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PHASE I STUDIES

A first-in-human phase 1a study of the bispecific anti-DLL4/anti-VEGF antibody navicixizumab (OMP-305B83) in patients with previously treated solid tumors

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Summary

Purpose Navicixizumab (OMP-305B83) is a bispecific antibody that inhibits delta-like ligand 4 and vascular endothelial growth factor. This Phase 1a trial assessed escalating doses of navicixizumab in refractory solid tumors patients. *Design* A 3 + 3 dose escalation design was used followed by the treatment of additional patients in an expansion cohort. Study objectives were determination of the maximum tolerated dose, safety, pharmacokinetics, pharmacodynamics, immunogenicity and efficacy. *Results* Sixty-six patients were treated once every 3 weeks in 8 dose-escalation cohorts (0.5, 1, 2.5, 3.5, 5, 7.5, 10, and 12.5 mg/kg) and an expansion cohort (7.5 mg/kg). The median age was 60 years and 68% of the patients were female. The most commonly enrolled tumor types were ovarian (12), colorectal (11) and breast, pancreatic, uterine and endometrial (4 each) cancers. As only 1 dose limiting toxicity occurred, the maximum tolerated dose was not reached, but 7.5 mg/kg was chosen as the dose for the expansion cohort. The treatment related adverse events (≥15% of patients) were hypertension (57.6%), headache (28.8%), fatigue (25.8%), and pulmonary hypertension (18.2%). Pulmonary hypertension was mostly asymptomatic at doses ≤5 mg/kg (6 Gr1, 1 Gr2), but was more severe at higher doses (4 Gr2, 1 Gr3). Navicixizumab's half-life was 11.4 days and there was a moderate (29%) incidence of anti-drug antibody formation. Four patients (3 ovarian cancer, 1 uterine carcinosarcoma) had a partial response and 17 patients had stable disease. Nineteen patients had a reduction in the size of their target lesions including 7/11 patients with ovarian cancer. Four patients remained on study for >300 days and 2 of these patients were on study for >500 days. *Conclusions* Navicixizumab can be safely administered with manageable toxicities and these data showed preliminary signs of antitumor activity in multiple tumor types, but was most promising in ovarian cancer. As a result these data justify its continued development in combination Phase 1b clinical trials.

Keywords Navicixizumab · OMP-305B83 · VEGF · DLL4 pathway

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Introduction

The Notch pathway mediates intercellular communication and plays a key role in regulating cell fate decisions in normal development and in many types of cancer [1]. There are four Notch receptors and five cell surface ligands that activate Notch signaling. One of the key ligands of the Notch pathway is Delta-Like Ligand 4 (DLL4). DLL4 plays a central role both in embryonic development (including the formation of the vasculature) and in adult cell regulation (including stem cell homeostasis, differentiation of the gastrointestinal tract and neo-angiogenesis). During neo-angiogenesis, DLL4 regulates the orderly formation of tip cells and sprouting and also serves as a feedback regulator of vascular endothelial growth factor (VEGF) signaling [2, 3]. Disruption of DLL4 signaling in the vasculature leads to an increase in VEGF activity, dysregulated sprouting and a VEGF-mediated hyperproliferation of endothelial cells that fail to form functional blood vessels, leading to disrupted angiogenesis [4, 5]. These mechanisms demonstrate a coordinated interplay between DLL4 and VEGF in neo-angiogenesis regulation.

The role of DLL4 has been evaluated in detail in solid tumors. Preclinical data demonstrate that DLL4 blockade elicits a potent anti-tumor effect in solid tumors such as colon, pancreatic, breast and lung cancers [6, 7]. The mechanisms underlying the anti-tumor activity of anti-DLL4 include the selective elimination of cancer stem cells (CSCs) and the inhibition of tumor angiogenesis, via mechanisms referenced above [8]. Multiple anti-DLL4 agents have advanced into clinical development [9, 10]. Single agent activity has been observed with anti-DLL4 therapy, but treatment duration was limited by cardiotoxicity that is believed to be due to the increased vascular sprouting which occurs when DLL4 is inhibited, but VEGF is not inhibited [10].

VEGF inhibition is a well-established anti-neoplastic therapy. Multiple inhibitors have been developed and approved for the treatment of solid tumors, including bevacizumab (Avastin®) [11]. Anti-VEGF treatment reduces endothelial cell proliferation and thereby inhibits angiogenesis and tumor growth. Targeting both DLL4 and VEGF would potentially augment the antitumor effects of DLL4 inhibition while limiting the VEGF-induced vascular sprouting.

There has been an increased focus in the development of agents targeting more than one pathway by a single molecule [12], given both developmental and biological advantages including inhibition of key proteins co-localized in tumors. Several bispecific antibodies have been granted regulatory approval including blinatumomab in the USA for relapsed or refractory B cell precursor Acute Lymphoblastic Leukemia in both adults and children [13], and catumaxomab in Europe for treatment of malignant ascites in patients with EpCAM positive cancer [13, 14] (later withdrawn by the manufacturer).

Navicixizumab is an IgG_2 humanized bispecific monoclonal antibody directed against both DLL4 and VEGF. This molecule was designed to enhance the anti-tumor effect that is obtained with inhibiting DLL4 or VEGF alone and also to avoid the cardiac toxicity that was observed with the anti-DLL4 agents. Preclinically it was tested in multiple human tumor xenograft studies, including colon, pancreatic, ovarian and gastric cancer and significant single agent activity was observed in every model tested as well as enhanced activity when combined with chemotherapy, which supported exploring navicixizumab in humans. In addition, inhibition of DLL4 decreases CSC frequency in human xenograft models, and has an immunomodulatory effect by decreasing myeloid derived suppressor cells. This first-in-human Phase 1 study reports the safety, tolerability, pharmacokinetics (PK), pharmacodynamics (PD), antitumor efficacy, and recommended Phase 2 dose (RP2D) of navicixizumab administered every 3 weeks in patients with advanced solid tumors.

Patients and methods

Patient eligibility

Eligible patients were ≥ 21 years old, had a histologically confirmed advanced solid tumor, an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1, adequate organ and marrow function, a tumor at least 1 cm in a single dimension on computerized tomography (CT) scan or magnetic resonance imaging (MRI) and their last chemotherapy, biologic, or investigational therapy was administered ≥4 weeks prior to enrollment. Exclusion criteria included receiving other investigational anticancer agents within 4 weeks of enrollment, receiving therapeutic doses of an anticoagulant; brain metastases; significant intercurrent illnesses or a history of abdominal fistula, gastrointestinal perforation, or intra-abdominal abscess. In addition, patients were excluded if they had a tumor involving the lumen of the gastrointestinal tract; a blood pressure of >140/90 mmHg, were receiving more than 2 antihypertensive agents, had a baseline brain natriuretic peptide (BNP) value of >100 pg/mL, had a left ventricular ejection fraction (LVEF) <50%, had a peak tricuspid velocity > 3.0 m/s on Doppler echocardiogram; had received a total cumulative dose of ≥400 mg/m^2 doxorubicin or had a significant history of cardiac disease. The study was approved by local Institutional Review Boards (IRBs) and was conducted in accordance with the declaration of Helsinki, the International Conference on Harmonization Good Clinical Practice guidelines, and all applicable local regulatory requirements and laws.

Study design and treatments

This phase 1, dose-escalation study (NCT02298387) was conducted at four centers in the United States. The primary endpoint was to determine the maximum tolerated dose. Secondary endpoints were to determine safety, pharmacokinetics (PK), immunogenicity and antitumor activity.

Patients were initially enrolled in escalating dose cohorts (3 + 3 design) and were treated with navicixizumab administered intravenously over 30 min once every 3 weeks at doses of 0.5, 1, 2.5, 3.5, 5, 7.5, 10, 12.5 mg/kg. Dose escalation was allowed if dose-limiting toxicity (DLT) occurred in 0/3 or ≤ 1/6 patients in each cohort during any time from the first dose to 21 days after the first dose (i.e., Study Days 0 through 21). DLTs were defined during the first 21 days of treatment, as any treatment emergent Grade ≥ 3 toxicities (per CTCAE version 4.03) unless clearly attributed to another cause, except for Grade 3 hypertension and Grade 3 proteinuria that reduces to ≤ Grade 1 prior to the next infusion. Following, completion of the dose escalation portion of the study, patients were enrolled in an expansion cohort and treated at the maximum tolerated dose (MTD) or a lower dose. Study treatment continued until disease progression, unacceptable toxicity (including DLT criteria above), or withdrawal of consent.

Study assessments

Safety assessments were conducted weekly throughout the study and for 30 days post-treatment. Adverse events (AEs) were graded using Common Terminology Criteria for Adverse Events (CTCAE), version 4.03. In addition to B-type natriuretic peptide (BNP) at screening, every 21 days while on study, and treatment termination, a complete cardiac assessment by Doppler echocardiograms were performed at screening, every 21 days, and at treatment termination. Patients with ≥ Grade 2 pulmonary hypertension were referred to a cardiologist or pulmonologist to determine what if any further evaluations and/or treatment for their pulmonary hypertension were warranted. A radiographic assessment of the tumor was performed every 63 days and response outcome was assessed according to RECIST version1.1. Tumor markers were obtained at baseline and every 63 days, if the patient's had an elevated value at baseline.

Pharmacokinetic analyses

Samples were obtained for pharmacokinetic analysis prior to and at the end of the infusion and 0.25, 2, 24 and 72 h and 7,14 and 21 days after infusion following the first and third infusions. Samples were also obtained prior to and 0.25 h post-infusion, and 7 and 14 days post- infusion on every other dose, and at treatment termination. Finally, samples were obtained every 3 weeks for 12 weeks following discontinuation of study drug. Serum was assayed for concentrations of navicixizumab by enzyme-linked immunosorbent assay (ELISA). For pharmacokinetic analyses, non-compartmental analysis was conducted for individual patients with evaluable pharmacokinetic data. Summary statistics including mean, standard deviation, median, minimum, and maximum of the pharmacokinetic parameters were reported by dose group.

Immunogenicity

Blood samples were collected for analysis of anti-navicixizumab antibodies (ADA) using an electrochemiluminescence bridging immunoassay: before every dose, every 3 weeks while receiving study drug, at treatment termination and every 3 weeks for the first 12 weeks after treatment termination.

Exploratory biomarker studies

Whole blood was drawn pre-dose and on study Days 0, 28, 49, 70 and every 12 weeks after Day 70 for subjects continuing on study drug t to evaluate changes in Notch and VEGF-related gene expression. RNAs were visualized on the Agilent 2100 Bioanalyzer and integrity was confirmed by the presence of intact 28S and 18S ribosomal peaks. Ribonucleic acid (RNA) from pretreatment and day 28 whole blood samples were quantified using Affymetrix Human GeneChip U133 plus 2 microarrays at Almac Diagnostics. The fold change represents the gene expression ratio was calculated by comparing post-treatment with pretreatment (day 0) samples. The *P* value for comparing gene expression changes was calculated using Limma duplicate. Correlation function by treating patient as a random effect and time-point as fixed effect. Genes were significantly modulated based on a p value less than 0.05. Gene Set Enrichment Analysis was performed to obtain the biological processes affected by navicixizumab. For genes with more than one probeset, the probeset with the greatest absolute value of the score was used.

Statistical methods

All analyses were conducted using SAS® software Version 9.1 or higher (SAS Institute, Inc., Cary, North Carolina). The general analytical approach for all endpoints was descriptive in nature. Demographic and analytical data were summarized using descriptive statistical methods. Summary statistics were presented for age, height, and weight by cohort and overall. Ethnicity, race, age category, and gender were summarized by cohort and overall. Duration of exposure in days, total number of infusions given, total dose received (mg), dose intensity, and number of patients by cohort were summarized for all patients. All safety analyses were conducted on all subjects who receive at least one dose of navicixizumab and who had

at least one post-dosing safety evaluation). Treatment-emergent adverse events (TEAEs) were coded using Version 13.1 or higher of the Medical Dictionary for Regulatory Activities (MedDRA). Toxicity grade was defined according to the Common Terminology Criteria for Adverse Events (CTCAE) 4.03. All laboratory tests, vital sign measurements, and electrocardiogram (ECG) data were presented in data listings. The Eastern Cooperative Oncology Group scores were summarized for the safety population as frequencies and percentages using a shift from baseline table by visit.

Results

Between December 2014 and May 2017 a total of 71 patients were enrolled in the study and 66 of these patients were treated with navicixizumab. The reasons for discontinuation of study treatment were disease progression (43 patients, 65.2%), adverse event (21 patients, 31.8%), withdrawal of consent (1 subject, 1.5%), and use of other anti-cancer therapy (1 subject, 1.5%). Patients' baseline characteristics are summarized in Table 1.

Dose escalation

The number of patients treated in each of the dose escalation cohorts are listed in Table 2. The 0.5 and 1 mg/kg dose levels enrolled 3 patients each without incidence. One DLT was reported in a subject receiving 2.5 mg/kg once every 3 weeks that was a Grade 3 large intestine perforation at the site of a diverticulum 5 days after receiving the first navicixizumab dose. The investigator considered the event to be related to navicixizumab. This dose level was expanded to 6 patients; of these, 4 patients discontinued from the study due to hypertension. As a result, a standardized protocol for hypertension management was established (initially using amlodipine or nifedipine at starting doses of 5 and 30 mg daily, when blood pressure was >140/90; ACE inhibitors or beta-blockers were added afterwards as needed) and 6 more patients were treated at 2.5 mg/kg without having to be withdrawn for hypertension. Six patients were then enrolled at 3.5 mg/kg, one of which developed elevated brain natriuretic peptide (BNP) outside of the DLT window. As a result, 6 more patients were enrolled at 3.5 mg/kg for safety, without further incidences. Cohorts of 6 patients were then enrolled at 5, 7.5 and 10 mg/kg without any DLTs. Enrollment at 12.5 mg/kg dose level was concluded after 5 patients, because of chronic toxicity (pulmonary hypertension) that emerged outside of the DLT window.

The maximum tolerated dose (MTD) for navicixizumab was not determined based on protocol-defined criteria. A dose of 7.5 mg/kg once every 3 weeks was chosen for the expansion cohort based on chronic toxicity that was observed at higher dose levels. However, as 3 patients receiving 7.5 mg/kg developed Grade 2 pulmonary hypertension and the maximal efficacy was observed at 3.5 mg/kg, the recommended Phase 2 dose is 5 mg/kg Q3W. The mean and median number of doses administered across all treatment cohorts was 4.2 and 3, respectively (range: 1 to 24).

Table 1 Characteristics of 66 enrolled patients

Characteristic	Number	%
Age (yrs)		
Median	60	
Range	32–79	
Gender		
Female	45	68
Male	21	32
ECOG performance status		
0	32	48
1	34	52
Tumor type		
Ovarian	12	18
Colorectal	11	17
Breast	4	6
Pancreatic	4	6
Uterine	4	6
Endometrial	4	6
Other	11	41

Abbreviations: ECOG, Eastern Cooperative Oncology Group

Tolerability

All 66 patients were evaluable for toxicity. Overall, 65/66 (98.5%) patients reported ≥1 TEAE regardless of relationship to study drug. The most frequent TEAEs regardless of relationship to study drug were hypertension (38 [57.6%] patients), fatigue (27 [40.9%] patients), diarrhea (23 [34.8%] patients), headache (23 [34.8%] patients), dyspnea (19 [28.8%] patients), nausea (17 [25.8%] patients), and constipation (16 [24.2%] patients) (Table 3).

There were 57 (86.4%) patients who experienced ≥1 navicixizumab-related TEAEs during the study. The most frequent navicixizumab-related TEAEs were hypertension (38 [57.6%] patients), headache (19 [28.8%] patients), fatigue (17 [25.8%] patients), and pulmonary hypertension (12 [18.2%] patients). Infusion reactions occurred in 7 (10.6%) patients.

Whereas the incidence of pulmonary hypertension was similar across the dose levels, its severity appeared to be dose dependent. Seven of 36 (19.4%) patients who received a dose 0.5 to 5 mg/kg developed pulmonary hypertension; 6 cases were asymptomatic Grade 1 pulmonary hypertension and 1

Table 2 Dose escalation

Dose level	Dose level (mg/kg)	Patients (n)	DLT	Median number of doses (range)
1	0.5	3		3 (3–6)
2	1	3		3 (2–3)
3	2.5	6	1	1 (1–8)
4	2.5	6		3 (2–13)
5	3.5	6		3 (2–23)
6	3.5	6		4 (2–24)
7	5	6		3 (3–11)
8	7.5	6		3 (1–12)
9	10	6		4 (3–9)
10	12.5	5		2 (1–3)
Expansion	7.5	13		3 (1–6)

Abbreviations: DLT = dose-limiting toxicity

was Grade 2. In contrast, 5 of the 30 (16.6%) patients who received a dose of 7.5 to 12.5 mg/kg developed pulmonary hypertension, but only 1 of the patients had asymptomatic Grade 1 pulmonary hypertension and the remaining 4 patients had Grade 2 ($N = 3$) or Grade 3 ($N = 1$) pulmonary hypertension.

There were 19 (28.8%) patients that had navicixizumab withdrawn because of an adverse event. The most common adverse event that resulted in navicixizumab being withdrawn were: pulmonary hypertension (7 [10.6%] patients); hypertension (4 [6.1%] patients); and infusion-related reactions, fatigue, and increased BNP (2 [3.0%] patients each).

Six patients had a BNP level > 250 pg/mL. Five of these patients had pulmonary hypertension which presumably caused the BNP elevation and 1 patient had a reduction in left ventricular ejection fraction (from 55 to 40%) without pulmonary hypertension. No clinically significant findings for hematology (except for anemia [5 patients]), serum chemistry, urinalysis, vital signs, physical examination, ECOG status score, echocardiogram or ECG were observed.

There were 46 (69.7%) deaths reported: 7 (10.6%) during the study and 39 (59.1%) during the survival follow-up period. None of the deaths were considered to be related to navicixizumab. One patient suffered a fatal bleeding event that was not related to navicixizumab. The majority of the deaths reported during the study and during the follow-up period were due to progressive disease (6 [85.7%] and 23 [59.0%] patients, respectively).

Pharmacokinetics

Navicixizumab pharmacokinetic data is shown in Supplementary Table 2 and Supplementary Figure 1. All 66 patients had sufficient data for estimation of pharmacokinetic parameters. Navicixizumab exhibited linear pharmacokinetic characteristics at 2.5 mg/kg and above, which is typical of a monoclonal antibody, with a mean clearance of 4.65 mL/day/kg and an 11.4-day terminal half-life. At the 0.5 and 1.0 mg/kg dose levels, the cohort mean clearances of 6.24 and 6.45 mL/day/kg, respectively, were slightly faster than those at 2.5 mg/kg and above, suggesting a moderate degree of target-mediated clearance, which again is one of the hallmarks of monoclonal antibody pharmacokinetics. The volume of central distributional compartment (V_c) was estimated to be 41.6 mL/kg, which is close to the physiological volume of serum in humans (40–45 mL/kg); the volume of peripheral distributional compartment (V_p) was estimated to be 26.4 mL/kg. Together, these estimates suggest navicixizumab distributes primarily within the vascular space, with modest extravasation into the peripheral tissues. The steady state volume of distribution (V_{ss}) of navicixizumab was estimated to be 66.0 mL/kg.

Immunogenicity

Navicixizumab resulted in treatment-induced anti-drug antibody (ADA) formation in 19 out of the 65 (29%) evaluable patients. The lower dose patients (≤ 2.5 mg/kg) had the highest incidence of ADA formation (10/18 [56%] patients were ADA positive). In contrast, only 2/18 (11%) patients treated at 3.5–5 mg/kg developed ADA. Roughly half of the patients with treatment-induced ADA had evidence of accelerated navicixizumab clearance and decreased exposure once ADAs were detected.

Biomarkers

Figures 1a-c provides the biomarker data. A reduction in the gene expression ratio (comparing post-treatment to pre-treatment) was observed for the Notch target gene *HEY1*. Specifically, the majority of patients treated at a dose ≥1 mg/kg had a reduction in *HEY1* expression. In addition, an increase in the gene expression ratio of *FOX03* that is a tumor

Table 3 Treatment-emergent adverse events occurring in ≥10% of patients in descending order of frequency ($N = 66$), regardless of attribution

System Organ Class/ Preferred Term	Dose Escalation						
	Cohort 10.5 mg/kg Q3W (N = 3) n (%)	Cohort 21.0 mg/kg Q3W (N = 3) n (%)	Cohort 32.5 mg/kg Q3W (N = 6) n (%)	Cohort 42.5 mg/kg Q3W ($N = 6$) n (%)	Cohort 53.5 mg/kg Q3W (N = 6) n (%)	Cohort 63.5 mg/kg Q3W (N = 6) n (%)	Cohort 75.0 mg/kg Q3W (N = 6) n (%)
Patients Reporting ≥1 TEAE	3 (100)	3 (100)	6 (100)	6 (100)	6 (100)	6 (100)	5 (83.3)
Hypertension	1 (33.3)	0	4 (66.7)	4 (66.7)	3 (50.0)	5 (83.3)	5 (83.3)
Fatigue	1 (33.3)	1 (33.3)	0	3 (50.0)	3 (50.0)	3 (50.0)	0
Diarrhoea	0	1 (33.3)	1 (16.7)	2 (33.3)	4 (66.7)	3 (50.0)	2 (33.3)
Headache	1 (33.3)	0	1 (16.7)	2 (33.3)	2 (33.3)	3 (50.0)	3 (50.0)
Dyspnoea	0	0	0	2 (33.3)	2 (33.3)	4 (66.7)	0
Nausea	0	0	1 (16.7)	0	1 (16.7)	3 (50.0)	2 (33.3)
Constipation	1 (33.3)	0	0	1 (16.7)	3 (50.0)	3 (50.0)	2 (33.3)
Abdominal pain	2 (66.7)	1 (33.3)	1 (16.7)	1 (16.7)	1 (16.7)	3 (50.0)	0
Cough	0	1 (33.3)	0	1 (16.7)	1 (16.7)	2 (33.3)	1 (16.7)
Dizziness	0	0	1 (16.7)	2 (33.3)	2 (33.3)	1 (16.7)	1 (16.7)
Oedema peripheral	1 (33.3)	0	2 (33.3)	2 (33.3)	1 (16.7)	3 (50.0)	1 (16.7)
Pulmonary hypertension	0	1 (33.3)	1 (16.7)	1 (16.7)	2 (33.3)	2 (33.3)	0
Anaemia	0	1 (33.3)	0	2 (33.3)	2 (33.3)	1 (16.7)	1 (16.7)
Decreased appetite	1 (33.3)	1 (33.3)	0	1 (16.7)	1 (16.7)	3 (50.0)	0
Vomiting	0	0	0	1 (16.7)	1 (16.7)	1 (16.7)	2 (33.3)
Weight decreased	0	1 (33.3)	1 (16.7)	0	1 (16.7)	3 (50.0)	0
Alkaline phosphatase	0	0	0	0	1 (16.7)	3 (50.0)	0
Hypophosphataemia	0	0	0	1 (16.7)	4 (66.7)	0	2 (33.3)

System Organ Class/ Preferred Term	Dose Escalation			Dose Expansion 7.5 mg/kgQ3W ($N = 13$) n (%)	Overall (N = 66) n (%)
	Cohort 87.5 mg/kg Q3W (N = 6) n (%)	Cohort 910 mg/kg Q3W (N = 6) n (%)	Cohort 1012.5 mg/kg Q3W (N = 5) n (%)		
Patients Reporting ≥1 TEAE	6 (100)	6 (100)	5 (100)	13 (100)	65 (98.5)
Hypertension	2 (33.3)	4 (66.7)	2 (40.0)	8 (61.5)	38 (57.6)
Fatigue	3 (50.0)	4 (66.7)	5 (100)	4 (30.8)	27 (40.9)
Diarrhoea	3 (50.0)	2 (33.3)	0	5 (38.5)	23 (34.8)
Headache	1 (16.7)	4 (66.7)	1 (20.0)	5 (38.5)	23 (34.8)
Dyspnoea	4 (66.7)	3 (50.0)	1 (20.0)	3 (23.1)	19 (28.8)
Nausea	4 (66.7)	1 (16.7)	0	5 (38.5)	17 (25.8)
Constipation	1 (16.7)	1 (16.7)	2 (40.0)	2 (15.4)	16 (24.2)
Abdominal pain	1 (16.7)	0	1 (20.0)	1 (7.7)	12 (18.2)
Cough	0	0	2 (40.0)	4 (30.8)	12 (18.2)
Dizziness	2 (33.3)	1 (16.7)	0	2 (15.4)	12 (18.2)

Table 3 (continued)

Oedema peripheral	1 (16.7)	1 (16.7)	0	0		12 (18.2)
Pulmonary hypertension	1 (16.7)	1 (16.7)	1 (20.0)	2 (15.4)		12 (18.2)
Anaemia	3 (50.0)	0	0	1 (7.7)		11 (16.7)
Decreased appetite	1 (16.7)	0	1 (20.0)	2 (15.4)		11 (16.7)
Vomiting	3 (50.0)	2 (33.3)	0	1 (7.7)		11 (16.7)
Weight decreased	1 (16.7)	1 (16.7)	1 (20.0)	2 (15.4)		11 (16.7)
Alkaline phosphatase	2 (33.3)	1 (16.7)	1 (20.0)	2 (15.4)		10 (15.2)
Hypophosphataemia	2 (33.3)	0	1 (20.0)	0		10 (15.2)

Abbreviations: AE = adverse event; N = number of patients per dose cohort or overall; n = number of patients in each category; Q3W = every 3 weeks; TEAE = treatment-emergent adverse event

For each preferred term summarization, patients reporting ≥1 AE were counted only once

suppressor and a mediator of endothelial cell morphogenesis and vascular homeostasis was observed at all dose levels. Finally, a gene set enrichment analysis of the blood demonstrated that there was significant down-regulation of both DLL4 and VEGF pathway related genes at Day 28.

Antitumor activity

Four of 66 (6.1%) patients achieved a partial response (PR), 17 (25.8%) achieved stable disease, 38 (57.6%) had progressive disease, and 7 (10.6%) were not evaluable (Fig. 2a shows a waterfall plot of the best percent change in target lesion size; Fig. 2b shows the ovarian cancer subset of patients). The overall response and clinical benefit rates (95% CIs) were 6.1% (1.7%, 14.8%) and 31.8% (20.9%, 44.4%). Three of the 4 PRs occurred in ovarian cancer patients (12 patients total) and the remaining PR occurred in a patient with uterine carcinosarcoma. Overall, 19 patients had a reduction in their RECIST target lesion size, including 7 of the 11 evaluable ovarian cancer patients. Figure 3a and b, show the duration on study for all 66 patients and the 12 patients with ovarian cancer, respectively. The patients achieving a PR remained on study for 132 (3.5 mg/kg), 505 (3.5 mg/kg), 512 (7.5 mg/kg), and 57 (12.5 mg/kg) days, respectively. Four patients remained on study for greater than 300 days and 2 of these patients remained on study for greater than 500 days.

Discussion

Angiogenesis is critical for the growth of many cancers and multiple anti-angiogenic inhibitors are in regular clinical use including the VEGF inhibitor bevacizumab, as well as sorafenib, sunitinib, regorafenib and pazopanib, that have anti-angiogenic activity. Several additional approaches are under development to further improve the inhibitory efficiency of the complex set of biologic events that result in tumor angiogenesis. Navicixizumab is a novel bispecific monoclonal antibody targeting both VEGF and delta like ligand 4 (DLL4) that inhibits angiogenic signaling and arrests tumor growth.

This first-in-human Phase 1a study evaluated navicixizumab in patients with refractory solid tumors. While most patients experienced at least one treatment-related adverse event, the majority of navicixizumab-related toxicities were mild, and included hypertension, fatigue and headache. In addition pulmonary hypertension occurred in 18% of patients, but the severity was dose dependent.. At doses of 5 mg/kg or less only one case of Grade 2 pulmonary hypertension occurred. While the MTD was not established a dose of 7.5 mg/kg was chosen for the expansion phase of the trial. However, as 3 patients receiving 7.5 mg/kg developed Grade 2 pulmonary hypertension and the maximal efficacy was observed at 3.5 mg/kg, the recommended Phase 2 dose is 5 mg/kg Q3W.

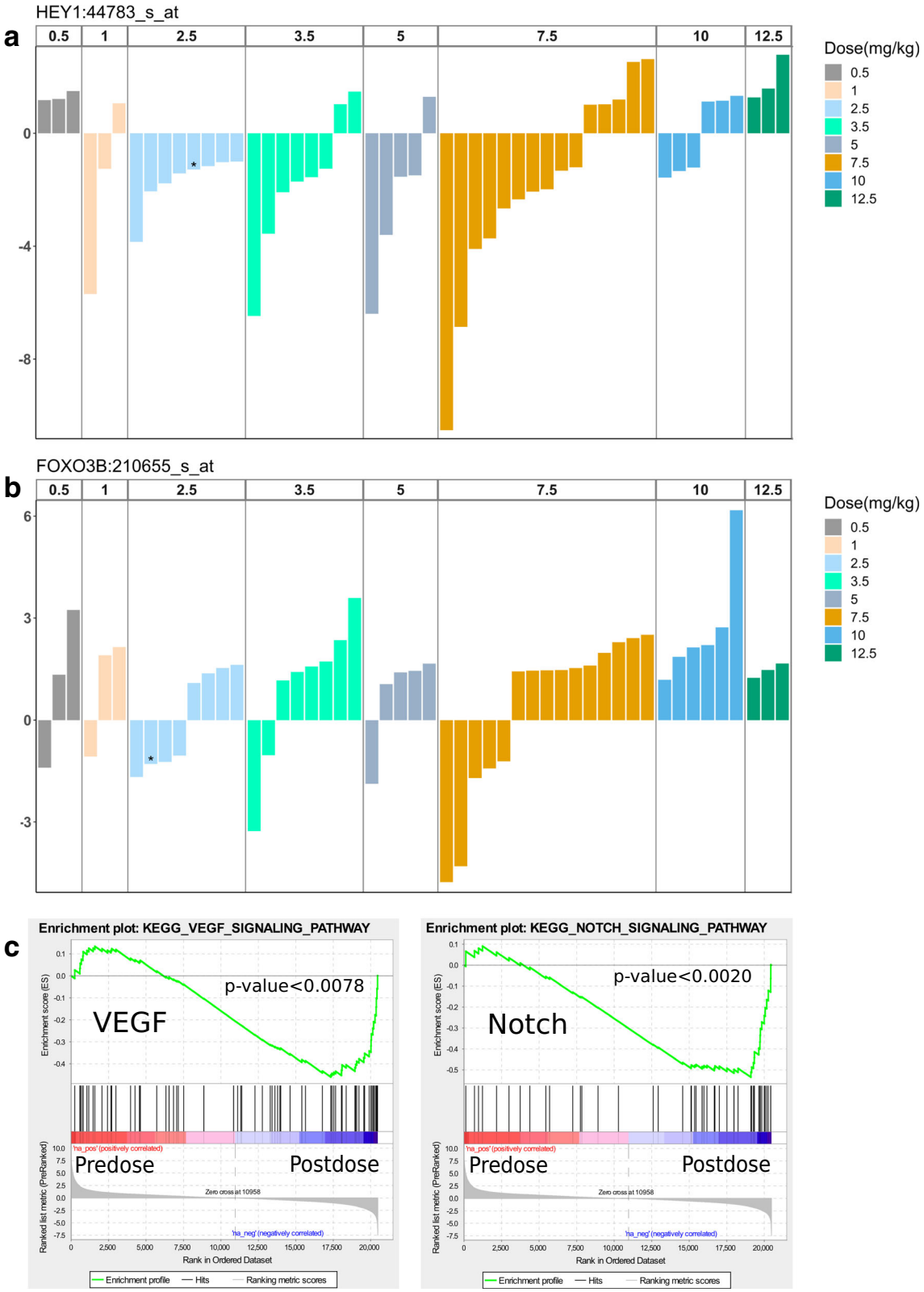


Fig. 1 Figures A and B. Dose effect on *HEY1* (**a**) and *FOX03* (**b**) genes. The fold change represents the gene expression ratio comparing posttreatment vs. predose (day 0) samples. Posttreatment time points were day 28 days except for 2 subjects, which were > 5 days before or after indicated days. Colors: dosing groups as indicated. Blood samples were collected seven (± 2) days after infusion except from subjects labeled with *, which were collected over two weeks after infusion. Fig. C. Gene set enrichment analysis (GSEA) of blood samples ($n = 54$ pts). VEGF and Notch signaling pathways were significantly down-regulated in Day 28 post-dose blood samples

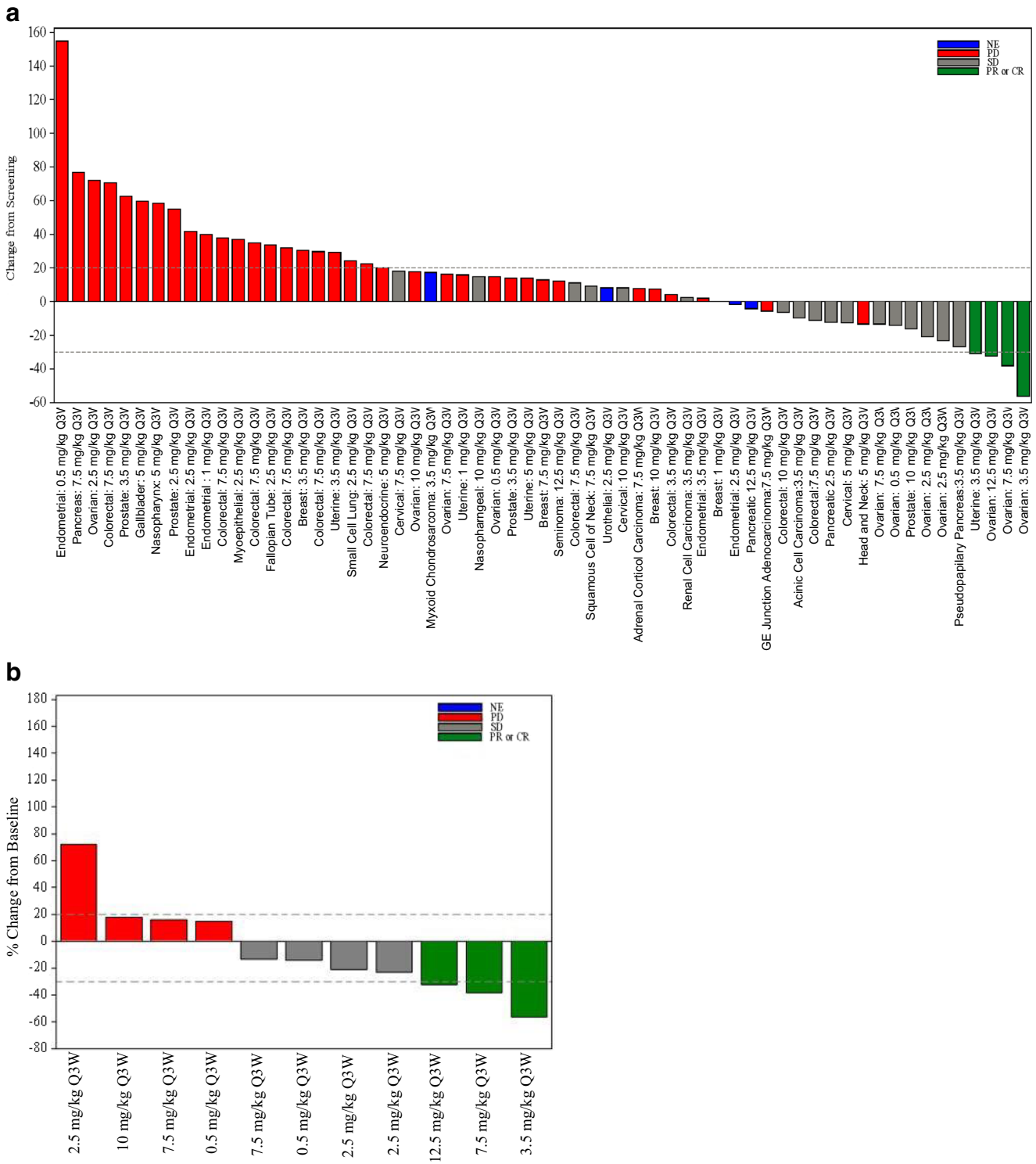
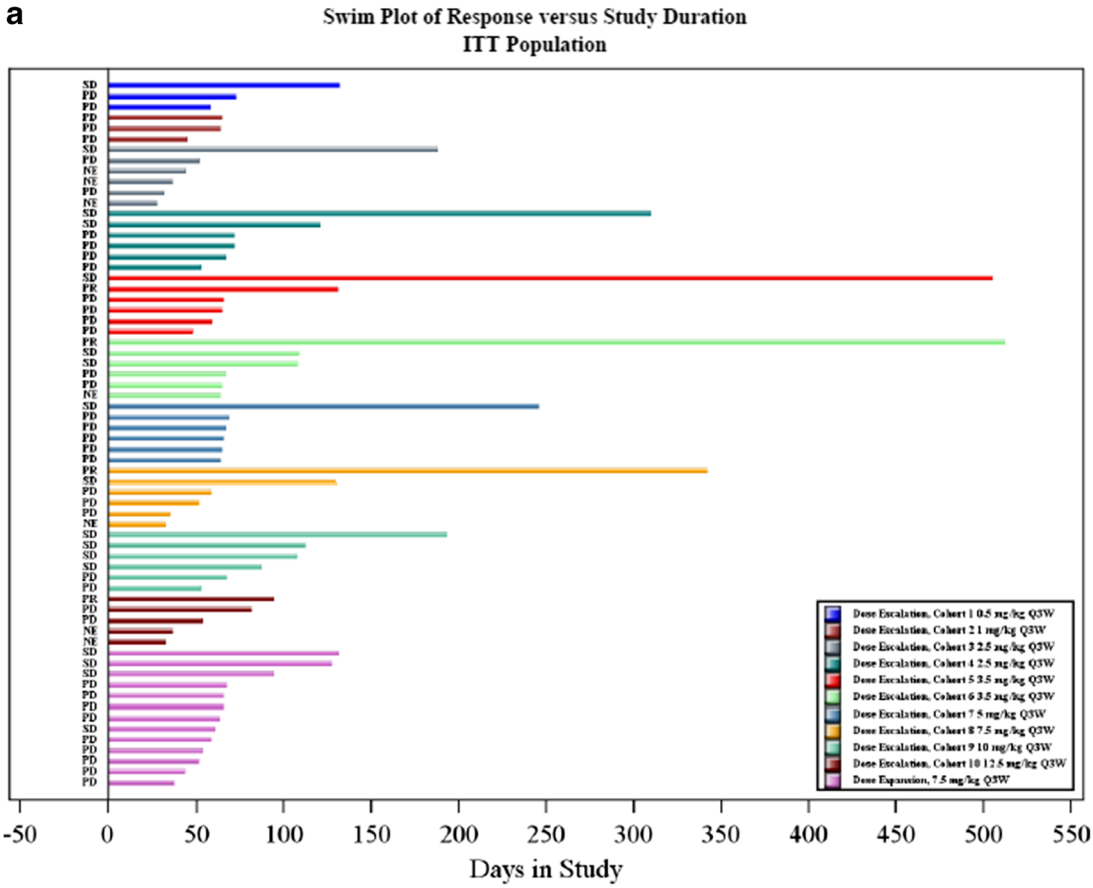
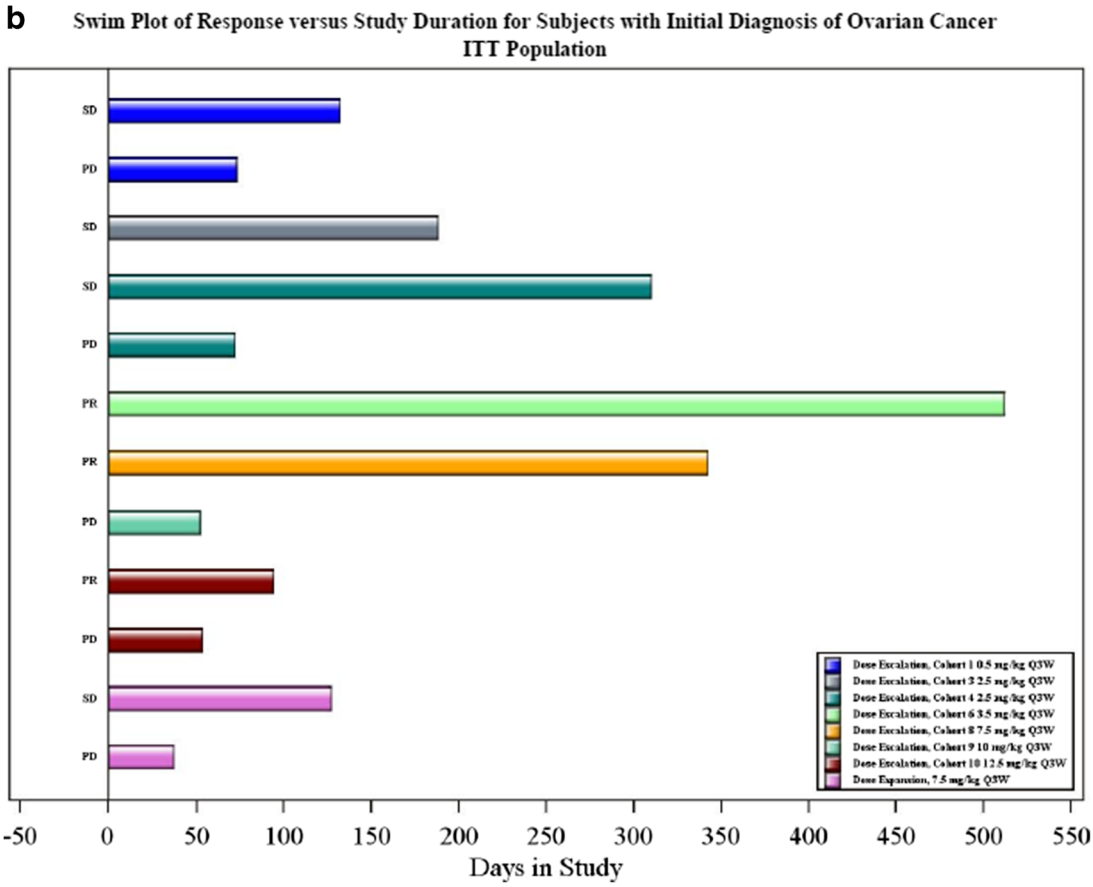


Fig. 2 **a** Waterfall plot of navicixizumab efficacy as measured by RECIST in all patients; **b** ovarian cancer patients

Navicixizumab exhibited linear pharmacokinetic characteristics at 2.5 mg/kg and above, which is typical of a monoclonal antibody. The estimated central (41.6 mL/kg) and peripheral (26.4 mL/kg) volume of distributions suggest navicixizumab distribution in humans is consistent with the conventional knowledge that monoclonal antibody therapeutics distribute primarily within the vascular space, with modest extravasation into the peripheral tissues. Navicixizumab had a steady state volume of distribution of 66.0 mL/kg and a 11.4-day terminal half-life, a PK profile that supports dosing once every 2 or 3 weeks.


a
Swim Plot of Response versus Study Duration
ITT Population
Dose Escalation, Cohort 1 0.5 mg/kg Q3W
Dose Escalation, Cohort 2 1 mg/kg Q3W
Dose Escalation, Cohort 3 2.5 mg/kg Q3W
Dose Escalation, Cohort 4 2.5 mg/kg Q3W
Dose Escalation, Cohort 5 3.5 mg/kg Q3W
Dose Escalation, Cohort 6 3.5 mg/kg Q3W
Dose Escalation, Cohort 7 5 mg/kg Q3W
Dose Escalation, Cohort 8 7.5 mg/kg Q3W
Dose Escalation, Cohort 9 10 mg/kg Q3W
Dose Escalation, Cohort 10 12.5 mg/kg Q3W
Dose Expansion, 7.5 mg/kg Q3W
-50 0 50 100 150 200 250 300 350 400 450 500 550
Days in Study


b
Swim Plot of Response versus Study Duration for Subjects with Initial Diagnosis of Ovarian Cancer
ITT Population
SD
PD
SD
SD
PD
PR
PR
PD
PR
PD
SD
PD
Dose Escalation, Cohort 1 0.5 mg/kg Q3W
Dose Escalation, Cohort 3 2.5 mg/kg Q3W
Dose Escalation, Cohort 4 2.5 mg/kg Q3W
Dose Escalation, Cohort 6 3.5 mg/kg Q3W
Dose Escalation, Cohort 8 7.5 mg/kg Q3W
Dose Escalation, Cohort 9 10 mg/kg Q3W
Dose Escalation, Cohort 10 12.5 mg/kg Q3W
Dose Expansion, 7.5 mg/kg Q3W
-50 0 50 100 150 200 250 300 350 400 450 500 550
Days in Study

◀ **Fig. 3** **a** Swim plot of time on study, with navicixizumab dose levels (in mg/kg, Q3W) by the left axis. **b** Swim plot of time on study for the ovarian cancer patients, with navicixizumab dose levels (in mg/kg, Q3W) by the left axis

Navicixizumab appears to have a toxicity profile consistent with other VEGF inhibitors such as bevacizumab, and investigational DLL4 inhibitors, such as demcizumab [10]. The most common navicixizumab toxicity was systemic hypertension that was well managed once the standard treatment algorithm was employed. Specifically, 38 (57.6%) patients experienced systemic hypertension that was manageable, especially when the standard treatment algorithm was employed. Twelve patients experienced pulmonary hypertension (6 asymptomatic Grade 1, 5 Grade 2 and 1 Grade 3) that was reversible upon drug discontinuation. Importantly, only 1 patient receiving 5 mg/kg or less developed symptomatic (Grade 2) pulmonary hypertension. This toxicity may be primarily related to the DLL4 inhibition, as this was one of the toxicities that was reported with DLL4 inhibitory drugs, although it has also been rarely occurred with anti-VEGF therapy [9, 10]. Bleeding was infrequently reported in this large cohort of patients, in contrast with the report of the anti-DLL4 antibody, demcizumab, where 5 patients were hospitalized with bleeding episodes (2 episodes of tumor-associated bleeding) [10]; it remains to be determined if the ability of navicixizumab to simultaneously inhibit VEGF prevents the bleeding that was observed after sole DLL4 inhibition. Left-sided heart failure was the primary toxicity which limited the therapeutic index of the anti-DLL4 agents. Importantly, no cases of left-sided heart failure were observed in this study of dual DLL4/VEGF inhibition. While these are potentially significant toxicities, their reversibility and/or potential preventability can help contextualize their risk/benefit ratio.

Both VEGF and DLL4 inhibitors have shown efficacy in a variety of tumors, including ovarian cancer. Bevacizumab is approved for use in both platinum-sensitive [15] and –refractory relapsed ovarian cancer [16], however the side effect profile can be limiting and the benefit is relatively modest, thus the need to improve anti-angiogenic therapy in this patient population. A recent Phase 1 study of the DLL4 inhibitor enoticumab showed sustained PRs and SD in multiple ovarian cancer patients [9]. Therefore, the observed navicixizumab efficacy in ovarian cancer patients is likely a result of both VEGF and DLL4 inhibition [9, 10].

The concept of bispecific antibodies came from the understanding that there are usually multiple factors contributing to disease and blockade of several of targets might result in enhanced efficacy [17]. There are four areas of focus for developing bispecific antibodies in cancer therapeutics: 1) inhibition of two cell surface receptors, 2) blocking of two ligands, 3) cross-linking two receptors, and 4) recruitment of T cells that typically do not carry an Fc receptor and therefore are not activated by antibodies [12]. Navicixizumab belongs to the second category, with the unique characteristic that since DLL4 is a cell-bound ligand, and therefore navicixizumab has the potential to localize to the tumor microenvironment and block both DLL4 and clear locally secreted VEGF.

To further explore the potential role of this novel VEGF and DLL4 bispecific inhibitor for the treatment of cancer, a Phase 1b study is currently ongoing which is assessing navicixizumab combined with paclitaxel in platinum resistant ovarian cancer patients who have received more than two prior therapies (NCT03030287). The dose being used in these trials is 3 mg/kg once every 2 weeks which produces an area under the curve that is similar to a dose of 5 mg once every 3 weeks (i.e., the dosing frequency used in this trial). The biomarker data demonstrates that navicixizumab modulated both Notch and VEGF biomarkers at the recommended Phase 2 dose.

In summary, navicixizumab can be safely administered with manageable toxicities and these data showed preliminary signs of antitumor activity in multiple tumor types justifying its continued development in combination Phase 1b clinical trials.

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Compliance with ethical standards

Conflicts of interest No conflicts were disclosed by any of the authors not affiliated with OncoMed. AMK, LX, and RS are employees of OncoMed, the study sponsor.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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