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ORIGINAL RESEARCH ARTICLE

Phase 1 Study of CEP-37250/KHK2804, a Tumor-specific Anti-glycoconjugate Monoclonal Antibody, in Patients with Advanced Solid Tumors

Monica M. Mita[1] · John Nemunaitis[2] · Juneko Grilley-Olson[3] · Bassil El-Rayes[4] · Tanios Bekaii-Saab[5] · R. Donald Harvey[4] · John Marshall[6] · Xiaoping Zhang[7] · Vincent Strout[7]

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Abstract

Background CEP-37250/KHK2804 is a recombinant, humanized, non-fucosylated, monoclonal antibody directed to sialic acid-containing glycoconjugates frequently found on certain tumor cell types.

Objective The objective was to determine the safety, tolerability, maximum tolerated dose (MTD), pharmacokinetics, potential immunogenicity, and preliminary clinical efficacy of CEP-37250/KHK2804 monotherapy in patients with advanced cancer in a first-in-human, phase 1 study.

Materials and Methods In phase 1a, patients ($n = 31$) with solid tumors received increasing doses of CEP-37250/KHK2804 (0.03–1.0 mg/kg) intravenously once weekly using a standard 3 + 3 dose-escalation design. In phase 1b, two dose-expansion cohorts of patients with colorectal ($n = 15$) and pancreatic ($n = 16$) cancer, respectively, received the maximum tolerated dose (MTD).

Results The MTD of CEP-37250/KHK2804 was 0.3 mg/kg weekly. Dose-limiting toxicities were infusion-related reactions and increased serum transaminases. In the overall population ($N = 62$), the most frequent treatment-related adverse event (AE) was an infusion-related reaction (45.2 %). Positive post-baseline CEP-37250/KHK2804 neutralizing antibodies were reported in 14 patients (22.6 %), almost exclusively in patients who developed infusion-related reactions. The most frequent treatment-related AE grade ≥3 was increased AST or ALT in six patients (9.7 %). Three patients experienced treatment-related serious cardiac events (grade 4

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✉ Monica M. Mita
Monica.Mita@cshs.org

John Nemunaitis
JNemunaitis@MaryCrowley.Org

Juneko Grilley-Olson
juneko_grilley-olson@med.unc.edu

Bassil El-Rayes
bassel.el-rayes@emoryhealthcare.org

Tanios Bekaii-Saab
Tanios.Saab@osumc.edu

R. Donald Harvey
donald.harvey@emory.edu

John Marshall
marshalj@georgetown.edu

Xiaoping Zhang
zhangxiaoping@aol.com

Vincent Strout
Vincent.Strout@kyowakirin.com

[1] Samuel Oschin Comprehensive Cancer Institute, Cedars-Sinai Medical Center, 8700 Beverly Boulevard, Suite MS31, Los Angeles, CA 90048, USA

[2] Mary Crowley Cancer Research, Dallas, TX, USA

[3] Department of Medicine, Division of Hematology-Oncology, and Lineberger Comprehensive Cancer Center, University of North Carolina School of Medicine, Chapel Hill, NC, USA

[4] Department of Hematology and Medical Oncology, Winship Cancer Institute, Emory University, Atlanta, GA, USA

[5] Gastrointestinal Disease Research Group, The Ohio State University – James Cancer Hospital, Columbus, OH, USA

[6] Lombardi Comprehensive Cancer Center, Georgetown University, Washington, DC, USA

[7] Kyowa Kirin Pharmaceutical Development, Inc., Princeton, NJ, USA

ECG abnormality, grade 4 atrial fibrillation, and grade 3 acute myocardial infarction, respectively). Pharmacokinetic exposure to CEP-37250/KHK2804 increased proportionally to dose, with accumulation up to two fold with repeated administration. Mean elimination half-life was 34.1 to 70.3 hours over the dose range from 0.03 to 1.0 mg/kg. No patient had a complete or partial best response. Thirteen of 40 (32.5 %) evaluable patients had unconfirmed stable disease, four of which were confirmed (10.0 %).

Conclusions The study was stopped early due to the lack of efficacy. Additionally, safety concerns (i.e., cardiac issues, hepatic toxicity, and infusion-related reactions) made the benefit-risk assessment unfavorable for continued development of CEP-37250/KHK2804, which was halted indefinitely. [Study registered at ClinicalTrials.gov #NCT01447732].

Key Points

CEP-37250/KHK2804, a monoclonal antibody directed at sialic acid-containing glycoconjugate expressed at high rates on some solid tumors, was evaluated in a first-in-human phase 1 study in patients with advanced cancer.

The study and development of CEP-37250/KHK2804 was stopped early because of lack of efficacy.

In addition, safety concerns (i.e. cardiac issues, hepatic toxicity, and infusion-related reactions) made the benefit-risk assessment unfavorable for continued developed of CEP-37250/KHK2804, which was halted indefinitely.

1 Introduction

CEP-37250/KHK2804 [Kyowa Kirin Pharmaceutical Development, Inc. and Teva Pharmaceuticals] is a recombinant, humanized, non-fucosylated immunoglobulin G1 mAb engineered from SC104, a parental murine mAb that binds to sialic acid-containing glycoconjugates found on the surface of colorectal cancer cells and certain other tumor cell types [1]. Non-fucosylated mAbs have been shown to have up to 100-fold higher ADCC activity against tumor cells compared to conventional fucosylated antibodies [2]. The humanized SC104 antibody (U5/78) was defucosylated using Potelligent® technology to generate KM8578, which was subsequently renamed CEP-37250/KHK2804.

Early work with SC104, the parental mouse antibody from which CEP-37250/KHK2804 was derived, implicated recognition of sialyltetraosylceramide glycolipids in colorectal tumor cell lipid extract. Desialylation resulted in complete loss of SC104 binding [1]. Later work performed with CEP-37250/KHK2804 based on immunoaffinity analyses of colorectal tumor cell lysates identified two classes of glycoprotein antigens: i) *O*-glycosylated mucin glycoproteins (e.g., mucin 13, bile salt-activated lipase) and ii) N-glycosylated glycoprotein galectin-3-binding protein (G3BP). Further characterization studies performed with purified G3BP revealed that the determinant recognized by the CEP-37250/KHK2804 antibody is a glycan, which has a Lewis-like composition with fucose and sialic acid attached to its N-acetyllactosamine units on the non-reduced end (manuscript in preparation). Despite this extensive analytical effort, the exact structure of the sialic acid containing antigen(s) remains unknown.

Pre-clinical studies of CEP-37250/KHK2804 [data on file, Kyowa Kirin Pharmaceutical Development, Inc.] showed that the mAb: recognizes and binds to a sialic acid-containing glycoconjugate that is expressed at high rates on the surface of primary cancers, e.g., colon (68 %), ductal pancreatic adenocarcinoma (86 %), and rectal (76 %), using tissue microarray analysis; exhibited ADCC activity against human colorectal, pancreatic, and gastric cancer cell lines, and CDC activity against colorectal cancer cell lines; exhibited direct killing or cell growth inhibition activity against human colorectal and pancreatic cancer cell lines; and exhibited antitumor activity against human colorectal tumor and pancreatic cancer xenographs in a SCID mouse model. CEP-37250/KHK2804 shows additive but no synergistic antitumor activity in combination with chemotherapeutic agents such as irinotecan, oxaliplatin, and 5-fluorouracil/leucovorin in the nude mouse model.

The aim of the current first-in-human phase 1 study was to determine the safety, tolerability, maximum tolerated dose (MTD), pharmacokinetics, potential immunogenicity, and preliminary clinical efficacy of CEP-37250/KHK2804 administered by intravenous (IV) infusion as monotherapy in patients with advanced cancer.

2 Materials and Methods

The study was conducted in accordance with the Declaration of Helsinki and International Conference for Harmonization of Good Clinical Practice guidelines, and registered at ClinicalTrials.gov (NCT01447732). All patients provided written informed consent prior to study registration. The protocol and its subsequent amendments were approved by the Institutional Review Board at each of the six participating study centers (Mary Crowley Cancer Research Centers, Dallas, TX; Department of Hematology Oncology, University of North Carolina at Chapel Hill, Chapel Hill, NC; Winship Cancer Institute, Emory University, Atlanta, GA; OSU Medical Center, The James Cancer Hospital and Solove Research Institute, Columbus, OH; Samuel Oschin Comprehensive Cancer Institute, Los Angeles, CA; and Medstar Georgetown University Hospital, Washington, DC).

2.1 Study Design

The primary objective was to determine the safety, tolerability, dose-limiting toxicity (DLT), maximum tolerated dose (MTD), and recommended phase 2 dose of CEP-37250/KHK2804 in patients with advanced solid tumors who no longer responded to standard therapy or for whom no standard therapy was available. Secondary objectives were to determine the pharmacokinetic profile of CEP-37250/KHK2804, to evaluate preliminary evidence of anti-tumor activity, and to screen for potential antibodies against CEP-37250/KHK2804.

As this was the first-in-human study of CEP-37250/KHK2804, the starting dose level was based on a toxicology study in cynomolgus monkeys (data on file, Kyowa Kirin Pharmaceutical Development, Inc.), which showed the no observed adverse effect level (NOAEL) was 1 mg/kg IV twice weekly for 4 weeks. The selected human starting dose of CEP-37250/KHK2804 0.03 mg/kg IV once weekly provided a safety factor of 66 compared to the NOAEL, which is sufficiently high for advanced cancer patients.

The study had a multicenter, open-label design consisting of two sequential parts: dose escalation to determine the MTD (phase 1a) followed by dose expansion at the MTD (phase 1b). CEP-37250/KHK2804 was administered as monotherapy on an out-patient basis. Phase 1a employed a standard 3 + 3 dose-escalation design in patients with advanced solid tumors. Increasing doses of CEP-37250/KHK2804 (0.03, 0.1, 0.3, and 1.0 mg/kg) were administered once weekly for 4 weeks (cycle 1) during which DLT was determined. Patients discontinuing because of adverse events (AEs) or any other reason, or not receiving all scheduled doses of CEP-37250/KHK2804 during cycle 1 were replaced by enrollment of new patients in the relevant cohort. Phase 1b included two disease-specific expansion cohorts of patients with advanced colorectal and pancreatic adenocarcinoma, respectively, each targeting recruitment of 16 patients.

CEP-37250/KHK2804 (Kyowa Kirin Pharmaceutical Development, Inc.) was administered by IV infusion in 0.9 % saline over 60–120 minutes using an infusion pump with a 0.22-μm low-protein-binding, in-line filter. Patients were continuously treated with CEP-37250/KHK2804 once weekly and a treatment period of 4 weeks was defined as one cycle. Patients were allowed to continue treatment for up to six cycles or until disease progression, increase in Eastern Cooperative Oncology Group (ECOG) performance status score ≥2, development of unacceptable toxicity, grade 3/4 infusion-related reaction, any potentially life-threatening therapy-related event, or any event requiring study therapy to be modified by more than one dose reduction or to be held for ≥4 weeks, protocol non-adherence, withdrawal of consent, or the patient was considered unsuitable for further treatment in the investigator's opinion. Patients could continue treatment beyond six cycles if they experienced a best response of at least stable disease (SD) and were not experiencing unacceptable toxicity.

Routine pre-medication for the prophylaxis of infusion-related reactions to CEP-37250/KHK2804 was not initially mandatory, but this was routinely instituted by protocol amendment following the occurrence of grade 2 infusion-related reactions in some of the initially recruited patients. The pre-medication regimen comprised a combination of dexamethasone, diphenhydramine, and ranitidine or famotidine. Details of the pre-medication regimen and the definition and treatment of infusion-related reactions are provided as supplementary data (available online).

DLT was defined as the occurrence of any of the following toxicities considered as possibly, probably, or definitely related to CEP-37250/KHK2804: grade 4 anemia or thrombocytopenia; grade 4 neutropenia for ≥5 days; grade 3/4 neutropenia with fever (≥38.5 °C) for ≥4 hours; grade ≥3 non-hematologic toxicity (except for grade 3 nausea/vomiting or diarrhea reduced to grade ≤2 within 24 hours with medical management, or any non-hematologic grade 3 laboratory AE that is asymptomatic and rapidly reversible [returning to baseline or grade ≤1 within 7 days or prior to next administration of CEP-37250/KHK2804]); and any other toxicity leading to treatment interruption for ≥2 weeks or representing a clinically significant hazard in the view of the investigator.

2.2 Patients

For phase 1a, eligible patients included adults (≥18 years) with histopathologically or cytologically documented, measurable or non-measurable, unresectable, advanced primary or recurrent metastatic solid tumor unresponsive to standard therapy or for which no standard therapy was available. Patients with tumor types that pre-clinical studies have shown no evidence of immunostaining for the target antigen for CEP-37250/KHK2804 were not included (see exclusion criteria). For phase 1b, eligible patients were restricted to those with measurable colorectal or pancreatic adenocarcinoma. All patients had to have an ECOG score ≤2 at entry, life expectancy ≥3 months, and preserved organ function. Full inclusion/exclusion criteria are detailed as supplementary data (available online).

2.3 Safety and Clinical Assessment

Demographic and medical/cancer histories were recorded at screening. Physical examination and laboratory value collection and assessment were undertaken at screening, on days 1, 8, 15, and 22 of cycle 1, on days 1 and 14 of subsequent cycles, at the end of treatment, and at 30-day follow-up. Vital signs were recorded at all visits. ECG was undertaken at screening, on days 1 and 22 of cycle 1, on day 1 of every second cycle from cycle 4, and at the end of

treatment. Multigated acquisition scans or echocardiograms were obtained at screening and repeated if considered clinically indicated. Tumor assessment (computed tomography and/or magnetic resonance imaging) was performed at screening and day 1 of cycle 2 and subsequent cycles. Tumor markers (serum carcinoembryonic antigen [CEA] and CA19-9 in patients with colorectal and pancreatic cancer, respectively) were determined on day 1 of cycles 1–6 during phase 1b. Archival biopsy tumor tissue, if available from either the primary or metastatic tumor, was subjected to immunohistochemistry (IHC) testing for the target antigen for CEP-37250/KHK2804, which was graded 0, 1+, 2+, and 3+ (negative, weak, moderate, and strong, respectively) depending on increasing staining intensity and fraction of positively stained tumor cells.

AEs were recorded following observation by the investigator during clinic visits or in response to non-leading questioning, spontaneous reporting by the patient, or on the basis of clinical or laboratory tests. They were graded by National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) v4.0. Treatment-related AEs were those classified as possibly, probably, or definitely related to CEP-37250/KHK2804. The safety analysis population included all patients who received at least one dose of CEP-37250/KHK2804. Serious AEs (SAEs) were reported in an expedited manner.

Blood samples were taken on days 1 and 15 of cycle 1, day 1 of cycles 2–6, day 1 of every third continuation cycles (e.g., cycles 7, 10, and 13), end of therapy, and 30 days after the last infusion of CEP-37250/KHK2804, from which serum was screened for the presence of anti-CEP-37250/KHK2804 antibodies using an electrochemiluminescent (ECL)-based ligand binding assay. A positive anti-CEP-37250/KHK2804 antibody response was confirmed using an immunodepletive assay.

2.4 Response assessment

Best overall response was determined in the efficacy evaluable population, which included those patients with baseline and at least one on-study tumor assessment. Response was assessed according to Response Evaluation Criteria in Solid Tumors (RECIST guidelines version 1.1) [3]. Patients with measurable disease were evaluated by imaging and physical examination at 8 weeks after the first dose of CEP-37250/KHK2804 and every 8 weeks thereafter. Confirmation of response was required not less than 4 weeks following initial response documentation. For confirmed SD, follow-up measurements must have met the SD criteria at least once after the first dose at a minimum interval of 8 weeks and confirmed by subsequent evaluation.

2.5 Pharmacokinetics

Blood samples were taken pre-dose, at the end of infusion, and at 1, 2, 4, 5–8, 24, 48, and 96 hours after the end of infusion of CEP-37250/KHK2804 after dosing on days 1 and 22 of cycle 1. Serum samples were analyzed for CEP-37250/KHK2804 using a validated sandwich ECL immunoassay. The quantification range was from 50 to 50,000 ng/mL. Pharmacokinetic parameters including area under the serum concentration-time curve from time zero to the time of the last measurable concentration (AUC_{0-t}) and to infinity ($AUC_{0-\infty}$), maximum serum concentration (C_{max}), time to C_{max} (T_{max}), and elimination half-life ($t_{1/2}$) were calculated using non-compartmental methods with Phoenix WinNonlin software (Version 6.3, Pharsight – A Certara Company, Mountain View, CA).

2.6 Statistics

Safety, efficacy, and pharmacokinetic data were summarized by descriptive statistics. From a safety perspective, 16 patients in each expansion cohort in phase 1b would provide a probability >80 % to detect at least one DLT for any true DLT rate of ≥10 %, and from an efficacy perspective, ensure a 90 % confidence interval on the observed response rate would be no wider than 0.25 for any true response rate of ≥10 %.

3 Results

3.1 Patient Characteristics

The study is complete and was conducted between 24 October 2011 and 21 January 2015. The baseline clinical and demographic characteristics of patients are summarized in Table 1. Patient disposition and drug exposure are summarized in Table 2. The safety and efficacy populations included 62 and 39 patients, respectively. The most common reasons for discontinuation from the study were progressive disease ($n = 31$, 50.0 %) and AEs ($n = 15$, 24.2 %).

3.2 Dose-limiting Toxicity and Safety

During phase 1a, DLT occurred in one patient in each of the first three dose cohorts: grade 2 infusion-related reaction in cohorts 1 and 2 (0.03 and 0.1 mg/kg weekly, respectively) and grade 3 ALT increase in cohort 3 (0.3 mg/kg weekly). In cohort 4 (1.0 mg/kg weekly) three of seven enrolled patients experienced DLT: grade 2 or 3 ALT increase ($n = 2$) and grade 2 infusion-related reaction ($n = 1$). The grade 2 infusion-related reactions and ALT increase were classed as DLTs as study drug was discontinued or in the former and led to dose reduction in the latter. CEP-37250/KHK2804 0.3 mg/kg weekly was subsequently established as the MTD for the dose-

Table 1 Baseline clinical and demographic characteristics

Characteristic	Total ($N = 62$)
Median age, years (min, max)	61.0 (28, 78)
Gender, *n* (%)	
Male	36 (58.1)
Female	26 (41.9)
Race, *n* (%)	
White	46 (74.2)
African American	12 (19.4)
Asian	2 (3.2)
Other	2 (3.2)
ECOG performance status, *n* (%)	
0	13 (21.0)
1	44 (71.0)
2	5 (8.1)
Primary tumor site, *n* (%)[a]	
Colorectal adenocarcinoma	23 (37.1)
Pancreatic adenocarcinoma	25 (40.3)
Other	14 (22.6)
Disease stage at study entry, *n* (%)	
III	3 (4.8)
IV	59 (95.2)
Median disease duration from first diagnosis, months (min, max)	29.9 (6.1, 103.3)
Prior cancer therapy, *n* (%)	
Systemic	61 (98.4)
Surgery	59 (95.2)
Radiotherapy	25 (40.3)
No. of prior systemic therapies, *n* (%)[b]	
0	1 (1.6)
1	2 (3.2)
2	8 (12.9)
3	14 (22.6)
4	14 (22.6)
≥ 5	23 (37.1)

ECOG, Eastern Cooperative Oncology Group

[a] The current disease site is presented for the patients in phase 1b expansion cohorts: colorectal adenocarcinoma ($n = 15$) and pancreatic adenocarcinoma ($n = 16$).

[b] Median 4 (range, 0–8).

Note: Total percentages may not equal 100 % exactly due to rounding.

expansion cohorts in phase 1b. In phase 1b, one patient in the colorectal cancer expansion cohort experienced DLT of a grade 3 ALT increase.

A summary of treatment-emergent AEs is provided in Table 3. The most frequent treatment-related AE was infusion-related reaction ($n = 28$, 45.2 %) and one patient experienced flushing, giving a combined rate of 46.8 %. Most were grade 1 or 2 and resolved within 24 hours. The most frequent treatment-related AE grade ≥3 was ALT increase in six patients (9.7 %). Three patients (4.8 %) had an AE with an outcome of death (sepsis in two, pleural effusion plus pneumonia in one). Death was not considered related to treatment with CEP-37250/KHK2804 in any of these patients. SAEs were reported in 32 patients (51.6 %), the most common of which were disease progression ($n = 14$), abdominal pain ($n = 6$), and pleural effusion ($n = 3$). Treatment-related SAEs occurred three 3 patients (4.8 %), including abdominal pain plus abnormal ECG, atrial fibrillation, and infusion-related reaction plus acute myocardial infarction in respective patients.

Ten patients experienced AEs classified as cardiac disorder, of which three were considered related to treatment (grade 1 tachycardia, grade 4 atrial fibrillation, and grade 3 acute myocardial infarction, respectively). An additional two patients had an AE classified as abnormal ECG, both of which were considered related to treatment. Of these five patients, three events (grade 4 ECG abnormality, grade 4 atrial fibrillation, and grade 3 acute myocardial infarction, respectively) were considered as treatment-related SAEs and occurred at a single study center during phase 1b.

Twenty patients (32.2 %) had at least one positive post-baseline confirmatory assay result for anti-CEP-37250/KHK2804 antibodies and 14 (22.6 %) had at least one positive post-baseline neutralizing assay result. All but one of these patients with neutralizing anti-CEP-37250/KHK2804 antibodies occurred in the patients who experienced infusion-related reaction ($n = 12$) or flushing ($n = 1$).

3.3 Anti-tumor Activity

No patient had a complete or partial response. Thirteen of 40 (32.5 %) evaluable patients had unconfirmed SD, four of which were confirmed (10.0 %). The duration of confirmed SD was 103, 330, 106, and 43 days in patients with colorectal adenocarcinoma, cholangiocarcinoma, anal squamous cell carcinoma, and ductal pancreatic adenocarcinoma treated with CEP-37250/KHK2804 0.03, 0.1, 0.3, and 0.3 mg/kg weekly, respectively. Confirmed SD occurred in phase 1a ($n = 3$) or phase 1b in the pancreatic expansion cohort ($n = 1$). Median PFS was 1.73 months (95 % confidence interval, 1.67–2.00 months).

In phase 1b, no patient in the colorectal cancer expansion cohort had a significant decrease (≥20 %) from baseline in serum CEA during treatment. Among the ten patients with elevated baseline serum CA19-9 in the pancreatic cancer expansion cohort, three (30 %) had a maximum decrease (≥50 %) from baseline in serum CA19-9 during treatment. For two of these patients, serum CA19-9 decreased to normal during treatment, of whom one had SD and one disease progression. The other patient

Table 2 Patient disposition and drug exposure

	CEP-37250/KHK2804 cohort						
	Cohort 1 0.03 mg/kg	Cohort 2 0.1 mg/kg	Cohort 3 0.3 mg/kg	Cohort 4 1.0 mg/kg	Colorectal expansion cohort 0.3 mg/kg	Pancreatic expansion cohort 0.3 mg/kg	Total
Patient disposition, *n* (%)							
Safety population	8	8	8	7	15	16	62 (100.0)
Efficacy population	5	5	5	5	10	10	40 (64.5)
Reason for withdrawal							
Disease progression	4	3	4	4	11	5	31 (50.0)
Adverse event	1	1	2	2	3	6	15 (24.2)
Consent withdrawal	1	1	1	0	1	2	6 (9.7)
Investigator discretion	1	1	0	0	0	2	4 (6.5)
Death	1	1	0	1	0	0	3 (4.8)
Other	0	1	1	1	3	5	12 (19.4)
Drug exposure, mean ± SD							
Cycles initiated, *n*	4.0 ± 2.5	3.0 ± 4.5	2.0 ± 1.3	1.6 ± 0.5	1.7 ± 0.5	2.0 ± 1.1	2.3 ± 2.1
Total CEP-37250/KHK2804 doses administered, *n*	14.3 ± 9.8	10.3 ± 17.1	6.8 ± 6.0	5.1 ± 3.3	4.7 ± 2.8	5.6 ± 4.4	7.2 ± 8.2
Actual CEP-37250/KHK2804 dose, mg	2.6 ± 0.53	8.6 ± 1.7	18.4 ± 10.9	75.5 ± 51.5	20.2 ± 7.9	15.9 ± 5.1	21.3 ± 26.6
Dose intensity, %[a]	98.9 ± 2.2	98.1 ± 3.4	79.6 ± 35.6	88.0 ± 26.6	82.0 ± 27.0	85.1 ± 15.9	87.4 ± 22.2

SD, standard deviation

[a] Calculated as actual dose/planned dose × 100 %

remained above the normal range and response was not evaluated. Pre-baseline archival biopsy tumor samples were available for 52 patients for testing CEP-37250/KHK2804 antigen positivity: scores were 0 ($n = 10$), 1+ ($n = 12$), 2+ ($n = 14$), and 3+ ($n = 14$). There was no correlation of CEP-37250/KHK2804 antigen expression with response.

3.4 Pharmacokinetics

Mean pharmacokinetic parameters are detailed as supplementary data (available online as Tables S1 and S2 for phase 1a and 1b, respectively). Mean $t_{1/2}$ for CEP-37250/KHK2804 ranged from 34.1 to 70.3 hours and was independent of dose and repeated administration. Exposure to CEP-37250/KHK2804 based on C_{max} and AUC values increased in a dose-related manner over the dose range from 0.03 to 1 mg/kg. CEP-37250/KHK2804 accumulated up to two fold in serum based on AUC_{0-t} values. *Post hoc* subgroup analysis showed a trend for lower exposure among patients with positive anti-CEP-37250/KHK2804 antibodies, which was more apparent in those who had a neutralizing antibody response. $AUC_{0-\infty}$ was lower in patients with a negative neutralizing antibody compared to those who had a positive neutralizing antibody (84,300 vs 321,000 μg•h/ml).

4 Discussion

The MTD for CEP-37250/KHK2804 was 0.3 mg/kg administered IV once weekly in patients with advanced solid tumors during dose-escalation in phase 1a of the study. Patients were excluded if they had tumor types that preclinical studies had shown no evidence of immunostaining for the sialic acid-containing glycoconjugate target antigen for CEP-37250/KHK2804. The exact structure of the target antigen(s) remains unknown. DLTs were grade 2 infusion-related reactions ($n = 3$) and grade 2 or 3 ALT increases ($n = 3$). The administration of therapeutic mAbs, even those that are humanized or fully human, may be associated with infusion-related reactions, especially with the first infusion [4, 5]. Routine pre-medication for the prophylaxis of infusion-related reactions to CEP-37250/KHK2804 was not mandatory in the original protocol, but this was subsequently instituted by protocol amendment following the occurrence of grade 2 infusion-related reactions in some of the initially recruited patients.

Two cohorts with colorectal and pancreatic adenocarcinoma, respectively, were recruited for dose expansion using the MTD dose of 0.3 mg/kg in phase 1b. These tumor types were selected given that more than two thirds of such primary cancers express the target antigen for CEP-37250/KHK2804 using tissue microarray analysis.

Table 3 Treatment-emergent adverse events

	No. of patients (%)						
	CEP-37250/KHK2804 cohort						
	Cohort 1 0.03 mg/kg ($n = 8$)	Cohort 2 0.1 mg/kg ($n = 8$)	Cohort 3 0.3 mg/kg ($n = 8$)	Cohort 4 1.0 mg/kg ($n = 7$)	Colorectal expansion cohort 0.3 mg/kg ($n = 15$)	Pancreatic expansion cohort 0.3 mg/kg ($n = 16$)	Total ($N = 62$)
AE	8	8	8	7	15 (100.0)	16 (100)	62 (100.0)
Treatment-related AE	5	5	7	7	12 (80.0)	14 (87.5)	50 (80.6)
AE grade ≥3	6	8	5	6	13 (86.7)	14 (87.5)	52 (83.9)
Treatment-related AE grade ≥3	0	0	2	3	6 (40.0)	5 (31.3)	16 (25.8)
Serious AE	6	2	3	2	9 (60.0)	11 (68.8)	33 (53.2)
Treatment-related serious AE	0	0	0	0	2 (13.3)	1 (6.3)	3 (4.8)
AE leading to discontinuation of CEP-37250/KHK2804	1	2	2	2	3 (20.0)	6 (6.3)	16 (25.8)
Death	3	2	0	1	5 (33.3)	5 (31.3)	16 (25.8)
Treatment-related[a] AE occurring in ≥3 patients overall by preferred term[b]							
Infusion-related reaction	3	2	2	5	8 (53.3)	8 (50.0)	28 (45.2)
Fatigue	0	2	2	2	4 (26.7)	6 (37.5)	16 (25.8)
AST increased	0	0	3	5	3 (20.0)	4 (25.0)	15 (24.2)
ALT increased	0	0	2	6	3 (20.0)	3 (18.8)	14 (22.6)
Nausea	1	1	3	1	1 (6.7)	4 (25.0)	11 (17.7)
Alkaline phosphatase increased	0	0	3	3	0	2 (12.5)	8 (12.9)
Vomiting	1	1	3	0	0	3 (18.8)	8 (12.9)
Diarrhea	0	0	0	2	1 (6.7)	3 (18.8)	6 (9.7)
Decreased appetite	0	0	1	1	1 (6.7)	1 (6.3)	4 (6.5)
Stomatitis	0	1	0	1	0	1 (6.3)	3 (4.8)
Pyrexia	1	0	2	0	0	0	3 (4.8)
Hyperglycemia	1	1	0	0	0	1 (6.3)	3 (4.8)
Anemia	0	1	1	0	0	1 (6.3)	3 (4.8)
Treatment-related[a] AE grade ≥3 occurring in ≥1 patient overall by preferred term[b]							
AST increased	0	0	1	3	2 (13.3)	0	6 (9.7)
ALT increased	0	0	0	0	1 (6.7)	1 (6.3)	2 (3.2)
Lymphopenia	0	0	0	0	0	2 (12.5)	2 (3.2)
Fatigue	0	0	0	0	2 (13.3)	0	2 (3.2)
Infusion-related reaction	0	0	0	0	0	2 (12.5)	2 (3.2)
Bilirubin increased	0	0	0	0	1 (6.7)	0	1 (1.6)
ECG abnormal	0	0	0	0	1 (6.7)	0	1 (1.6)
Acute myocardial infarction	0	0	0	0	0	1 (6.3)	1 (1.6)
Atrial fibrillation	0	0	0	0	1 (6.7)	0	1 (1.6)
Abdominal pain	0	0	0	0	1 (6.7)	0	1 (1.6)
Hyperglycemia	0	0	0	0	0	1 (6.3)	1 (1.6)
Hypertension	0	0	1	0	0	0	1 (1.6)

AE, adverse event; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ECG, electrocardiogram

[a] Considered by the investigator as possibly, probably, or definitely related to treatment

[b] Coded by MedDRA version 14.1

The most common treatment-related AE across all patients in phase 1a and 1b was infusion-related reactions (45.2 %). Most were grade 1 or 2 (in all but two patients), resolved within 24 hours, and were similar to what has been observed with other therapeutic mAbs. Positive post-baseline CEP-37250/KHK2804 neutralizing antibodies were reported in 14 patients (22.6 %), all but one of which occurred in patients who developed infusion-related reactions and/or flushing. After fatigue (25.8 %), increased AST and/or ALT (25.8 % and 24.2 %, respectively) were the next most frequent treatment-related AEs. Treatment-related increased AST and/or ALT (9.7 % and 3.2 %) were the most frequent treatment-related grade ≥3 AEs. Based on an independent hepatologist's review of the results for all patients, the increase in hepatic function values did not meet Hy's Law as the ALT or AST increases were not associated with bilirubin increases. Preclinical studies showed mild to marked biliary epithelium binding on tissue cross-reactivity panels for human and cynomolgus monkeys and hepatic enzyme and correlative histopathologic hepatotoxicity at high doses (10 and 30 mg/kg) in animal toxicology [data on file, Kyowa Kirin Pharmaceutical Development, Inc.]. Soon after recruitment into phase 1b, three patients developed cardiac events at a single study center. All these events were considered treatment-related SAEs

and led to withdrawal of CEP-37250/KHK2804. This led to a temporary hold of recruitment into the clinical trial. Based on an independent cardiologist's assessment, the cardiac events were considered to be related to infusion-related reactions. The trial was reopened following protocol amendment (necessitating more frequent ECG and vital sign monitoring, and exclusion of patients with atrial fibrillation and other prior arrhythmia requiring treatment). The following factors were taken into consideration in making this conclusion. There appeared to be no apparent dose relationship across all cardiac events that occurred during dose escalation in phase 1a. Mandatory pre-medication for prophylaxis of infusion-related reactions was instituted during dose expansion in phase 1b. Comparison of all cardiac events that occurred during phase 1a versus phase 1b revealed a lower incidence of cardiac events with mandatory pre-medication for infusion-related reactions in phase 1b. Furthermore, preclinical studies had not revealed a potential for cardiac toxicity with CEP-37250/KHK2804: tissue panels from three humans and cynomolgus monkeys showed no specific binding against heart tissue, and there were no cardiac changes in either single or multiple dose toxicology studies in cynomolgus monkeys [data on file, Kyowa Kirin Pharmaceutical Development, Inc.].

Thirteen of 40 (32.5 %) evaluable patients had unconfirmed SD, four of which were confirmed (10.0 %). No patient had a complete or partial best response. The study was therefore terminated early due to the lack of efficacy. In addition, continued safety concerns (i.e., cardiac issues, hepatic toxicity, infusion-related reactions, and management of glucose levels because the steroid pre-medication regimen necessitated the addition of metformin for glucose control in many patients) made the benefit-risk assessment unfavorable for continued development of CEP-37250/KHK2804, which was halted indefinitely.

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Compliance with Ethical Standards

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Conflict of Interest JN is a founder of, holds stocks/stock options and patents of, and receives royalties from Gradalis Inc., and has received consulting fees or honoraria and payment for lectures including service on speakers bureaus from Amgen. JG-O received consulting fees or honoraria from Sanofi-Aventis. BE-R received honoraria for advisory boards from Genentech and Merrimack Pharmaceutical, and has conducted contracted research for AVEO, Genentech, Synta, Novartis, Pfizer, Hoosier Cancer Research Network, Boston Biomedical Inc., Cleave Biosciences, Taiho, and Bristol-Meyers Squibb outside the submitted work. TB-S has received consulting fees from Genentech, Bayer, and Taiho. XZ was and VS is an employee of Kyowa Kirin Pharmaceutical Development, Inc. JM, DH, and MM declare no conflict of interest.

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