disease (SD) as BOR, reflecting a clinical benefit rate of 52.5% (**Fig. 1A**). At the time of cutoff, the duration of SD for these patients ranged from 1.9 to 11.9 months. The median duration of exposure to DFF332 was 18 weeks (range, 1–76 weeks; **Fig. 1B**).

PK and pharmacodynamic assessments

DFF332 demonstrated fast oral absorption, with a median time to peak drug concentration of ~1 to 2 hours, and very slow elimination (effective half-life estimated by population PK analysis was ~85 days). Significant accumulation of DFF332 in plasma was observed with both weekly and daily dosing schedules (**Fig. 2A** and **B**). At DFF332 100 mg daily, plasma exposure [based on DFF332 mean AUC from time 0 to 24 hours (AUC_{0-24})] was 10.1-fold higher on cycle 2 day 1 than on cycle 1 day 1. There was nearly dose-proportional exposure with both weekly and daily dosing regimens. Simulations of DFF332 exposure in plasma with repeated daily dosing suggested that steady state will be achieved after ~1 year of treatment (**Fig. 2C**).

A positive dose-response relationship was also observed for pharmacodynamic effects through assessments of plasma erythropoietin levels. At a dose of 50 mg weekly, increased levels of erythropoietin were observed on day 8. At doses of 100 mg weekly and 25 mg daily, erythropoietin levels remained relatively stable for this duration. By contrast, at doses of 50, 100, and 150 mg daily, decreases in erythropoietin levels were noted, in which the decrease ranged from 27% to 51% on day 8 (**Fig. 3**). Of the two patients with PR, the first patient in the 25-mg once-daily cohort had undergone three prior lines of therapy: pembrolizumab, followed by nivolumab and lenvatinib, and finally nivolumab plus cabozantinib. At the time of enrollment, he had metastases in the lung, pancreas, lymph nodes, and bones. This patient showed an erythropoietin reduction of about 15% from baseline and reached a PR by cycle 6 and remained on treatment for 13 cycles before discontinuing due to PD. The second patient, treated with 100 mg once daily, had metastases in the adrenal gland, lymph nodes, lung, and bones at the

Table 2. TRAE observed in ≥5% of the treated patients.

TRAE by preferred term, *n* (%)	50 mg once weekly (*n* = 3)		100 mg once weekly (*n* = 6)		25 mg once daily (*n* = 9)		50 mg once daily (*n* = 5)		100 mg once daily (*n* = 12)		150 mg once daily (*n* = 5)		All patients (*N* = 40)	
Grade	**1–2**	**3**	**1–2**	**3**	**1–2**	**3**	**1–2**	**3**	**1–2**	**3**	**1–2**	**3**	**1–2**	**3**
Patients with ≥1 AE	0	0	5 (83.3)	0	7 (77.8)	0	4 (80.0)	0	8 (66.7)	2[a] (16.7)	1 (20.0)	0	25 (62.5)	2 (5.0)
Anemia	0	0	0	0	2 (22.2)	0	2 (40.0)	0	1 (8.3)	0	0	0	5 (12.5)	0
Increased blood cholesterol	0	0	0	0	2 (22.2)	0	0	0	3 (25.0)	0	0	0	5 (12.5)	0
Fatigue	0	0	1 (16.7)	0	3 (33.3)	0	1 (20.0)	0	0	0	0	0	5 (12.5)	0
Increased ALT	0	0	1 (16.7)	0	1 (11.1)	0	0	0	1 (8.3)	0	1 (20.0)	0	4 (10.0)	0
Hypertriglyceridemia	0	0	1 (16.7)	0	1 (11.1)	0	1 (20.0)	0	1 (8.3)	0	0	0	4 (10.0)	0
Increased AST	0	0	1 (16.7)	0	1 (11.1)	0	0	0	0	0	1 (20.0)	0	3 (7.5)	0
Dizziness	0	0	1 (16.7)	0	0	0	1 (20.0)	0	1 (8.3)	0	0	0	3 (7.5)	0
Dyspnea	0	0	1 (16.7)	0	0	0	1 (20.0)	0	0	0	0	0	2 (5.0)	0
Hypertension	0	0	0	0	0	0	1 (20.0)	0	1 (8.3)	1 (8.3)	0	0	2 (5.0)	1 (2.5)
Increased lipase	0	0	1 (16.7)	0	0	0	0	0	1 (8.3)	0	0	0	2 (5.0)	0
Nausea	0	0	1 (16.7)	0	0	0	0	0	1 (8.3)	0	0	0	2 (5.0)	0
Peripheral edema	0	0	0	0	1 (11.1)	0	0	0	1 (8.3)	0	0	0	2 (5.0)	0

Abbreviations: AE, adverse event; ALT, alanine transaminase; AST, aspartate aminotransferase.
[a]Three grade 3 TRAE were reported in one patient each: hypertension and increased weight in the 100-mg once daily group and lymphopenia in the 150-mg once daily group.

time of enrollment. His prior treatments included ipilimumab plus nivolumab, followed by cabozantinib, and finally axitinib. This patient showed an erythropoietin reduction of about 80% from baseline and achieved PR by the first follow-up scan (cycle 2), with the deepest response of 51% by cycle 10. At data cutoff, he was still ongoing in cycle 19. The difference in percentage change from baseline to cycle 3 day 1 erythropoietin levels between responders (PR + SD) and nonresponders (PD) was not significant ($P = 0.551$). There was no clear correlation between erythropoietin and hemoglobin levels (Supplementary Fig. S2). Given the small number of

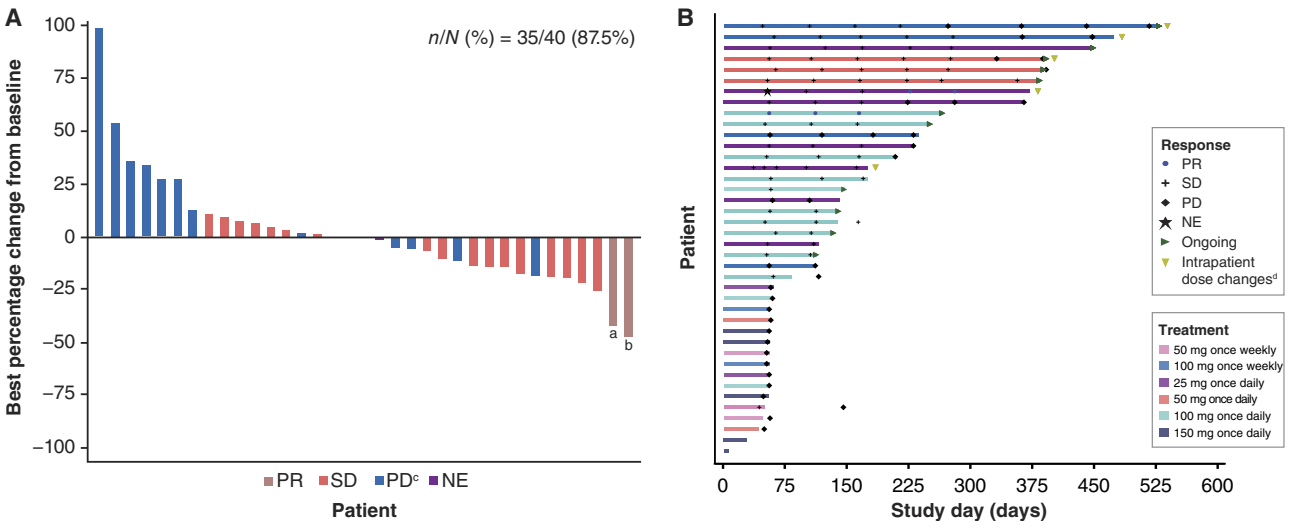

Figure 1.
Antitumor activity. (**A**) Best percentage change from baseline in tumor size per RECIST version 1.1 criteria and (**B**) duration of exposure to DFF332. [a]25 mg once daily; [b]100 mg once daily; [c]four patients had target tumor shrinkages as reflected in the plot and had an overall response of PD due to either new lesions or worsening of nontarget lesions at that same RECIST evaluation. All patients were permitted to continue treatment at the discretion of the physician who assessed that they benefited from the study treatment, and none are ongoing at the data cutoff; [d]intrapatient dose escalations or reductions are reported in five patients: (i) from 100 mg once weekly to 25 mg once daily on study day 251; (ii) from 100 mg once weekly to 25 mg once daily on study day 232, to 50 mg once daily on study day 288, to 100 mg once daily on study day 400; (iii) from 25 mg once daily to 12.5 mg once daily on study day 113; (iv) from 25 mg once daily to 50 mg once daily on study day 110, and (v) from 50 mg once daily to 100 mg once daily on study day 337. *n*, number of patients with RECIST data; *N*, total number of patients treated; NE, not evaluable; PD, progressive disease; PR, partial response; RECIST, Response Evaluation Criteria in Solid Tumors; SD, stable disease.

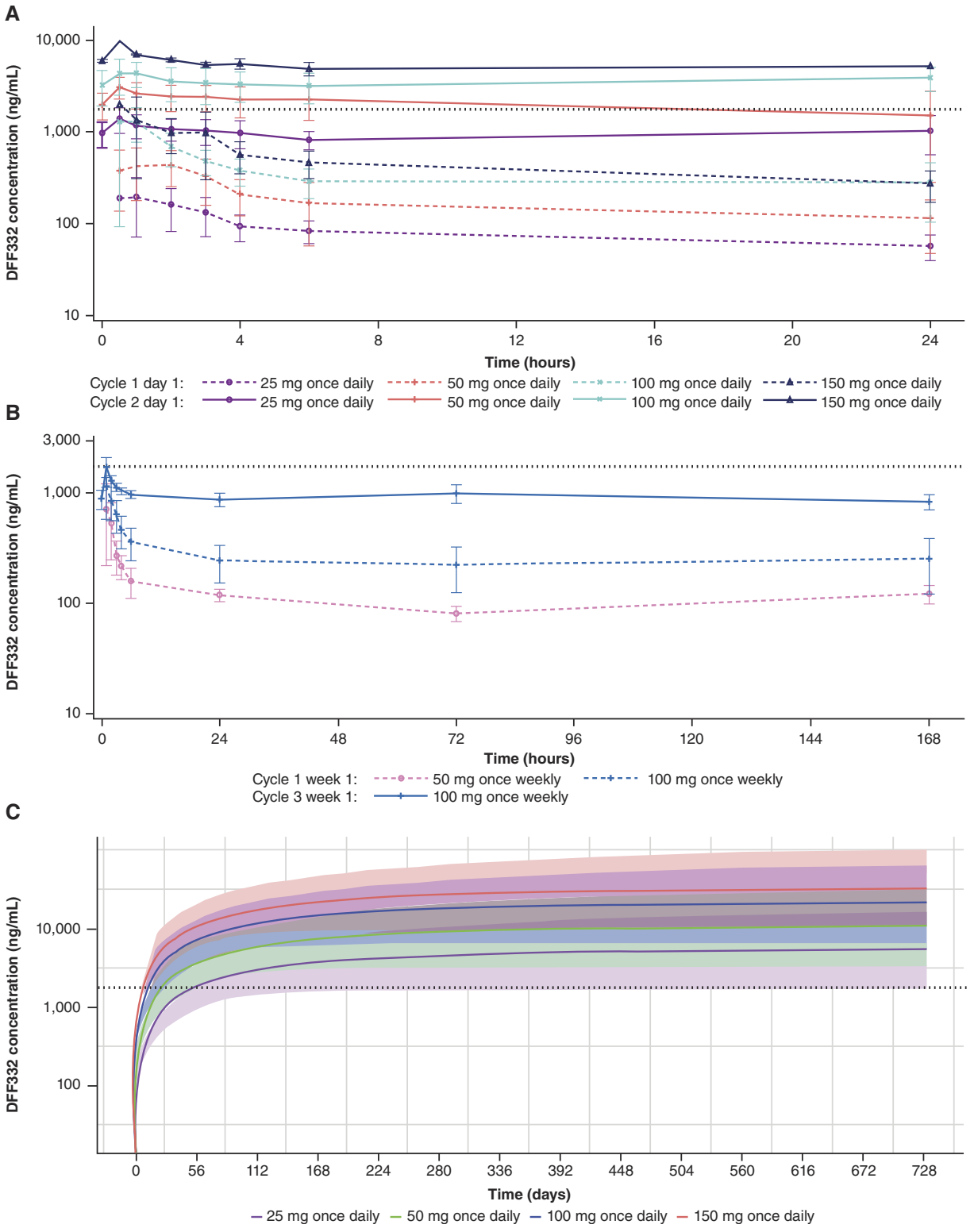

Figure 2.

PK profile mean (SD) plasma concentration–time profiles for DFF332 at varying doses on cycle 2 day 1 and cycle 3 day 1 for (**A**) once daily and (**B**) once weekly, respectively. (**C**) Simulated DFF332 plasma concentration–time profiles. Black dotted lines represent estimated trough DFF332 concentration (1,760 ng/mL) at the dose of maximum efficacy (10 mg/kg/d) in 786-O and SKRC01 xenograft mouse models; in (**C**), curved lines show mean DF332 concentration; shaded regions indicate interpatient variability.

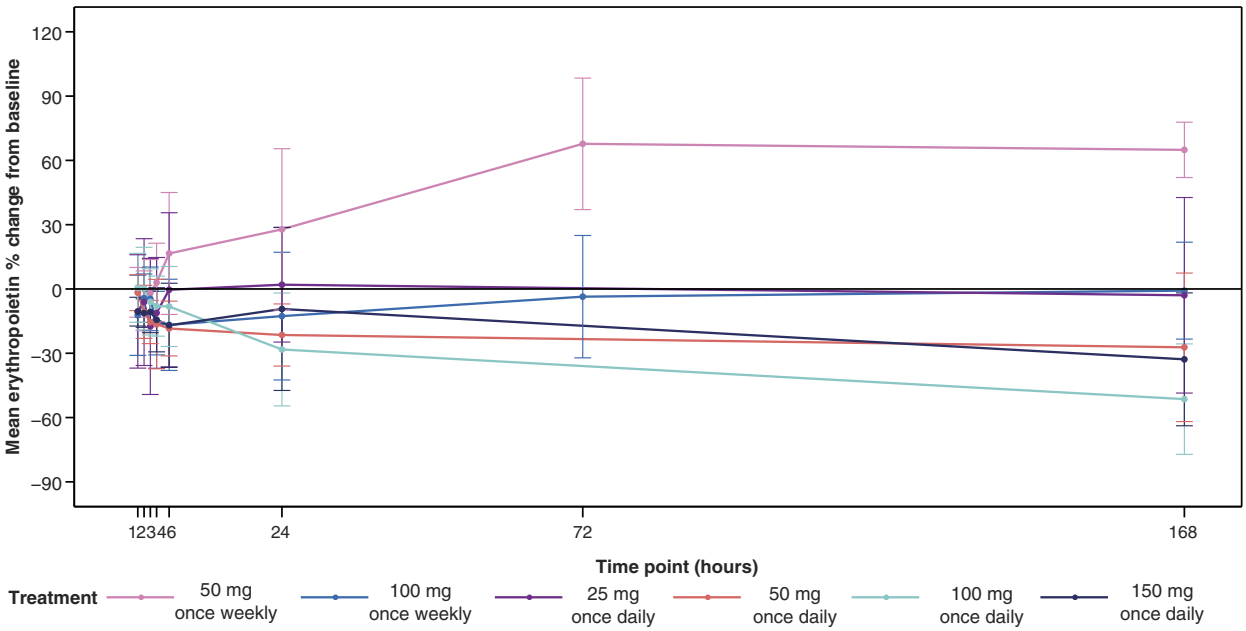

Figure 3.
Change in erythropoietin levels from before the first dose to cycle 1 day 8.

responders in this study, no conclusions could be drawn about the exposure-response relationship or the target optimum exposure range in humans.

Assessment of tumor expression of HIF-1α and HIF-2α

Both HIF-1α and HIF-2α were detectable by IHC analysis in most available specimens; however, no obvious correlation was observed between baseline expression levels of these moieties and response to DFF332 (**Fig. 4A**). In the examination of paired baseline and on-treatment biopsies, a trend toward decreased HIF-2α expression and increased HIF-1α expression was observed relative to baseline (**Fig. 4B** and **C**).

Discussion

In the current study, we report the safety, tolerability, and preliminary antitumor activity of DFF332, a novel HIF-2α inhibitor, in patients with advanced, heavily pretreated ccRCC. Relevant treatment-related on-target AE (e.g., hypoxia and anemia) occurred in a small proportion of patients. These AE occurred at rates lower than those with the approved HIF-2α inhibitor belzutifan. The two responses observed in this study (amounting to a response rate of 5%) occurred in one patient with DFF332 25 mg once daily and in another patient with DFF332 100 mg once daily. The study sponsor decided to halt further enrollment of patients for business reasons before the maximum tolerated/recommended dose could be determined. This was not predicated on any safety concerns with the compound. Thus, there was no opportunity to further explore the efficacy of DFF332 at higher doses in an expansion cohort. Further investigation would be necessary to accurately characterize the antitumor activity of the drug. In addition, the extended half-life poses significant challenges in drug development, as it prolongs the time required to reach steady-state concentrations. An alternative dosing strategy, such as administering a loading dose during the first cycle followed by a maintenance dose for the remainder of the treatment, could expedite the achievement of target exposure while minimizing the risk of toxic accumulation. For instance, population PK model-based simulations suggest that a regimen of 200 mg once daily for the initial cycle, followed by a maintenance dose of 75 mg once daily, may effectively shorten the time needed to achieve target efficacious exposures.

It is tempting to indirectly compare DFF332 with belzutifan, an agent approved by the FDA as of December 2023 for use as monotherapy in patients with advanced ccRCC. Both agents bind to the PAS-B domain of HIF-2α and inhibit further transcriptional activity of the moiety (6, 7, 11). Clinically, belzutifan is associated with high rates of anemia (88%); in contrast, in the present study, the overall rate of anemia with DFF332 was 33%, and it further decreased to 13% when only TRAE were considered (4, 5). Moreover, we observed no grade ≥3 anemia events with DFF332, as compared with >30% with belzutifan in a phase III study. Anemia is an on-target effect, mainly due to decreased erythropoietin production, perhaps leading one to believe that an agent that results in less anemia may have lesser potency (12). In the current study, erythropoietin levels showed somewhat dose-dependent variability. Higher doses of DFF332 (50 mg daily and above) resulted in decreased levels of erythropoietin, but lower doses had minimal effect on the levels. Our findings revealed no significant association between the modulation of erythropoietin by DFF332 and clinically significant changes in hemoglobin levels. Further studies using these higher doses would be necessary to confirm any correlation with study drug activity.

Another distinction in the toxicity profile between DFF332 and belzutifan is the absence of hypoxia with DFF332. Belzutifan is associated with hypoxia (grade 3/4) in approximately 15% of patients (13). Although the mechanism of belzutifan-associated hypoxia

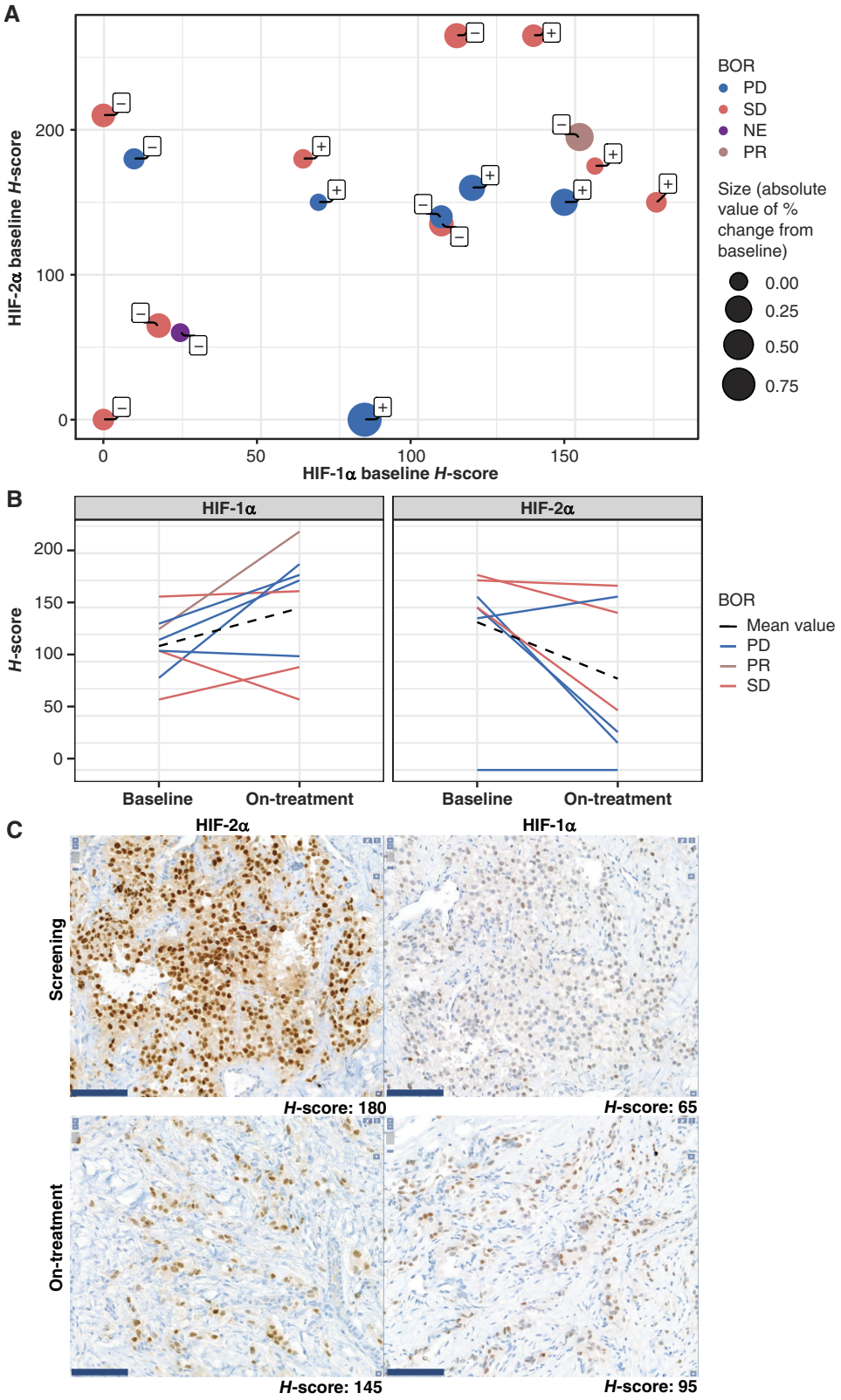

Figure 4.

IHC expression analysis of HIF-1α and HIF-2α: (**A**) HIF-1α versus HIF-2α baseline H-score in patients receiving DFF332, (**B**) evolution of HIF-1α and HIF-2α with DFF332 therapy, and (**C**) representative images. In (**A**), the size of the bubble is proportional to the percentage change in tumor sum of longest diameters; "±" indicates increase/decrease. IHC analysis is reported for patients for whom the full dataset (IHC on the baseline biopsy, baseline and on-treatment staining, or both) is available. In (**C**), tumor sections were stained using a fully automated system (Ventana Discovery Benchmark, Ventana Medical System) with a rabbit monoclonal anti-HIF-2α, clone D6T8V (Cat. No. 59973; Cell Signaling Technology), or with a rabbit monoclonal anti-HIF-1α, clone EP118 (Cat. No. BSB2520; Bio SB), primary antibody. The percentage of stained tumor cells and tumor positivity was scored using an H-score (0–300) by a certified pathologist. Scale bar, 80 μm. IHC, immunohistochemical; NE, nonevaluable; PD, progressive disease; PR, partial response; SD, stable disease.

remains unclear, it can be dose-limiting in patients. Thus, the safety profile of DFF332 may lend itself to being useful in combination strategies; however, this needs to be confirmed in a larger series. Belzutifan is currently approved for patients with previously treated disease, and multiple definitive studies are ongoing to evaluate the agent in earlier disease settings. In the front-line setting, belzutifan is being assessed in combination with lenvatinib (a VEGF-directed therapy) and pembrolizumab (a PD-1 inhibitor), whereas in the adjuvant setting, belzutifan with pembrolizumab is being compared with placebo plus pembrolizumab (13, 14). Although combination therapies may offer synergistic or additive antitumor activity, increased AE may complicate the effective delivery of the therapy. The exploration of DFF332 in combination with other therapies (e.g., immunotherapy, everolimus) was planned in the present study (ClinicalTrials.gov ID: NCT04895748) before it was halted (15).

A robust and validated biomarker of response in HIF-2α–directed therapies for advanced ccRCC remains elusive. Alteration in VHL alone does not seem to be sufficient. In the phase III trial evaluating belzutifan, a response rate of 22% falls short of expectations if approximately 50% of ccRCC is driven by HIF-2α in patients possessing VHL alterations (2). In our study, we aimed to determine whether HIF-2α could predict clinical benefit, but we found no association. Intriguingly, HIF-1α expression seemed to increase, and HIF-2α expression decreased with DFF332 therapy. Although HIF-2α has been described as necessary and sufficient for the development of VHL-deficient RCC, HIF-1α may possess tumor-suppressive properties (2, 16). Interestingly, in preclinical pharmacology experiments conducted during the development of DFF332, we observed that HIF-1α transcripts and HIF-1α–dependent targets increased upon HIF-2α inhibition in ccRCC models, in which both HIF-1α and HIF-2α are expressed, and HIF-2α–dependent efficacy seemed to be less prominent (7). With respect to these results, one might speculate that the antitumor activity of DFF332 may drive the restoration of a less malignant phenotype.

Limitations of this study include the small sample size. In our dose-escalation study, only about half of the patients received DFF332 at doses known to generate the on-target effect of decreased erythropoietin production (50 mg daily or higher). This gives us a limited opportunity to explore the antitumor activity effectively. Moreover, in our study, patients were heavily pretreated, with 50% receiving ≥3 prior lines of therapy, which makes inferring activity a challenge. Furthermore, the PK of DFF332 may be considered a limitation because elimination seems to be very slow. The dose–biomarker relationship in this study cannot be interpreted as the plasma concentrations on day 8 are much lower than at steady state. Although the investigators believe that combinations are feasible, given the favorable toxicity profile of the compound, low clearance could pose a challenge. This gives us a limited opportunity to explore the antitumor benefits effectively. There may also be mechanisms of resistance (e.g., increased signaling through AXL, MET, and other pathways) that render the tumor less dependent on HIF (17). In addition to belzutifan and DFF332, other small-molecule agents inhibiting HIF-2α, including AB521 and NK2152, are under development (18, 19). Clinical evaluation of these compounds in patients with advanced ccRCC is ongoing. Recently, data for ARO-HIF2 (an siRNA drug directed at HIF-2α) were reported: the response rate was 8% with a disease control rate of 39% (20). As multiple HIF-2α–directed strategies emerge, the onus will be on the scientific community to identify agents with the greatest activity (given the crowded landscape of therapies in advanced RCC) that also pair best with existing therapies.

In summary, we present data for a novel HIF-2α inhibitor, DFF332, in patients with advanced ccRCC. The safety and tolerability of the compound seemed to be favorable when compared with those of existing drugs with a similar mechanism of action. Although our data were not sufficient to fully characterize the efficacy of the drug, some antitumor activity was observed. HIF-2α is clearly vital to the biology of ccRCC, and more efforts are needed to combine this approach with existing therapeutic strategies. Further exploration of this agent is warranted, first with an appropriate dose expansion and then in combination with distinct classes of RCC therapy.

Authors' Disclosures

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Authors' Contributions

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Note

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