Journal of Hepatocellular Carcinoma Dovepress

Open Access Full Text Article

CLINICAL TRIAL REPORT

A Phase 1a/1b Study of Fostroxacitabine Bralpamide (Fostrox) Monotherapy in Hepatocellular Carcinoma and Solid Tumor Liver Metastases

Ruth Plummer[1], Alastair Greystoke[1], Gregory Naylor[2], Debashis Sarker[3,4], ANM Kaiser Anam[4], Hans Prenen[5], Laure-Anne Teuwen[5], Eric Van Cutsem[6], Jeroen Dekervel[6], Beate Haugk[1], Thomas Ness[1], Sujata Bhoi[7], Malene Jensen[7], Tom Morris[7], Pia Baumann[7], Niclas Sjögren[8], Karin Tunblad[7], Hans Wallberg[7], Fredrik Öberg[7], Thomas R Jeffry Evans[2]

[1]Newcastle University and Newcastle Hospitals NHS Foundation Trust, Newcastle Upon Tyne, UK; [2]Beatson West of Scotland Cancer Centre, University of Glasgow, Glasgow, UK; [3]School of Cancer and Pharmaceutical Sciences, King's College London, London, UK; [4]Department of Medical Oncology, Guy's Hospital, London, UK; [5]Department of Oncology, Antwerp University Hospital, Edegem, Belgium; [6]Department of Oncology, University Hospitals Gasthuisberg Leuven and KU Leuven, Leuven, Belgium; [7]Medivir AB, Huddinge, Sweden; [8]SDS Life Science, Stockholm, Sweden

Correspondence: Pia Baumann, Medivir AB, Box 1086, SE-141 22, Huddinge, Sweden, Tel +46 739163897, Email pia.baumann@medivir.com

Purpose: To evaluate safety, preliminary efficacy, pharmacokinetics, and pharmacodynamics, of fostroxacitabine bralpamide (fostrox, MIV-818), a novel oral troxacitabine nucleotide prodrug designed to direct exposure to the liver, while minimizing systemic toxicity.

Patients and Methods: Fostrox monotherapy was administered in an open-label, single-arm, first-in-human, phase 1a/1b study, in patients with hepatocellular carcinoma (HCC), intrahepatic cholangiocarcinoma, or solid tumor liver metastases. The first part (1a) consisted of intra/inter-patient escalating doses (3 mg to 70 mg) QD for up to 5 days, and the second part (1b), doses of 40 mg QD for 5 days, in 21-day cycles. Safety and tolerability were evaluated by the Safety Review Committee, and efficacy was assessed every 6 weeks with CT or MRI using RECIST 1.1 and mRECIST.

Results: Nineteen patients were treated with fostrox. Most common adverse events (AEs) were hematological and increased AST. Grade 3 treatment related AEs (TRAE) were seen in 53% of the patients, with transient neutropenia and thrombocytopenia as the most common. No grade 5 AE was observed. Recommended Phase 2 dose of fostrox was 40 mg QD for 5 days in 21-day cycles. Preliminary efficacy showed a clinical benefit rate in the liver of 53% and stable disease (SD) as best response in 10 patients. Liver targeting with fostrox was confirmed with higher exposure of troxacitabine and its metabolites in liver compared to plasma. Systemic exposure of fostrox was generally low with troxacitabine as main analyte. Biopsies demonstrated tumor-selective, drug-induced DNA damage.

Conclusion: The phase 1a/1b monotherapy study of fostrox, in patients with liver tumors, showed a tumor selective effect in the liver and that 40 mg QD for 5 days in 21-day cycles is safe and tolerable. Safety and preliminary efficacy in patients with advanced HCC supports clinical development of fostrox in combination with other modes of action in HCC.

Keywords: phase 1, fostrox, hepatocellular carcinoma, nucleotide prodrug, pharmacokinetics, pharmacodynamics

Introduction

The occurrence of tumor lesions in the liver is generally associated with poor outcome independently of primary tumor type. This is partly due to the negative impact the lesions have on vital liver functions but also since a tumor with the ability to metastasize to the liver is commonly associated with a more aggressive cancer. Primary liver cancer, hepatocellular carcinoma (HCC), has a particularly poor prognosis, differing in certain aspects from liver metastasis. Not only is the major tumor burden located in the liver but more importantly, almost all HCC patients have an underlying liver cirrhosis and many die due to liver failure caused by the combination of tumor progression and lack of normal tissue reserve in the liver.[1] The incidence of HCC is increasing dramatically, attributed to viral hepatitis, alcohol excess, and in particular obesity and type II diabetes-related non-alcoholic steatohepatitis (NASH).[2] The incidence of intrahepatic cholangiocarcinoma (iCCA) is also increasing together with

Received: 6 June 2024
Accepted: 11 October 2024
Published: 24 October 2024

liver metastasis from other primary tumor types, most commonly carcinomas of the colon, pancreas, and breast.[3] Despite progress in the development of new treatments in recent years, the overall prognosis for patients with lesions in the liver is still poor, constituting a large unmet medical need.[3]

For patients with locally advanced unresectable or metastatic HCC, systemic treatment has evolved to a current standard of care with immunotherapy combinations.[4,5] With this change in the first line, there are currently no regulatory approved treatments in second line post an immunotherapy combination and treatment guidelines recommend clinical studies for this population.[6] In several tumor types with metastasis to the liver, chemotherapy has an important role and is the backbone partner in many combinations, particularly in tumors lacking defined molecular targets. In contrast, conventional systemic chemotherapy is not routinely used in HCC due to lack of proven survival benefits.[7] In general, the clinical efficacy of chemotherapy in both liver metastatic disease and HCC is limited by systemic intolerance of the doses required to achieve efficacious exposure in the liver. By increasing the loco-regional exposure of chemotherapy, eg, by transarterial chemoembolization (TACE) or hepatic artery infusion chemotherapy (HAIC), increased efficacy in the liver and reduced systemic toxicities can be achieved.[8] However, due to their broad action chemotherapeutic drugs are in many cases associated with substantial hepatic toxicities, limiting their use in patients with poor liver function.[9]

Fostrox is a prodrug of troxacitabine monophosphate (MP) with oral administration, enhancing the delivery to the liver. Bioactivation of fostrox to the active metabolite troxacitabine triphosphate (TP) is an intracellular multistep process dependent on liver function. The intention is to maximize the exposure to the liver by first-pass metabolism while minimizing systemic exposure to fostrox and troxacitabine (TRX), and thus limit adverse effects in other organs. Nonclinical in vivo data on rats has shown 100-fold increased delivery of the active metabolite to liver by oral administration of fostrox compared to intravenous administration of troxacitabine.

Troxacitabine itself is an unnatural stereochemical deoxycytidine analogue, making it resistant to deamination by deoxycytidine deaminase, with potent activity against HCC[10] and other tumor types.[11] The active metabolite is a substrate for DNA polymerases[12] and is incorporated into DNA during replication, which results in complete DNA chain termination.[10] Once incorporated into the DNA, the troxacitabine nucleotide is inefficiently excised, which leads to DNA double strand breaks, DNA damage responses, and cytotoxicity.[13,14] Cytotoxic effects are strictly dependent on cells undergoing DNA replication, which also creates a therapeutic index favoring toxicity to cancer cells versus normal cells due to the differences in proliferation rate.[14]

Troxacitabine administered intravenously was evaluated in several clinical trials for different cancer indications[15–19] and progressed into Phase 2/3 in acute myelocytic leukemia. The development of troxacitabine was, however, discontinued due to insufficient efficacy within its relatively narrow therapeutic window, when given intravenously.

A first-in-human, phase 1 study with fostrox as monotherapy in escalating doses was conducted in patients with HCC, iCCA, and liver metastases from other solid tumors. The primary objective was safety and tolerability, and preliminary efficacy, pharmacokinetics, liver targeting, and tumor selectivity were also explored. This report discusses the dose finding and safety data, and additionally highlights results in patients with HCC, the patient population selected for the next phase of fostrox development.

Materials and Methods

Clinical Study Design and Patients

The ongoing multicenter, single-arm, open-label, first-in-human phase 1/2 clinical trial is evaluating the safety and tolerability, and as exploratory objectives pharmacokinetics/pharmacodynamics, and preliminary antitumor activity of fostrox in HCC, iCCA and liver metastases from other solid tumors. As the primary objective was safety and tolerability in a patient group with diverse primary cancers, no formal sample size calculation was performed. The study is being conducted in several parts. Monotherapy part: phase 1a/1b monotherapy with fostrox intra/inter-patient dose escalation in 19 patients with HCC, iCCA or liver metastases from other solid tumors. Combination part 1: phase 1b combination with fostrox inter-patient dose escalation in combination with lenvatinib or pembrolizumab in 16 patients with up to two prior treatment lines for locally advanced unresectable or metastatic HCC to identify the recommended phase 2 dose (RP2D) for the combinations. Combination part 2: phase 2a with fostrox RP2D expansion in combination with lenvatinib in 15 patients with up to two prior treatment lines for

locally advanced unresectable or metastatic HCC. With the change in first-line treatment to an immunotherapy combination, moving TKI to the second line setting, the decision was taken to focus on the lenvatinib combination and not to proceed with the expansion of the combination of fostrox plus pembrolizumab.

The study was performed in accordance with the principles of the Declaration of Helsinki, the International Conference on Harmonization guideline for Good Clinical Practice, and applicable local regulatory requirements. The protocol and protocol amendments were approved by the relevant ethics committees. Written informed consent was obtained from all patients before the initiation of any study procedure. The study was registered at ClinicalTrials.gov (NCT03781934), and at EudraCT (2018–000995-14).

The phase 1a/b fostrox monotherapy part has been completed and is reported here. This part of the study was conducted at six investigational sites in the UK and Belgium. Patients (≥18 years) diagnosed with HCC, iCCA, or metastases from other solid tumors with adequate organ function, Eastern Cooperative Oncology group (ECOG) performance status ≤1 and measurable disease by Response Evaluation Criteria in Solid Tumors version 1.1 (RECIST v1.1), were eligible for inclusion. The main exclusion criteria included; tumor volume exceeding 50% of liver, history of solid organ transplant or bone marrow transplant, poorly controlled ascites and/or requirement for therapeutic paracentesis more frequently than once every 3 months, symptomatic encephalopathy within 3 months prior to Screening and/or requirement for medication for encephalopathy, esophageal variceal bleeding within 2 weeks prior to screening, receiving anticancer therapy within 4 weeks prior to first dose of fostrox.

Treatment

Phase 1a constituted an intra-patient dose-escalation, in which fostrox monotherapy was administered orally as capsules on one occasion in Cycle 0 (on Day −7 or Day −6), and once daily (QD) for up to 5 consecutive days from Cycle 1 and onwards. One cycle comprised a 21-day period where each patient could receive a maximum of two fostrox dose escalations (Figure 1). Cumulative safety data, up to and including completion of each treatment cycle for each patient prior to the next patient being enrolled, were reviewed by the Safety Review Committee (SRC), comprising of representatives from the sponsor and study site investigators.

Phase 1b constituted an inter-patient dose-escalation, in which fostrox monotherapy was administered orally as capsules QD for 5 days in a 21-day cycle (Figure 1). The starting dose was based on the data from phase 1a.

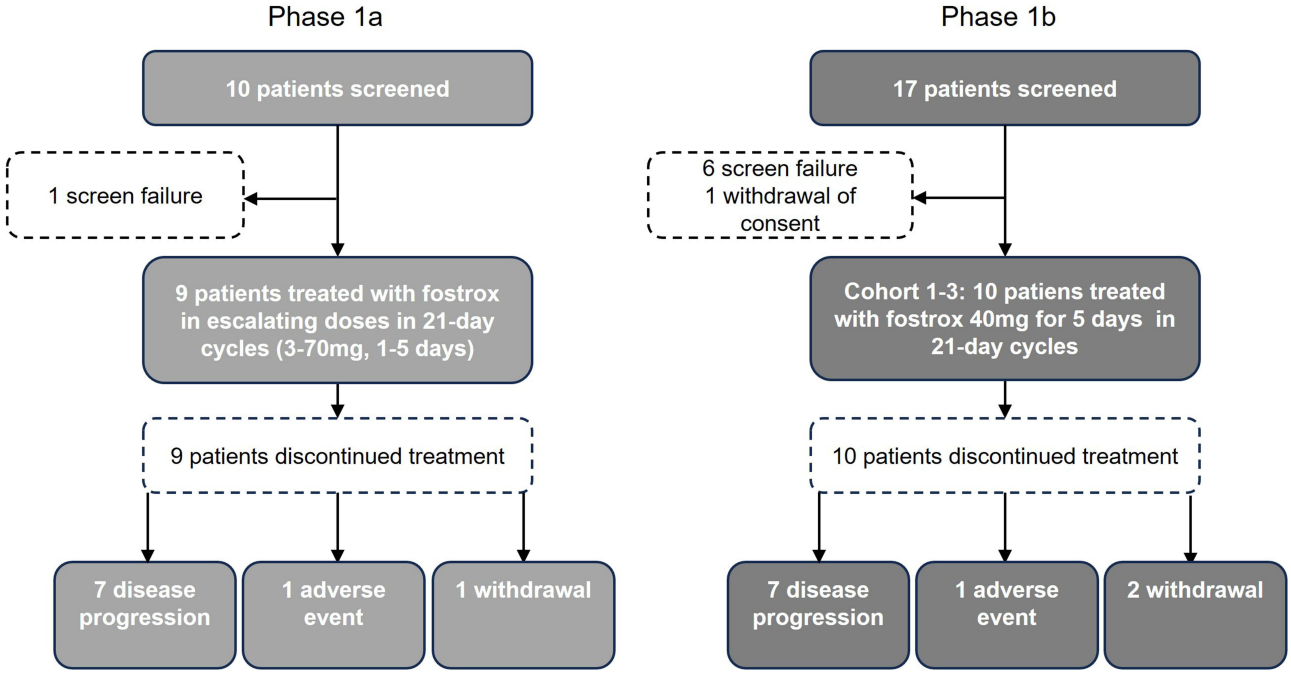

Figure 1 Study overview.

The dose could be adjusted by the SRC based on their continuous evaluation of emerging safety data. Patients were treated until disease progression, death, unacceptable toxicity, or withdrawal of consent.

Safety Evaluation

Treatment-emergent adverse events (TEAE) were assessed according to the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) version 5.0, coded using the Medical Dictionary for Regulatory Activities (MedDRA) version 21.0, and assessed for relatedness by the investigators. The definition of the dose limiting toxicities (DLT) occurring during the first 21-day treatment cycle is described in the Supplementary Information.

Anti-Tumor Activity

The tumor response was assessed as complete response (CR), partial response (PR), stable disease (SD), or progressive disease (PD), via contrast-enhanced computed tomography (CT) or magnetic resonance imaging (MRI) every 6 weeks by an independent reviewer, according to RECIST 1.1 and mRECIST criteria. The objective response rate (ORR) was defined as the proportion of patients with the best response (either CR or PR), and the clinical benefit rate (CBR) was defined as CR, PR, or SD lasting > 6 weeks.

Pharmacokinetic and Pharmacodynamic Analysis

Blood samples for pharmacokinetic (PK) analysis were collected at pre-defined time-points (Supplementary Table 1). The samples were centrifuged to obtain plasma and stored at −80°C until bioanalysis. A liver biopsy for PK analysis was collected from 7 patients in phase 1a and 5 patients in phase 1b, 2 to 4 hours after fostrox administration in Cycle 2 and stored at −80°C until analysis.

The plasma samples were analyzed according to good laboratory practice (GLP) applying protein precipitation and liquid chromatography with tandem mass spectrometric detection to quantify the fostrox and the metabolites TRX and the alanine metabolite (AM) (scheme for bioactivation of fostrox in Supplementary Figure 1). The liver biopsy samples were analyzed using an analytical method involving sample homogenization and liquid chromatography with tandem mass spectrometric detection. The method was used to quantify AM, TRX, TRX-MP, TRX-DP, and TRX-TP.

Here we present PK results from Day 1 in cycles 1 and onwards where PK samples were collected. A complete analysis of all PK data using population PK modelling will be presented separately.

For the pharmacodynamics samples, needle biopsies containing both tumor and normal liver tissue were collected in Cycle 2, 2 to 4 hours after fostrox treatment, fixed in 10% neutral buffered formaldehyde and embedded in paraffin. Slides from the on-treatment biopsy and, if present, an archival/predose sample, were stained with hematoxylin/eosin (H&E), and immunohistochemistry analysis of DNA damage (pH2AX), proliferation (Ki-67), and hypoxia (GLUT1). Double staining for pH2AX/GLUT1 was performed to detect co-localization of DNA damage and hypoxia. See Supplementary Table 2 for detailed information on antibodies.

Results

Patient Characteristics

Between October 2018 and June 2021, a total of 19 patients received fostrox monotherapy in the phase 1a/1b monotherapy part. One patient did not complete the first cycle and could only be evaluated for safety. Overall, the median age was 64 years (range 47 to 84 years), with 74% being males. A total of 8 patients were diagnosed with HCC (including one with mixed HCC and iCCA) with a median age of 72 years (range 54 to 84 years), and 11 patients presented with liver metastases from other tumor types (2 patients with iCCA, and 9 patients with metastases from other solid tumors). All but one patient had progressed on prior systemic treatment. Demographic and other baseline characteristics are described in Table 1.

Treatment

In phase 1a, 9 evaluable patients, 2 patients with HCC, received escalating doses of fostrox monotherapy, ranging from 3 mg to 70 mg daily for 1–5 days in 21-day cycles (Table 2).

Table 1 Demographic and Other Baseline Characteristics by Primary Cancer Type

	HCC	Other Cancer Types[a]	Total
Number of patients	8 (42%)	11 (58%)	19 (100%)
Age (years, median [min; max])	72 (54; 84)	57 (47;74)	64 (47;84)
Gender			
Male	7 (88%)	7 (64%)	14 (74%)
Female	1 (12%)	4 (36%)	5 (26%)
Time since diagnosis (year)			
1-<2	3 (38%)	4 (36%)	7 (37%)
2-<3	3 (38%)	3 (27%)	6 (32%)
3-<4	1 (12%)	2 (18%)	3 (16%)
>4	1 (12%)	2 (18%)	3 (16%)
Prior lines of systemic therapy			
0	1 (12%)	0	1 (5%)
1	4 (50%)	3 (27%)	7 (37%)
2	2 (25%)	2 (18%)	4 (21%)
3	1 (12%)	3 (27%)	4 (21%)
4	0	3 (27%)	3 (16%)
Prior loco-regional therapy			
0	4 (50%)	11 (100%)	15 (79%)
TACE	3 (38%)	0	3 (16%)
Laparotomy and resection	1 (12%)	0	1 (5%)
Performance status at screening (ECOG)			
0	5	4	9
1	3	7	10
Study phase			
Phase 1a	2	7	9
Phase 1b	6	4	10

Notes: Data are presented as number of subjects with percentages in parentheses. [a]Patients with intrahepatic cholangiocarcinoma (iCCA) or liver metastasis from colorectal, gastric, esophageal, pancreas, and melanoma.
Abbreviation: TACE, transarterial chemoembolization.

Each patient received a single dose at their set starting level (Cycle 0), either 3, 10, 24, 40, 50, 60, or 70 mg, followed by a Cycle 1 of either the dose from Cycle 0 or a dose at the next level. There were up to 2 intra-patient dose escalations for 1 to 4 treatment cycles. The 2 patients with HCC received a starting dose (Cycle 1) of 50 and 60 mg for 4 and 5 days and received 1 and 4 cycles, respectively. The maximum total dose per cycle for all patients was 300 mg (60 mg once per day for 5 days), and the maximum cumulative dose was 960 mg over a period of 11 weeks (Table 2).

In 4 out of 5 patients enrolled in phase 1a with doses > 40 mg for 4 or 5 days in 21-day cycles, grade ≥ 3 hematological adverse events were reported leading to a phase 1b dose expansion with 40 mg QD for 5 days in 21-day cycles being opened

Table 2 Fostrox Dosing per Patient Dosing in Phase 1a and Phase 1b

Patient id	Primary Cancer Diagnosis	Number of Cycles*	Days Per Cycle	Dose (mg)	Cumulative Dose (mg)
A5	HCC	5*	4	50/60	960
A9	HCC	2*	5	60	360
B2	HCC	9	5	40/30	1750
B4	HCC	3	5	40/30	550
B5	HCC	1	5	40	200
B7	HCC	4	5	40/30	650
B8	HCC	3	5	40	600
B9	HCC	4	5	40/20	700
A1	Esophageal carcinoma	3*	1/3	3/10	22
A2	Rectal carcinoma	3*	3	10/20	100
A3	ICCA	3*	3	20/30	160
A4	Colorectal carcinoma	3*	4	24/40	280
A6	Melanoma	3*	4/5	70/30	500
A7	Pancreatic carcinoma	3*	5	50	550
A8	Colorectal carcinoma	3*	5	60	660
B1	Rectal carcinoma	2	5	40	400
B3	ICCA	3	5	40/30	500
B6	Gastric carcinoma	2	5	40	400
B10	Colorectal carcinoma	1	5	40	200

Notes: *Cycle 0 considered as 1 cycle. Patients marked "A" were included in phase 1a, patients marked "B" were included in phase 1b.
Abbreviations: HCC, hepatocellular carcinoma; ICCA, intrahepatic cholangiocarcinoma.

and enrolling in total 10 patients in 3 cohorts of 3 patients each. One patient discontinued before the end of the first cycle and was replaced. The maximum exposure over one cycle was 200 mg (40 mg for 5 days), and the maximum cumulative dose was 1750 mg administered over 25 weeks. Six patients with HCC were enrolled in phase 1b.

Dose reductions in phase 1a/b due to adverse events were done in 5 patients (3 HCC patients). In 3 patients from 40 mg to 30 mg, in one patient from 40 mg to 20 mg, and in one patient from 70 mg to 30 mg (Table 3).

Safety

In phase 1a/1b all 19 patients had at least one treatment emergent adverse event (TEAE) that was also judged as either related or possibly related to fostrox by the investigator. The most commonly reported all grade TEAE was anemia and increased levels of aspartate aminotransferase (AST). All grades of neutropenia and thrombocytopenia were reported in 42% and 42%, respectively (Table 4) and were mostly transient and in 21% of the overall patient population managed with dose interruption and/or dose reduction.

Grade 3 treatment related AEs (TRAE) were seen in 53% of patients with transient neutropenia and thrombocytopenia as the most common event. No patients had a grade 5 AE.

TEAEs leading to treatment discontinuation were reported in 4 patients, of which 2 were judged to be related and possibly related to fostrox; a grade 2 thrombocytopenia in an HCC patient at day 186 in the study and a grade 2 hyperbilirubinemia in a patient with liver metastases at day 11, respectively. The additional 2 patients discontinued

Table 3 Dose Reductions and Reasons for Discontinuations in Phase 1a and Phase 1b

Primary Cancer Diagnosis / Patient id	Starting Dose (mg)	Dose Reduction to (mg)	Cycle and Day of Dose Reduction	Reason for Drug Discontinuation	Cycle and Day of Drug Discontinuation
HCC					
A5	50			Disease progression	C4D22
A9	60			Disease progression	C1D50
B2	40			AE; thrombocytopenia	C8D39
B4	40	30	C3D1	Withdrawal by subject	C3D37
B5	40			Disease progression	C2D9
B7	40	30	C2D1	Withdrawal by subject	C4D29
B8	40			Disease progression	C3D15
B9	40	20	C4D1	Disease progression	C4D15
Other diagnosis					
A1	3			AE, esophageal hemorrhage	C2D14
A2	10			Disease progression	C2D20
A3	20			Disease progression	C2D18
A4	24			Disease progression	C2D22
A6	70	30	C2D1	AE, prolonged hem toxicity	C2D33
A7	50			Disease progression	C2D18
A8	60			Disease progression	C2D22
B1	40			Disease progression	C2D24
B3	40	30	C2D1	Disease progression	C3D13
B6	40			Disease progression	C2D17
B10	40			Disease progression	C1D16

Notes: Patients marked "A" were included in phase 1a, patients marked "B" were included in phase 1b.

Table 4 All Treatment Emergent and Treatment Related Adverse Events in Phase 1a and Phase 1b

System Organ Class Preferred Term	TEAEs, All Grade #patients (%)	TEAEs Grade ≥3 #patients (%)	TRAEs, All Grade #patients (%)	TRAEs Grade ≥3 #patients (%)
Blood and lymphatic system disorders				
Anemia	10 (53%)	4 (21%)	7 (37%)	4 (21%)
Thrombocytopenia	8 (42%)	5 (26%)	8 (42%)	5 (26%)
Neutropenia	8 (42%)	7 (37%)	8 (42%)	7 (37%)
Lymphopenia	3 (16%)	2 (11%)	3 (16%)	2 (11%)

(*Continued*)

Table 4 (Continued).

System Organ Class Preferred Term	TEAEs, All Grade #patients (%)	TEAEs Grade ≥3 #patients (%)	TRAEs, All Grade #patients (%)	TRAEs Grade ≥3 #patients (%)
General disorders and administration site conditions				
Fatigue	8 (42%)	–	6 (32%)	–
Chest pain	3 (16%)	1 (5%)	1 (5%)	1 (5%)
Investigations				
Aspartate aminotransferase increased	8 (42%)	2 (11%)	5 (26%)	–
Blood alkaline phosphatase increased	6 (32%)	–	4 (21%)	–
Alanine aminotransferase increased	6 (32%)	2 (11%)	4 (21%)	1 (5%)
Gamma-glutamyl transferase increased	4 (21%)	2 (11%)	3 (16%)	1 (5%)
White blood cell count decreased	3 (16%)	3 (16%)	3 (16%)	3 (16%)
Platelet count decreased	3 (16%)	2 (11%)	3 (16%)	2 (11%)
Blood creatinine increased	3 (16%)	–	–	–
Skin and subcutaneous tissue disorders				
Pruritus	7 (37%)	–	5 (26%)	–
Palmar-plantar erythrodysaesthesia syndrome	4 (21%)	–	3 (16%)	–
Rash	5 (26%)	1 (5%)	5 (26%)	1 (5%)
Gastrointestinal disorders				
Nausea	6 (32%)	–	6 (32%)	–
Constipation	5 (26%)	–	2 (11%)	–
Diarrhea	4 (21%)	–	2 (11%)	–
Vomiting	4 (21%)	1 (5%)	–	–
Abdominal pain upper	3 (16%)	–	–	–
Metabolism and nutrition disorders				
Decreased appetite	6 (32%)	–	4 (21%)	–
Nervous system disorders				
Lethargy	4 (21%)	–	2 (11%)	–
Hepatobiliary disorders				
Hyperbilirubinemia	3 (16%)	2 (11%)	1 (5%)	–

Abbreviations: TEAE, treatment emergent adverse event; TRAE, treatment related/possibly related adverse event.

treatment due to serious adverse events (SAEs); grade 3 esophageal hemorrhage at day 48 and grade 3 spinal cord compression at day 38, which were both considered by the investigator not to be related to study treatment.

Two DLTs occurred during the study, in phase 1a a DLT with neutropenic sepsis was reported in one patient receiving fostrox at a dose of 70 mg QD for 4 days in 21-day cycles. Dose reduction to 30 mg QD for 5 days in a 21-day cycle was applied after this AE. In phase 1b, a DLT with grade 3 rash was reported in one patient in the first 3 patients' cohort on

fostrox 40 mg QD for 5 days in a 21-day cycle. No dose modification was applied. This DLT triggered the expansion of the next cohort of 3 patients at the 40 mg level. A further expansion with a third cohort of 3 patients, at the same 40 mg dose level, was added to characterize hematological events over a longer period of time. In the absence of a formal MTD as defined by the study protocol (≥2DLT at a given dose level), all available accumulated safety, tolerability, and PK was considered, and the recommended phase 2 dose was defined as 40 mg for 5 days in a 21-day cycle.

There were in total 14 SAEs reported (7 in 5 patients in phase 1a and 7 in 4 patients in phase 1b), out of which 4 were related to treatment: febrile neutropenia, neutropenic sepsis, hyperbilirubinemia, and chest pain (Table 5). There was no clear correlation of SAEs to fostrox dose level or cumulative exposure. From the known safety profile of troxacitabine, certain AEs are considered of special interest and were reported as follows: neutropenia (9 patients, grade ≥3 in 7 patients), hyperbilirubinemia (5 patients, grade ≥3 in 2 patients), hand and foot syndrome (4 patients, no patients with grade ≥3), rash (4 patients, grade ≥3 in 1 patient), and stomatitis (1 patient, no patients with grade ≥3).

During follow-up, 14 patients died, with no death considered by the investigator to be related to study treatment. Assessments of vital signs, physical examination, and electrocardiogram did not provide any safety concerns.

Anti-Tumor Activity

In phase 1a, the 9 patients evaluable for response had a clinical benefit rate (CBR) of 44% with RECIST 1.1. Four patients had stable disease (SD), and 5 patients had best response of progressive disease (PD).

In phase 1b, the 9 evaluable patients all received a dose of 40 mg QD for 5 days in 21-day cycles. The CBR was 40% with RECIST v.1.1 (4 patients with SD), 30% with mRECIST (3 patients with SD) and 60% with RECIST 1.1 in the liver (6 patients with SD).

In phase 1a + 1b taken together, ORR, CR, PR, SD, and PD were 0%, 0%, 0%, 44%, and 55%, respectively, with RECIST v.1.1. The CBR in the liver was 53% and stable disease (SD) as best response seen in 10 patients. In patients with HCC, CBR was 63% with both 2 patients in phase 1a having SD and 3 out of 6 patients in phase 1b having SD, with two patients having SD for >12 weeks.

Table 5 Summary of Serious Adverse Events (SAE), in Phase 1a and Phase 1b, by Preferred Term During the Overall Study Period (Safety Analysis Set)

Preferred Term	HCC n=8	Other Diagnosis n=11	Total N=19	Total Possibly Related/Related
Spinal cord compression		1	1	
Neutropenic sepsis		1	1	1
Esophageal hemorrhage		1	1	
Hematemesis		1	1	
Campylobacter infection	1		1	
Procedural pain		1	1	
Aspartate aminotransferase increased	1		1	
Hyperbilirubinemia	1	1	2	1
Back pain	1		1	
Chest pain	1		1	1
Platelet count decreased	1		1	
Febrile neutropenia	1		1	1

Pharmacokinetics

Fostrox was quickly absorbed and eliminated in accordance with the expectations from a prodrug (Figure 2A). Maximum plasma concentration was generally observed within 2 hours after fostrox administration, and in most patients, fostrox was only quantifiable up to 4 hours post dose.

In patients dosed with 40 mg fostrox (phase 1b), the total exposure in plasma on Day 1 in Cycle 1 was low with an average estimated $AUC_{0\text{-}t}$ of 0.024 µM*h (Supplemental Table 2). Similarly, the exposure to alanine metabolite (AM) was low (Figure 2B), and generally not quantifiable pre-dose in repeat dose administration. TRX was the main analyte in plasma (Figure 2C), with the total exposure and half-life estimated to 0.48 µM*h and 24 hours, respectively, based on Cycle 1 Day 1 data (Supplemental Table 3). A 2-fold accumulation of TRX was observed after repeated dosing of fostrox (Figure 2D). The exposure to fostrox, TRX, and AM in patients enrolled in phase 1a generally increased when increasing the fostrox dose (Supplemental Table 4).

PK assessment in liver biopsies was evaluable in 8 patients, of which one patient had HCC, and 7 had metastatic liver disease. TRX, AM, and TRX-MP were quantified in 7, 4, and 5 of the biopsies, respectively. Patients who had quantifiable concentrations of at least one metabolite received doses of at least 20 mg for 3 days. Exposure to the active metabolite (TRX-TP) was confirmed in one patient, dosed with 30 mg for 5 days. At the time of liver biopsy collection, the exposure to TRX was 1.3 to 11-fold higher in liver than in plasma. Corresponding data for the AM was 19 to 40-fold higher concentration in liver than in the plasma. The exposure to fostrox and its metabolites in liver and in plasma in 2 patients is presented in Figure 3.

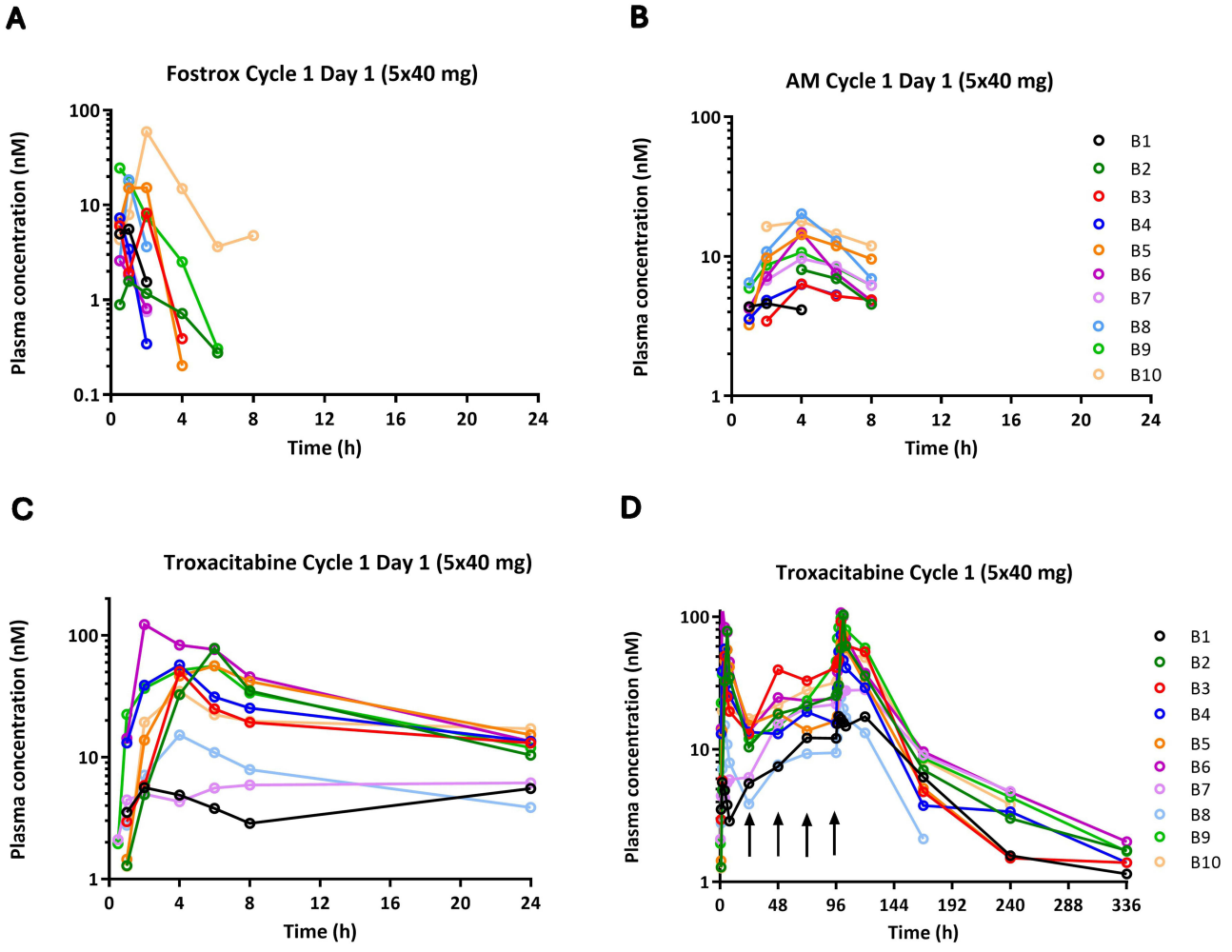

Figure 2 Observed concentrations of fostrox in Cycle 1 Day 1 (**A**), the alanine metabolite (AM) Cycle 1 Day 1 (**B**), troxacitabine in Cycle 1 Day 1 (**C**) and troxacitabine in Cycle 1 (**D**) in phase 1b. The arrows indicate fostrox dose administrations. Each curve presents individual patient data (eg, 1B, 2B etc.). The lower limit of quantification (LLOQ) was 0.2 nM, 1.0 nM, and 3.0 nM for fostrox, TRX, and AM, respectively.

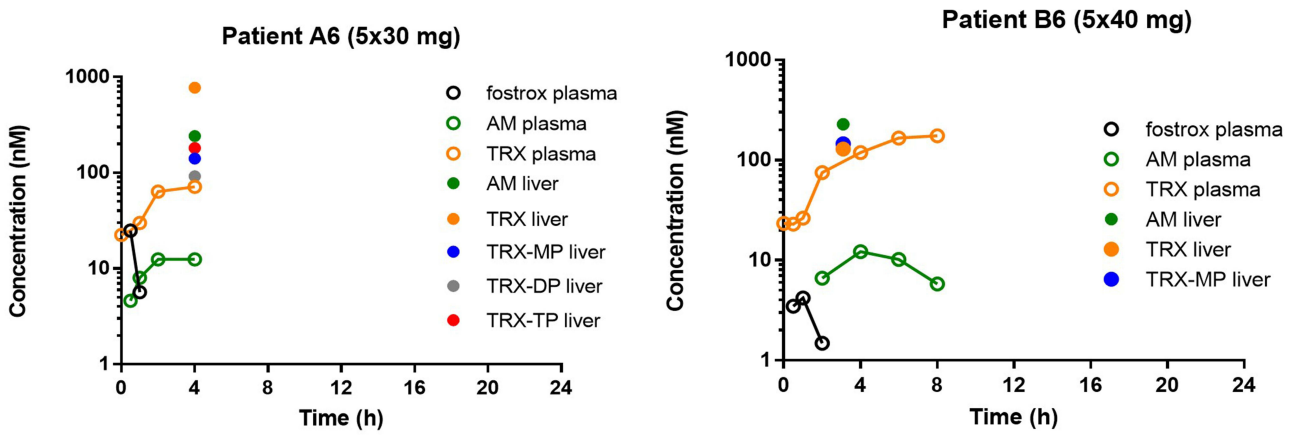

Figure 3 Concentrations of fostrox and its metabolites in plasma and liver biopsies in cycle 2 in two patients diagnosed with metastatic liver disease. For a sample amount of 10 mg, the LLOQ was 100 nM for AM, 40 nM for TRX and 50 nM for TRX-MP, TRX-DP and TRX-TP.
Abbreviations: TRX, troxacitabine; AM; alanine metabolite; TRX-MP, TRX-monophosphate; TRX-DP, TRX-diphosphate; TRX-TP, TRX-triphosphate.

Pharmacodynamics

Liver biopsies were obtained from 12 patients (7 in phase 1a and 5 in phase 1b) with HCC (n = 3), iCCA (n = 2), or metastatic liver disease (n = 7).

Liver biopsies taken on Day 4 or 5 in Cycle 2 presented with clear DNA damage in tumor tissue, as measured by pH2AX staining. In contrast, adjacent normal liver tissue from the same biopsy showed no evidence of DNA damage, with an almost complete lack of staining (Figure 4A). For 3 patients, A2, B2, and B5, an archival/pre-dose sample was obtained, in which an increased level of DNA damage (pH2AX%) was observed upon fostrox treatment as compared to the pre-dose sample (Figure 4B).

The tumor biopsies showed varying levels of Ki-67 staining, ranging from 10 to 90%, suggesting different proliferation rates in this rather heterogeneous group of cancers. A significantly higher percent Ki67 was observed in tumor vs normal liver tissue (Figure 4C). When comparing the level of DNA damage (% pH2AX) with proliferation (% Ki-67) a clear correlation was observed (R^2=0.683) (Figure 4D).

With the exception of two tumor biopsies, one HCC (patient B5) and one liver metastatic lesion from melanoma (patient A6), hypoxic regions in the tumor tissue were observed, as evidenced by strong membrane staining for GLUT1. Double-staining showed co-localized expression of pH2AX and GLUT1, indicating DNA damage in hypoxic regions of the tumor. The level of pH2AX staining was not significantly different in the normoxic (GLUT1 negative) and hypoxic (GLUT1 positive) regions of the tumors (Figure 4E). GLUT1 staining was not observed in the normal liver tissue (data not shown).

Expression of the tumor suppressor TP53 was exclusively detected in tumor tissue, whereas normal liver tissue displayed low or non-detectable staining (Figure 4F).

Discussion

In this multicenter, single-arm, open-label, first-in-human phase 1/2a study, safety and tolerability together with preliminary efficacy was evaluated with fostrox monotherapy in patients with manifestation of cancer in the liver. Eight out of the 19 patients had primary HCC, two patients had intrahepatic cholangiocarcinoma, and the remaining patients had metastases from different solid tumor types including colorectal, pancreatic, gastric, and melanoma. All, except one HCC patient with only prior locoregional treatment, had progressed on at least one prior systemic treatment. Escalating doses from 3 mg to 70 mg were given for 1–5 days in a 21-day cycle in phase 1a and in phase 1b, 40 mg for 5 days (one patient received only 4 days) in a 21-day cycle. Fostrox monotherapy was well tolerated at doses ≤40 mg, with mainly low grade hematological adverse events including transient neutropenia and thrombocytopenia. Four patients discontinued due to adverse events, where one was evidently related to fostrox with grade 2 thrombocytopenia at Cycle 4.

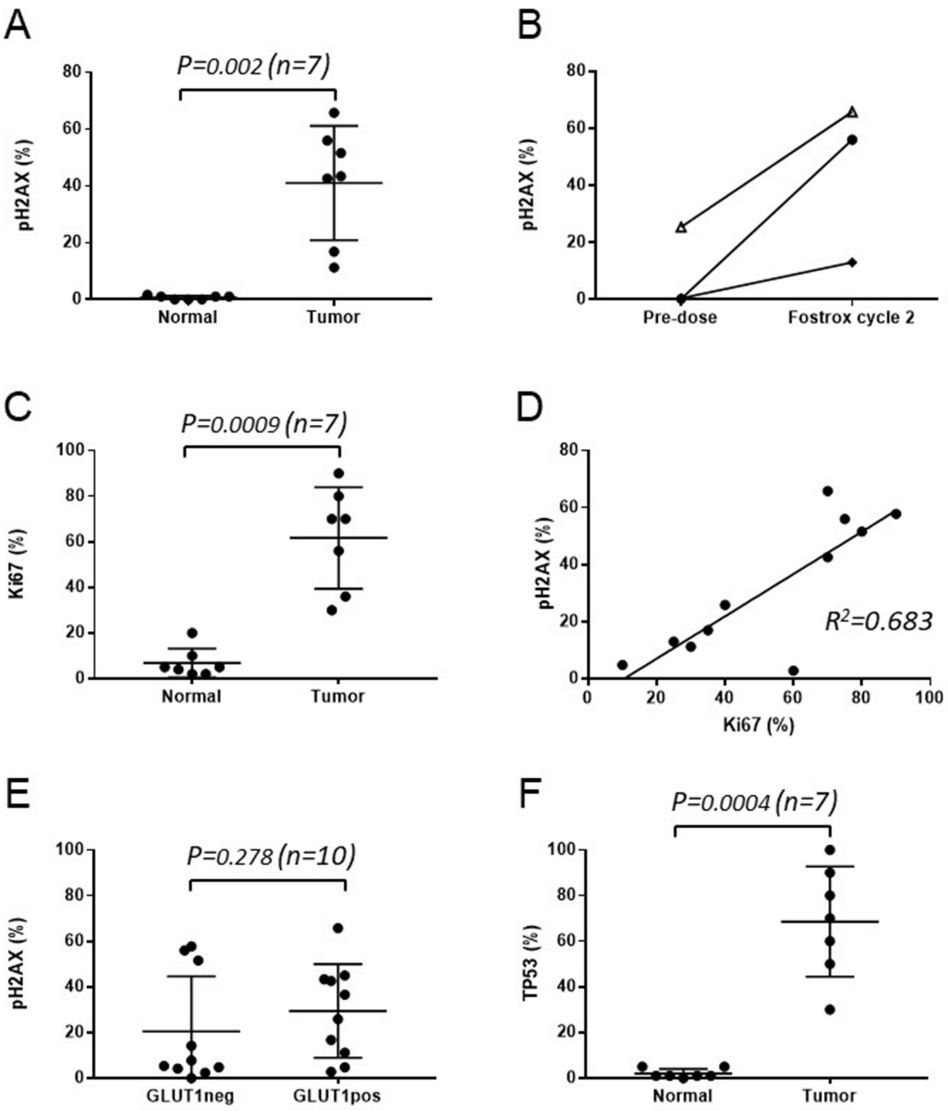

Figure 4 Liver tumor biopsies. (**A**) DNA damage (pH2AX %) in normal liver tissue compared to tumor tissue; (**B**) DNA damage before fostrox and in cycle 2 of fostrox treatment in tumor biopsies from 3 patients; (**C**) Proliferation (Ki67%) in normal liver tissue compared to tumor; (**D**) Correlation between proliferation (Ki67%) and DNA-damage (pH2AX %); (**E**) Comparison of the level of DNA-damage (pH2AX %) in normoxic (GLUTneg) and hypoxic (GLUT1pos) tumor regions; (**F**) Level of TP53 positive cells in normal liver tissue compared with tumor.

Abbreviations: pH2AX, histone protein H2AX phosphorylated on serine 139, GLUT1, glucose transporter 1, TP53, tumor protein 53.

Due to the variation in duration of dosing, type of manifestation of cancer in the liver, as well as the small numbers of patients at each dose level, the results of toxicity in relation to fostrox dose and exposure should be interpreted with caution. However, in phase 1a neutropenia and/or thrombocytopenia of grade 3 or above were only seen in those patients receiving doses greater than 40 mg daily. In phase 1b, all patients started on a 40 mg dose for 5 consecutive days in 21-day cycles, and neutropenia and thrombocytopenia were seen in 5 out of 10 patients, and even if evaluation of safety by dose level was not possible, incidence of neutropenia and thrombocytopenia were examined in relation to troxacitabine AUC. There was a potential correlation of increased incidence of thrombocytopenia and neutropenia with exposure, with all 4 patients with troxacitabine AUC above or at the median level plus at least 1 cycle of follow-up had either thrombocytopenia or neutropenia compared to 1 out of 5 patients with troxacitabine AUC below the median level. The results might though be impacted by variation in duration of dosing and follow-up time. In addition, with underlying liver cirrhosis present, substantial heterogeneity in liver function might affect the ability to activate fostrox. Some leakage of troxacitabine from the liver into systemic circulation is expected and is causing the mainly transient hematological side effects seen in this study. A similar pattern, with

the onset of neutropenia observed on days 8–15 of troxacitabine treatment and a median time for haematological recovery of 7 days, was previously reported[20]. However, compared to conventional systemic use of troxacitabine, toxicity seen with fostrox was as expected distinctly reduced and did not lead to the need for dose modifications in most patients.[21] Treatment with fostrox was in general tolerable with gastro-intestinal and skin toxicity seen in only few patients, and the hematological adverse events were manageable and transient.

Delivery of fostrox to the liver was confirmed by the detection of significantly higher (19 to 40-fold) levels of the alanine metabolite in tumour biopsies compared with plasma. The alanine metabolite is an intermediate step which could only have been derived from the fostrox prodrug. Exposure to the active metabolite (TRX-TP) in liver biopsies was confirmed in one patient, dosed with 30 mg for 5 days. In mouse xenograft models, the cytostatic concentration of TRX-TP was estimated to be 0.025 to 0.13 µmol/L (unpublished data). This suggests that relatively low levels of active metabolite, close to or below the LLOQ (0.05 µmol/L) of the assay used to analyze the human biopsies, may be required for anti-tumor effects.

Consistent with this notion increased pH2AX levels in tumor tissue were detected in liver biopsies from the three patients, treated with 20 mg for 3 days, 40 mg for 5 days and 40 mg for 5 days, respectively, for which pre-dose samples were available (Figure 4B).

Systemic chemotherapy is not an effective treatment option in HCC with clinically effective doses hampered by systemic toxicity, and the risk of harming normal liver tissue.[7,9,22] Fostrox, by its prodrug mechanism, delivers cell-killing activity selectively to the liver. In addition, the DNA-damage, measured as pH2AX, was observed in tumor but not normal liver tissue (Figure 4A) is consistent with a tumor selective effect. This unique, liver targeted approach builds on the experience of antiviral nucleotide pro-drugs in hepatitis, harnessing the phosphoramidate prodrug approach to target tumor lesions in the liver.[23]

Preliminary efficacy seen in the HCC patients showed a CBR of 63% with stable disease in 5 patients. Three of 6 patients that received fostrox 40 mg daily for 5 days in 21-day cycles showed stable disease ongoing at 12 weeks as measured by RECIST 1.1. Similar activity with fostrox monotherapy that was seen in HCC was also seen in patients with cholangiocarcinoma, while no activity was seen in patients with liver metastases from other tumor types. Fostrox enters the liver via vena porta and the difference in arterial and venous blood supply between HCC/iCCA and liver metastases could potentially partly explain the difference in preliminary efficacy. Liver metastases tend to be more addicted to arterial blood supply, while HCC relies on a more mixed vascular supply.[24]

In addition to reducing the tumor burden, it is of major importance to avoid harming the remaining healthy liver tissue and preserve the vital functioning of the liver. The tumor itself damages the liver together with comorbidities causing progress of the cirrhosis. An optimal treatment in advanced HCC would therefore be liver targeted, have a selective tumor cell killing activity to avoid damage to normal cells and have a good safety profile. The results from the phase 1a/1b monotherapy study with fostrox showed an acceptable tolerability and safety profile together with preliminary efficacy in HCC. The next part of this study includes a phase 1b/2a combination with fostrox plus lenvatinib or pembrolizumab in locally advanced or metastatic HCC.

Conclusion

The phase 1a/1b monotherapy study of fostrox, in patients with tumor lesions in the liver, showed that a dose of 40 mg QD for 5 days in 21-day cycles is safe and tolerable. Preliminary anti-tumor effect was seen in patients with advanced HCC and supports clinical development of fostrox in combination with drugs with other modes of action in this patient population.

Data Sharing Statement

Deidentified participant data may be shared upon relevant request and according to local regulations.

For requests please contact: Dr Pia Baumann (pia.baumann@medivir.com). Medivir AB, Box 1086, SE-141 22 huddinge, Sweden.

Ethics Approvals

West of Scotland REC 1, Research Ethics. Ref 18/WS/0121. Ethics Committee Research UZ / KU Leuven. Ref S61640. Antwerp University Hospital Ethics Committee Administrative Office. Ref 19/47/545.

Acknowledgments

We thank the patients who volunteered to participate in this trial and their families, all the investigators and study site personnel, for their contribution to this study.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. The UK sites (Newcastle, Glasgow, Guys) are supported by Experimental Cancer Medicine Centre (ECMC) funding from Cancer Research UK and the National Institute for Health Research (NIHR, England) and the Chief Scientist's Office (Scotland).

Disclosure

Professor Ruth Plummer reports personal fees from Pierre Faber, Bayer, Novartis, BMS, Ellipses, Immunocore, Genmab, Astex Therapeutics, MSD, Nerviano, AmLo, Incyte, Cybrexa, Benevolent AI, and Sanofi Aventis, outside the submitted work. Dr Debashis Sarker reports personal fees from MSD, Eisai, AstraZeneca, Ipsen, Bayer; SIRTEX, AAA, ROCHE, SERVIER, AbbVie, Incyte; non-financial support from Medivir; grants from UCB, during the conduct of the study. Dr Eric Van Cutsem reports participation to advisory boards for AbbVie, Agenus, ALX, Amgen, Arcus Biosciences, Astellas, AstraZeneca, Bayer, Beigene, Bexon Clinical, Biontech, Boehringer Ingelheim, Bristol-Myers Squibb, Canfour, Daiichi, Debiopharma, Elmedix, Eisai, Galapagos, GSK, Hookipa Biotech, Incyte, Ipsen, Lilly, Merck Sharp & Dohme, Merck KGaA, Mirati, Novartis, Nordic, Pierre Fabre, Pfizer, Roche, Seattle Genetics, Servier, Simcere, Takeda, Taiho, Terumo, outside the submitted work. Dr Beate Haugk reports royalties from Springer publication, outside the submitted work. Dr Malene Jensen is an employee and stockholder of Medivir AB. Dr Tom Morris reports personal fees from Medivir, during the conduct of the study; personal fees from AstraZeneca, Calliditas Therapeutics AB, Ascelia Pharma AB, Oxford Vacmedix Ltd, outside the submitted work. Dr Pia Baumann and Dr Karin Tunblad are employees at Medivir. Professor Fredrik Öberg is an employee and shareholder of Medivir AB. In addition, Professor Fredrik Öberg has a patent WO 2016/030335 issued to Medivir AB. Professor Thomas Evans reports grants, personal fees from Medivir, during the conduct of the study; grants, personal fees, and/or non-financial support from Astra Zeneca, Bayer, Bicycle Therapeutics, Bristol-Myers Squibb, Celgene, Eisai, MSD, Nucana, Nurix, Seagen, Pfizer, Adaptimmune, Astellas, Amgen, Avacta, Beigene, Basilea, BioNTech, Boehringer Ingelheim, Codiak, Exelixis, CytomX, Immunocore, iOnctura, Johnson & Johnson, Lilly, MiNa Therapeutics, Moderna, Novartis, Sapience, Starpharma, T3P, UCB, Verastem, Sanofi, Ascelia, Immodulon, Roche, Genmab, and Springer Nature, outside the submitted work. The authors report no other conflicts of interest in this work.

References

1. Senthilnathan S, Memon K, Lewandowski RJ, et al. Extrahepatic metastases occur in a minority of hepatocellular carcinoma patients treated with locoregional therapies: analyzing patterns of progression in 285 patients. *Hepatology*. 2012;55(5):1432–1442. doi:10.1002/hep.24812
2. Llovet JM, Kelley RK, Villanueva A, et al. Hepatocellular carcinoma. *Nat Rev Dis Primers*. 2021;7(1):6. doi:10.1038/s41572-020-00240-3
3. Waninger JJ, Ma VT, Chopra Z, Pearson AN, Green MD. Evaluation of the prognostic role of liver metastases on patient outcomes: systematic review and meta-analysis. *Cancer J*. 2023;29(5):279–284. doi:10.1097/PPO.0000000000000683
4. Detail G NCCN guidelines. 2023. Available from: https://wwwnccnorg/guidelines/guidelines-detail. Accessed October 14, 2024.
5. Ducreux M, Abou-Alfa GK, Bekaii-Saab T, et al. The management of hepatocellular carcinoma. current expert opinion and recommendations derived from the 24th ESMO/World Congress on gastrointestinal cancer. *ESMO Open*. 2022;8(3):101567. doi:10.1016/j.esmoop.2023.101567
6. Reig M, Forner A, Rimola J, et al. BCLC strategy for prognosis prediction and treatment recommendation: the 2022 update. *J Hepatol*. 2022;76(3):681–693. doi:10.1016/j.jhep.2021.11.018
7. Nowak AK, Stockler MR, Byrne MJ. Assessing quality of life during chemotherapy for pleural mesothelioma: feasibility, validity, and results of using the European organization for research and treatment of cancer core quality of life questionnaire and lung cancer module. *J Clin Oncol*. 2004;22(15):3172–3180. doi:10.1200/JCO.2004.09.147

8. Hou JP, Shi YB, Fu YF, Li H. I-125 seeds insertion with TACE for advanced HCC: a meta-analysis of randomized controlled trials. *Minim Invasive Ther Allied Technol*. 2022;31(6):848–855. doi:10.1080/13645706.2022.2033786
9. Grigorian A, O'Brien CB. Hepatotoxicity secondary to chemotherapy. *J Clin Transl Hepatol*. 2014;2(2):95–102. doi:10.14218/JCTH.2014.00011
10. Grove KL, Guo X, Liu SH, Gao Z, Chu CK, Cheng YC. Anticancer activity of beta-L-dioxolane-cytidine, a novel nucleoside analogue with the unnatural L configuration. *Cancer Res*. 1995;55(14):3008–3011.
11. Gourdeau H, Jolivet J. Troxacitabine. *Bull Cancer*. 2004;91(3):213–218. La troxacitabine.
12. Kukhanova M, Liu SH, Mozzherin D, Lin TS, Chu CK, Cheng YC. L- and D-enantiomers of 2',3'-dideoxycytidine 5'-triphosphate analogs as substrates for human DNA polymerases. Implications for the mechanism of toxicity. *J Biol Chem*. 1995;270(39):23055–23059. doi:10.1074/jbc.270.39.23055
13. Ewald B, Sampath D, Plunkett W. ATM and the Mre11-Rad50-Nbs1 complex respond to nucleoside analogue-induced stalled replication forks and contribute to drug resistance. *Cancer Res*. 2008;68(19):7947–7955. doi:10.1158/0008-5472.CAN-08-0971
14. Grove KL, Cheng YC. Uptake and metabolism of the new anticancer compound beta-L-(-)-dioxolane-cytidine in human prostate carcinoma DU-145 cells. *Cancer Res*. 1996;56(18):4187–4191.
15. Dent SF, Arnold A, Stewart DJ, et al. Phase II study of troxacitabine (BCH-4556) in patients with advanced non-small-cell lung cancer. *Lung*. 2005;183(4):265–272. doi:10.1007/s00408-004-2539-7
16. Giles FJ, Feldman EJ, Roboz GJ, et al. Phase II study of troxacitabine, a novel dioxolane nucleoside analog, in patients with untreated or imatinib mesylate-resistant chronic myelogenous leukemia in blastic phase. *Leukemia Res*. 2003;27(12):1091–1096. doi:10.1016/s0145-2126(03)00094-8
17. Giles FJ, Garcia-Manero G, Cortes JE, et al. Phase II study of troxacitabine, a novel dioxolane nucleoside analog, in patients with refractory leukemia. *J Clin Oncol*. 2002;20(3):656–664. doi:10.1200/JCO.2002.20.3.656
18. Lapointe R, Letourneau R, Steward W, et al. Phase II study of troxacitabine in chemotherapy-naive patients with advanced cancer of the pancreas: gastrointestinal tumors. *Ann Oncol*. 2005;16(2):289–293. doi:10.1093/annonc/mdi061
19. Vose JM, Panwalkar A, Belanger R, et al. A phase II multicenter study of troxacitabine in relapsed or refractory lymphoproliferative neoplasms or multiple myeloma. *Leukemia Lymphoma*. 2007;48(1):39–45. doi:10.1080/10428190600909578
20. Swords R, Giles F. Troxacitabine in acute leukemia. *Hematology*. 2007;12(3):219–227. doi:10.1080/10245330701406881
21. de Bono JS, Stephenson J, Baker SD, et al. Troxacitabine, an L-stereoisomeric nucleoside analog, on a five-times-daily schedule: a Phase I and pharmacokinetic study in patients with advanced solid malignancies. *J Clin Oncol*. 2002;20(1):96–109. doi:10.1200/JCO.2002.20.1.96
22. Verslype C, Rosmorduc O, Rougier P. Hepatocellular carcinoma: ESMO-ESDO clinical practice guidelines for diagnosis, treatment and follow-up. *Ann Oncol*. 2012;23(Suppl 7):vii41–8. doi:10.1093/annonc/mds225
23. Mehellou Y, Rattan HS, Balzarini J. The protide prodrug technology: from the concept to the clinic. *J Med Chem*. 2018;61(6):2211–2226. doi:10.1021/acs.jmedchem.7b00734
24. Griscom JT, Wolf PS. Liver Metastasis. *StatPearls*. StatPearls Publishing; 2024.