



Praluzatamab Ravtansine, a CD166-Targeting Antibody-Drug Conjugate, in Patients with Advanced Solid Tumors: An Open-Label Phase I/II Trial

Valentina Boni¹, Mary J. Fidler², Hendrik-Tobias Arkenau³, Alexander Spira⁴, Funda Meric-Bernstam⁵, Nataliya Uboha⁶, Rachel E. Sanborn⁷, Randy F. Sweis⁸, Patricia LoRusso⁹, Misako Nagasaka¹⁰, Javier Garcia-Corbacho¹¹, Shadia Jalal¹², James J. Harding¹³, Stella K. Kim¹⁴, Iris H.C. Miedema¹⁵, Danielle J. Vugts¹⁶, Marc C. Huisman¹⁶, Gerben J.C. Zwezerijnen¹⁶, Guus A.M.S. van Dongen¹⁶, C. Willemien Menke van der Houven van Oordt¹⁵, Song Wang¹⁷, Tam Dang¹⁷, Ivan A. Zein¹⁷, Olga Vasiljeva¹⁷, Susan K. Lyman¹⁷, Virginia Paton¹⁷, Alison Hannah¹⁷, and Joyce F. Liu¹⁸

ABSTRACT

Purpose: Praluzatamab ravtansine (CX-2009) is a conditionally activated Probody drug conjugate (PDC) comprising an anti-CD166 mAb conjugated to DM4, with a protease-cleavable linker and a peptide mask that limits target engagement in normal tissue and circulation. The tumor microenvironment is enriched for proteases capable of cleaving the linker, thereby releasing the mask, allowing for localized binding of CX-2009 to CD166. CX-2009 was evaluated in a phase I/II clinical trial for patients with advanced solid tumors.

Patients and Methods: Eligible patients had metastatic cancer receiving ≥ 2 prior treatments. CX-2009 was administered at escalating doses every 3 weeks (0.25–10 mg/kg) or every 2 weeks (4–6 mg/kg). Primary objective was to determine the safety profile and recommended phase II dose (RP2D).

Results: Of 99 patients enrolled, the most prevalent subtype was breast cancer ($n = 45$). Median number of prior therapies

was 5 (range, 1–19). Dose-limiting toxicities were observed at 8 mg/kg every 3 weeks and 6 mg/kg every 2 weeks. On the basis of tolerability, the RP2D was 7 mg/kg every 3 weeks. Tumor regressions were observed at doses ≥ 4 mg/kg. In the hormone receptor-positive/HER2-nonamplified breast cancer subset ($n = 22$), 2 patients (9%) had confirmed partial responses, and 10 patients (45%) had stable disease. Imaging with zirconium-labeled CX-2009 confirmed uptake in tumor lesions and shielding of major organs. Activated, unmasked CX-2009 was measurable in 18 of 22 posttreatment biopsies.

Conclusions: CD166 is a novel, ubiquitously expressed target. CX-2009 is the first conditionally activated antibody-drug conjugate to CD166 to demonstrate both translational and clinical activity in a variety of tumor types.

Introduction

Probody therapeutic candidates (Pb-Tx) are conditionally activated mAb-based therapeutics. A Pb-Tx consists of three modular components [a mAb directed against a tumor antigen, a peptide that masks the complementarity-determining regions (CDR), and a protease-cleavable substrate linking the peptide mask to the mAb] produced as a single protein by standard recombinant mAb methodology. A Probody drug conjugate (PDC) is a Pb-Tx that includes a toxin conjugated to the mAb component. Pb-Txs remain largely intact

(i.e., masked) in circulation and in normal tissue, but upon reaching the tumor, upregulated protease activity in the tumor microenvironment promotes cleavage of the substrate linker and subsequent release of the CDR-masking peptide. This generates a fully activated Pb-Tx (i.e., unmasked molecule) that can bind to its tumor target (1–3). CD166 is a transmembrane type-1 glycoprotein expressed in both normal and tumor tissues that plays a role in angiogenesis, inflammation, tumor propagation and invasiveness, migration of monocytes across endothelial tissues, intravasation of leukocytes in the central nervous system, T-cell activation, and hematopoiesis (4). Given the

¹START Madrid HM CIOCC (Centro Integral Oncológico Clara Campal), Hospital Universitario HM Sanchinarro, HM Hospitales, Madrid, Spain. ²Rush University Medical Center, Chicago, Illinois. ³Sarah Cannon Research Institute UK Limited, London, United Kingdom. ⁴Virginia Cancer Specialists, Fairfax, Virginia. ⁵MD Anderson Cancer Center, Houston, Texas. ⁶University of Wisconsin-Carbone Cancer Center, Madison, Wisconsin. ⁷Earle A. Chiles Research Institute, Providence Cancer Institute, Portland, Oregon. ⁸University of Chicago Medicine, Chicago, Illinois. ⁹Yale University School of Medicine, New Haven, Connecticut. ¹⁰Barbara Ann Karmanos Cancer Institute, Detroit, Michigan. ¹¹Hospital Clinic Barcelona, Barcelona/IDIBAPs, Spain. ¹²Indiana University Melvin and Bren Simon Cancer Center, Indianapolis, Indiana. ¹³Memorial Sloan Kettering Cancer Center and Weill Cornell Medical College, New York, New York. ¹⁴Robert Cizik Eye Clinic, Houston, Texas. ¹⁵Department of Medical Oncology, Cancer Center Amsterdam, Amsterdam UMC, Vrije Universiteit, Amsterdam, the Netherlands. ¹⁶Department of Radiology and Nuclear Medicine, Cancer Center Amsterdam, Amsterdam UMC, Vrije Universiteit, Amsterdam, the Netherlands. ¹⁷CytomX Therapeutics, Inc., South San Francisco, California. ¹⁸Dana-Farber Cancer Institute, Boston, Massachusetts.

Note: Supplementary data for this article are available at Clinical Cancer Research Online (<http://clincancerres.aacrjournals.org/>).

Current address for V. Boni: NEXT Oncology Madrid, University Hospital Quironsalud, Madrid, Spain.

Corresponding Author: Valentina Boni, Phase 1 Unit Oncology Department, NEXT Madrid, Madrid 28223, Spain. Phone: 0034-6662-09969; E-mail: vboni@nextoncology.eu

Clin Cancer Res 2022;28:2020–9

doi: 10.1158/1078-0432.CCR-21-3656

This open access article is distributed under Creative Commons Attribution-NonCommercial-NoDerivatives License 4.0 International (CC BY-NC-ND).

©2022 The Authors; Published by the American Association for Cancer Research

Translational Relevance

This is the first publication of a clinical trial reporting the safety and preliminary activity of praluzatamab ravtansine (CX-2009), a Pb-Tx antibody–drug conjugate (ADC) targeting CD166. These data support converting a target antigen from “undruggable” due to its wide distribution on tumor cells and healthy tissues to one that is “druggable” with the use of a conditionally activated ADC. The preliminary findings support further exploration. CX-2009 appears to be generally well tolerated, with signs of anticancer activity in patients with advanced, refractory cancers. First-in-human imaging with zirconium-labeled CX-2009 (^{89}Zr]Zr-CX-2009) confirmed uptake in tumor lesions and shielding of major organs known to express CD166. Analysis of on-treatment biopsies shows CX-2009 is activated/unmasked in the tumor micro-environment. The results of this study support future investigations of CX-2009 in patients with advanced, previously treated breast cancer and other tumors that express CD166.

expression of CD166 in normal tissues, it was considered “undruggable” until the advent of Probody technology because of the expected potential high rate of off-tumor/on-target toxicities. Praluzatamab ravtansine (CX-2009) is a CD166-targeting PDC where the mAb moiety is conjugated via a disulfide cleavable linker to the potent microtubule inhibitor N2'-deacetyl-N2'-(4-mercapto-4-methyl-1-oxopentyl)-maytansine (DM4) with an average drug antibody ratio of approximately 3.5 for the conjugated species (5).

This phase I/II study (CTMX-M-2009-001; NCT03149549) was designed to evaluate the tolerability, activity, and pharmacokinetics of CX-2009 to identify the recommended phase II dose (RP2D). Results from dose escalation of CX-2009 regimens at every 2 weeks and every 3 weeks are presented here, including translational analyses of on-treatment biopsies assessing activation of CX-2009 in the tumor, and a visual and quantitative assessment of ^{89}Zr]Zr-CX-2009 distribution in tumor and nontumor tissues by immuno-PET.

Patients and Methods

Study design and participants

This multicenter, open-label, phase I/II study was designed as a phase I dose-escalation/expansion trial followed by a modified toxicity probability interval-2 (mTPI-2) design (refs. 6, 7; i.e., a rule-based design similar to the 3+3 design that allows for dose escalation and deescalation) to guide patient allocation from observed safety outcomes at different dose levels.

Eligible patients [age ≥ 18 years; Eastern Cooperative Oncology Group (ECOG) 0, 1] had either histologically confirmed, metastatic or locally advanced unresectable solid tumors with progressive disease (PD) after standard treatment or known intolerance to available treatment. The allowed tumor types, based on the predicted prevalence of CD166 expression, were breast cancer, castration-resistant prostate cancer, non–small cell lung cancer (NSCLC), epithelial ovarian cancer, head and neck squamous cell cancer (HNSCC), cholangiocarcinoma, and endometrial carcinoma. The online Supplementary Appendix lists all inclusion/exclusion criteria.

Submission of either archival or fresh tumor tissue was required for evaluation of CD166 expression by a qualified IHC assay performed in a central Clinical Laboratory Improvement Amendments–certified laboratory. The original protocol did not require CD166 expression for

eligibility or prophylaxis for DM4-associated corneal toxicity. These two requirements were implemented with subsequent protocol amendments.

This study was conducted in compliance with the Declaration of Helsinki, Good Clinical Practice guidelines, the principles of informed consent, and the requirements of public registration of clinical trials. Written informed consent was obtained from each patient before screening. The protocol was approved by the institutional ethics committees.

Procedures

CX-2009 doses, ranging from 0.25 to 10 mg/kg based on the patient's adjusted ideal body weight, were given intravenously every 3 weeks, starting with a single-patient accelerated titration cohort (0.25 mg/kg), followed by a 3+3 dose escalation with doses ranging from 0.5 to 10 mg/kg. Subsequently, a 14-patient mTPI-2 cohort was planned to further refine the RP2D. For the every 2 weeks schedule, a mTPI-2 escalation design started at 6 mg/kg, with potential escalation/deescalation to 8–10 or 4 mg/kg. Expansion cohorts at the RP2D followed the escalation phase.

Dose-limiting toxicity (DLT) criteria occurring in cycle 1 (i.e., first 3 weeks schedule or first 4 weeks schedule) included grade ≥ 3 treatment-related adverse events (AE). Detailed DLT criteria can be found in the online Supplementary Materials and Methods. The decision to proceed through the dose escalation was overseen by a safety review committee that was composed of study investigators and the sponsor's medical monitor and pharmacovigilance team. Enrollment continued in each cohort until the MTD or maximum administered dose was reached.

Safety assessments at every visit included physical examinations and monitoring of AEs and vital signs. Electrocardiograms, ophthalmologic examinations, and clinical laboratory investigations were done according to the protocol-mandated schedule or as clinically indicated. AEs were graded by severity using National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) version 4.03, seriousness, and relation to study treatment (as determined by the treating investigators). An independent Data Safety Monitoring Board (DSMB) reviewed data from the ongoing trial every 6 months.

Antitumor activity was assessed by standard radiographic imaging at screening and every 8 weeks thereafter. Patients who received study drug for ≥ 12 months had tumor assessments every 12 weeks. Tumor response was evaluated locally by the treating investigator according to RECIST v1.1. An analysis of safety and antitumor activity outcomes for patients with hormone receptor–positive/HER2–nonamplified breast cancer (HR⁺/HER2⁻) and triple-negative breast cancer (TNBC) was performed because the protocol had prospectively determined that these subtypes of breast cancer would be studied as separate expansion cohorts.

Sample collection and assays

Measurement of CX-2009 in circulation

The following analytes were measured in human plasma by validated assays using LC/MS-MS: intact CX-2009 (masked prodrug form of CX-2009), total CX-2009 (intact and activated forms of CX-2009), Probody conjugated-DM4, free DM4, and DM4-Me (S-Methyl metabolite of DM4).

IHC analysis of CD166

The study sponsor, CytomX Therapeutics, Inc., in collaboration with MolecularMD (now ICON Specialty Laboratories), developed a

novel IHC CD166 assay with a pathologist-based algorithm and scoring method to evaluate CD166 expression in patient tumor biopsies (Supplementary Materials and Methods). The CD166 scoring method comprises a semiquantitative evaluation of the percentage of tumor cells with membranous staining at different intensities (0, 1+, 2+, and 3+) in the tumor samples; these data are used to generate a composite staining score (H-score; Supplementary Materials and Methods).

Collection of on-treatment biopsies

Starting at 4 mg/kg, additional patients with high-expressing CD166 tumors could provide consent for on-treatment biopsies and enroll into a previously declared safe dose. Biopsies were obtained in cycle one on day 4 following the first CX-2009 infusion (C1D4) and were analyzed to determine levels of activated CX-2009 and CD166. Activated CX-2009 in tumor biopsies was measured by a qualified capillary immunoelectrophoresis (CEI) assay on the PeggySue instrument (ProteinSimple) using a custom anti-idiotypic antibody (developed by CytomX) that was directed against the light chain of CX-2009. CD166 in on-treatment biopsies also was measured by a qualified CEI assay on the PeggySue instrument, using a commercial anti-CD166 antibody (ab233750; Abcam). It is important to note that this assay measures the amount of activated light chain, and results are interpolated against standard curves consisting of the parental antibody of CX-2009 (lacking the DM4 conjugate) in which either both light chain arms are intact or both are activated (two-arm-activated). However, that the most probable state in vivo is a mixture of one-arm-activated and two-arm-activated CX-2009 molecules because cleavage of one arm may not necessarily be followed immediately by cleavage of the second arm. See Supplementary Materials and Methods for full assay details

PET/CT imaging with [⁸⁹Zr]Zr-CX-2009

To understand the distribution of CX-2009 in healthy and tumor tissues, immuno-PET imaging was performed with zirconium-89 (⁸⁹Zr) radiolabeled CX-2009. Thirty-seven MBq/10 mg of [⁸⁹Zr]Zr-CX-2009 was administered followed by four sequential PET/CT scans at 2, 26, 90, and 162 hours after injection. A Patlak analysis was performed to extract irreversible uptake (Ki) in organs of interest. These results were compared with validated baseline uptake of antibodies in organs lacking target expression, which was based on four radiolabeled mAbs (8). Details on the imaging substudy procedures and analyses are provided in the Supplementary Materials and Methods. After completing imaging procedures, patients could enroll to the actively enrolling dosing cohort.

Outcomes

The primary objectives of this dose-escalation/expansion study were to assess the safety and tolerability of CX-2009, including the incidence and nature of DLTs, and the determination of the MTD and RP2D on both the every 3 weeks and every 2 weeks schedules. Secondary objectives were antitumor response outcomes, including objective response rate (ORR), clinical benefit rate [CBR, i.e., patients who achieved a best response of complete response (CR), partial response (PR; confirmed or unconfirmed), or had stable disease (SD) lasting ≥16 or ≥24 weeks].

Statistical analysis

Although no formal power calculations were done, the sample size of each dose cohort was guided by the mTPI-2 design (6). The safety analysis population, which included all enrolled patients who received

at least one dose of CX-2009, was used to describe patient characteristics, CX-2009 doses and duration of treatment, safety endpoints, and activity analyses. The response-evaluable population, which included all patients who had an adequate baseline disease assessment and at least one postbaseline disease assessment occurring prior to any new anticancer treatment, was used to evaluate ORR and CBR.

SAS (version 9.4) was used for statistical analysis of patient data. Descriptive statistics were used to summarize all study data. Continuous variables were summarized by number, mean (SD), and median interquartile range (IQR) values. Categorical variables were summarized by frequency and percentage of patients. Exact 95% confidence intervals were calculated for all proportion estimates. Estimates of time-to-event endpoints (progression-free survival and overall survival) were obtained using the Kaplan-Meier method.

The Spearman rank-order test was used to assess the correlation of intratumoral CD166 with activated CX-2009 (TIBCO Spotfire) because the data were not normally distributed. Normality of the datasets was assessed using the D'Agostino-Pearson test (GraphPad Prism 9.1).

This trial is registered with ClinicalTrials.gov, NCT03149549, and with the EU Clinical Trials Register, EudraCT 2017-000625-12.

Data sharing

All data relevant to the study are included in the article or uploaded as online Supplementary Data. The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Role of the funding source

The funder, in collaboration with the study investigators, developed the study protocol. The funder was also involved in data collection, analysis, interpretation of results, and writing of this article. The corresponding author had full access to all the study data and had final responsibility for the decision to submit for publication.

Results

Enrollment and patient demographics

Of 138 patients evaluated for eligibility between June 14, 2017 and April 9, 2020, 99 patients were enrolled from 27 academic and community oncology centers in the United States, Spain, the United Kingdom, and the Netherlands (Supplementary Fig. S1). Supplementary Table S1 summarizes reasons for screen failures. Because of logistical difficulties associated with the COVID-19 pandemic, study enrollment was stopped at a point when the dose-escalation phase was completed, and there was limited enrollment into the dose-expansion phase. As of November 17, 2020, the median duration of follow-up was 18.4 weeks (IQR, 9–49 weeks). All 99 enrolled and treated patients contributed to the safety-analysis population; 80 patients met the criteria for the response-evaluable population. Those excluded from the response evaluation either did not have a postbaseline disease assessment ($n = 17$) or started a new anticancer treatment prior to the postbaseline tumor assessment ($n = 2$). Reasons for treatment discontinuation included progressive disease ($n = 54$), symptomatic deterioration ($n = 16$), AEs ($n = 12$), investigator or patient decision ($n = 11$), or death ($n = 6$). On-treatment biopsies were collected from 27 patients at doses ≥4 mg/kg; 22 of these were evaluable for measurement of intratumoral CX-2009 and CD166. Two patients participated in the imaging substudy.

Table 1. Baseline characteristics of patients (safety population).

	All cohorts (n = 99)	HR ⁺ /HER2- nonamplified breast cancer and TNBC (n = 39)
Age, years	59 (51-67)	53 (44-67)
Sex		
Female	78 (79%)	39 (100%)
Male	21 (21%)	—
Race		
White	81 (82%)	30 (77%)
Asian	5 (5%)	1 (3%)
Black or African American	2 (2%)	—
Native Hawaiian or other Pacific Islander	2 (2%)	1 (3%)
Other	3 (3%)	2 (5%)
Unknown	6 (6%)	5 (13%)
ECOG performance status		
0	33 (33%)	17 (44%)
1	66 (67%)	22 (56%)
Tumor type		
Breast carcinoma	45 (46%)	
HR-positive, HER2- nonamplified	28	28
HER2 positive	6	—
Triple negative	11	11
CRPC	2 (2%)	—
NSCLC	13 (13%)	—
Epithelial ovarian carcinoma	22 (22%)	—
Endometrial carcinoma	3 (3%)	—
HNSCC	9 (9%)	—
Cholangiocarcinoma	5 (5%)	—
CD166 status		
High	80 (81%)	32 (82%)
Low	13 (13%)	5 (13%)
Unknown	6 (6%)	2 (5%)
No. of prior cancer treatment regimens	5 (4-8)	8 (5-9)
Types of prior treatments		
Platinum-containing compound or microtubule inhibitor	96 (97%)	37 (95%)
Platinum-containing compounds	71 (72%)	15 (39%)
Microtubule inhibitor	81 (82%)	37 (95%)
Anti-PD-1/PD-L1 treatment	36 (36%)	6 (15%)
CDK4/6 inhibitor	21 (21%)	17 (44%)

Note: Data are median (IQR) and *n* (%).

Abbreviations: CRPC, castration-resistant prostate cancer; HNSCC, head and neck squamous cell carcinoma; HR⁺, hormone receptor positive; NSCLC, non-small cell lung cancer; TNBC, triple-negative breast cancer.

Most patients were women (*n* = 78/99) and White (*n* = 81/99) with an ECOG score of 1 (*n* = 66/99; **Table 1**). The median age of the study population was 59 years (IQR, 51–67). The most common tumor types were breast cancer [*n* = 45; HR⁺/HER2[−] (*n* = 28), TNBC (*n* = 11), and HER2⁺ (*n* = 6)], epithelial ovarian carcinoma (*n* = 22), and NSCLC (*n* = 13). The most common prior therapies included treatment with a platinum-containing compound or with a microtubule inhibitor (*n* = 97), endocrine therapy (*n* = 44), or an anti-PD1/PD-L1 agent (*n* = 36). The median number of prior therapies was five (IQR, 4–8) for the total study population and eight (IQR, 5–9) for the HR⁺/HER2[−] and TNBC subgroups.

IHC analysis

IHC analysis of CD166 in archival or predose samples showed that of 99 tumor samples, 80 were classified as high for CD166, 13 were low for CD166, and six samples were insufficient for IHC analysis.

PET/CT imaging with [⁸⁹Zr]Zr-CX-2009

Two patients, 1 with NSCLC and the other with metastatic breast cancer, were evaluated for tumor uptake and biodistribution using [⁸⁹Zr]Zr-CX-2009 PET/CT imaging (**Fig. 1A**). Results are available for the patient with NSCLC. Patlak analysis was performed to extract irreversible uptake values (Ki) of [⁸⁹Zr]Zr-CX-2009 in major organs using validated dataset of four mAbs (8). CX-2009 performed identically to a nonspecific/nonbinding mAb with respect to uptake in lung, spleen, and kidney (**Fig. 1B**). In contrast, liver uptake was increased, most likely due to the attached cytotoxic payload, as previously demonstrated in preclinical studies (9, 10). Tumor uptake was confirmed despite the challenge of assessing uptake in the low-cellularity context of this specific tumor type (**Fig. 1C**).

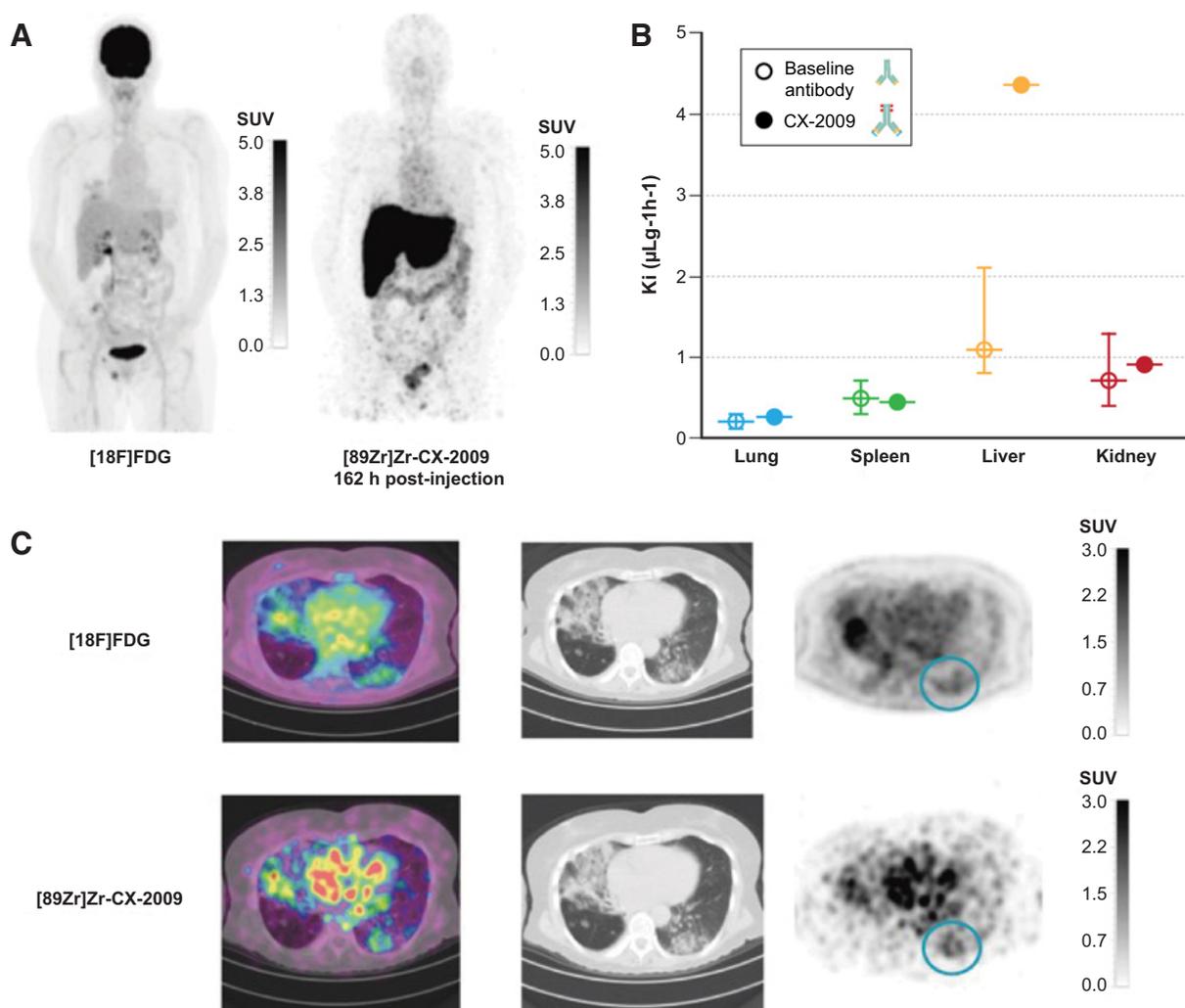
Safety

Patients received a median of three doses (IQR, 2–4) of CX-2009 during a median of 8.9 weeks (IQR, 6–14) in all cohorts. The maximum administered dose was 10 mg/kg. One DLT (vomiting) occurred in the 8 mg/kg every 3 weeks cohort and two DLTs (peripheral neuropathy and increased liver transaminases) occurred in the 6 mg/kg every 2 weeks cohort. All DLTs were reversible.

Table 2 summarizes treatment-emergent AEs (TEAE) by dosing cohort and in the total study population. Of 99 enrolled patients, 98 patients experienced at least one TEAE; 90 patients experienced a treatment-related AE (TRAE). Infusion-related reactions were reported in 22 patients; one was grade 3 and the others were grades 1 or 2. Premedications, including corticosteroids, acetaminophen or antihistamines, were allowed if needed for infusion reaction prophylaxis. Twelve patients (12%) experienced 12 TRAEs that led to CX-2009 discontinuation. Five patients discontinued treatment due to ocular AEs. Two patients discontinued treatment for peripheral neuropathy and one patient each discontinued treatment for nausea, sepsis, back pain, dyspnea, and urticaria.

Across all dosing cohorts, 37% of patients had at least one grade ≥3 TRAE (**Table 2**). The most commonly reported grade ≥3 TRAEs were keratitis (9%), increased aspartate aminotransferase (8%), increased alanine aminotransferase (5%), and anemia (5%). One patient with NSCLC had grade 5 sepsis, which the investigator considered to be treatment related and complicated by pancytopenia. This 60-year-old individual died 11 days after receiving the first dose of CX-2009 8 mg/kg. Upon DSMB review, the event was not considered to meet the criteria for a DLT during cycle 1 because the patient, although eligible, had a history of bone marrow failure that began before receiving the first dose of CX-2009. For this patient, pharmacokinetic parameters were similar to the other patients receiving this dose, and a gastrointestinal (GI) source of infection was considered likely given the initial presentation with diarrhea and pyrexia. Severe hematologic toxicity remains uncommon with CX-2009.

Supplementary Table S2 lists all-grade TRAEs that were reported in ≥15% of patients in any cohort. The aggregate safety data from this study demonstrate that the safety profile of CX-2009 was dose dependent with more frequent and severe TEAE occurring at doses ≥8 mg/kg every 3 weeks (**Table 2**). Ocular AEs, mainly keratitis or other corneal AEs were reported in 49 (49%) of all treated patients but occurred more frequently at higher doses of CX-2009 (Supplementary Table S3). Among 22 grade ≥3 TEAEs reported in patients treated with

**Figure 1.**

^{89}Zr -CX-2009 evaluation and comparison with mAbs evaluated in previous clinical studies (baseline). **A**, A total of 10 mg, 37 MBq of ^{89}Zr -CX-2009 was administered to a patient with micropapillary adenocarcinoma of the lung. **B**, Using Patlak analysis, irreversible uptake (Ki) of ^{89}Zr -CX-2009 in major organs was compared with a validated set of four mAbs. **C**, Tumor uptake was confirmed in this tumor type with low cellularity (images 162 hours after injection, top = ^{18}F FDG, bottom = ^{89}Zr -CX-2009, left images = PET/CT fusion, middle = CT scan in lung setting, right = PET). A tumor lesion in the left lower lung is indicated with a blue circle. Note: additional tumor tissue is present in the right lower lung. However, due to the close proximity of this lesion with the liver, uptake of ^{89}Zr -CX-2009 is not reliably distinguishable.

doses ≤ 7 mg/kg, one was an ocular toxicity (grade 3). In contrast, patients treated with doses ≥ 8 mg/kg ($n = 39$) experienced eight grade ≥ 3 ocular events, primarily keratitis, vision blurred, and dry eye. Seven patients had doses discontinued because of ocular events; 18 patients (28 events) had doses interrupted; and 2 patients required a dose reduction. Most ocular events occurred after cycle 2 and resolved within 2 to 3 weeks of onset. Ocular prophylaxis (vasoconstrictors, corticosteroids, artificial tears) was implemented during the 8 mg/kg dosing cohort and appeared to have some effectiveness in preventing ocular adverse events given there were more ocular events reported in patients who did not receive prophylactic medications.

On the basis of the absence of DLTs during cycle 1, the low rate of ocular AEs, and the observed tolerability for chronic administration, the CX-2009 RP2D was determined to be 7 mg/kg every 3 weeks. For

the every 2 weeks mTPI-2 dose escalation, the 6 mg/kg dose was not tolerable because 2 of 6 patients experienced DLTs during cycle 1; however, there were no DLTs in the 4 patients who enrolled in the CX-2009 4 mg/kg dose cohort.

Pharmacokinetics

Preliminary single-dose CX-2009 pharmacokinetic data in 85 patients suggested dose-proportionality following administration of 1.0 to 10.0 mg/kg CX-2009 every 3 weeks. CX-2009 circulated predominantly in the intact form ($>90\%$), with a median terminal half-life of 5.24 to 9.03 days (Fig. 2A). Figure 2B shows the median plasma concentration versus time curves for Probody-conjugated-DM4, total CX-2009, DM4-Me, and free DM4 following administration of a single dose of CX-2009 at 7 mg/kg every 3 weeks ($n = 9$). Both DM4-Me and free DM4 circulated as the minority species at $\leq 4.0\%$ of total CX-2009.

Table 2. Summary of TEAE incidence (safety population).

	CX-2009 dose (mg/kg) every 3 weeks							CX-2009 dose (mg/kg) every 2 weeks		All cohorts (n = 99)
	≤4 (n = 20)	5 (n = 9)	6 (n = 9)	7 (n = 12)	8 (n = 22)	9 (n = 9)	10 (n = 8)	4 (n = 4)	6 (n = 6)	
TEAE	19 (95%)	9 (100%)	9 (100%)	12 (100%)	22 (100%)	9 (100%)	8 (100%)	4 (100%)	6 (100%)	98 (99%)
TEAE related to CX-2009	14 (70%)	9 (100%)	9 (100%)	12 (100%)	21 (96%)	9 (100%)	7 (88%)	3 (75%)	6 (100%)	90 (91%)
TEAE grade ≥3	9 (45%)	4 (44%)	4 (44%)	8 (67%)	17 (77%)	7 (78%)	7 (88%)	1 (25%)	5 (83%)	62 (63%)
TRAE grade ≥3	1 (5%)	3 (33%)	3 (33%)	4 (33%)	14 (64%)	5 (56%)	4 (50%)	0	3 (50%)	37 (37%)
IRRs	4 (20%)	2 (22%)	3 (33%)	3 (25%)	5 (23%)	0	1 (13%)	2 (50%)	2 (33%)	22 (22%)
IRRs CTCAE grade ≥3	0	0	0	0	1 (5%)	0	0	0	0	1 (1%)
TRAE leading to CX-2009 discontinuation	0	3 (33%)	2 (22%)	1 (8%)	3 (14%)	2 (22%)	1 (13%)	0	0	12 (12%)
Dose-limiting toxicity	0	0	0	0	1 (4%)	0	0	0	2 (33%)	3 (3%)
SAE	3 (15%)	2 (22%)	3 (33%)	5 (42%)	10 (46%)	5 (56%)	3 (38%)	1 (25%)	1 (17%)	33 (33%)
SAE related to CX-2009	0	0	0	2 (17%)	6 (27%)	2 (22%)	1 (12%)	0	0	11 (11%)
TEAE leading to death	0	0	0	1 (8%)	1 (5%)	1 (11%)	0	0	0	3 (3%)
TEAE leading to death related to CX- 2009	0	0	0	0	1 (5%)	0	0	0	0	1 (1%)
Ocular toxicity related to CX-2009	2 (10%)	6 (67%)	3 (33%)	3 (25%)	12 (55%)	5 (56%)	6 (75%)	1 (25%)	5 (83%)	43 (43%)
Ocular toxicity CTCAE grade ≥3 related to CX-2009	0	1 (11%)	1 (11%)	0	3 (14%)	3 (33%)	1 (13%)	0	2 (33%)	11 (11%)
Incidence of grade ≥3 CX-2009-related TEAEs reported in ≥10% of patients in any dosing cohort										
≥1 event	1 (5%)	3 (33%)	3 (33%)	4 (33%)	14 (64%)	5 (56%)	4 (50%)	0	3 (50%)	37 (37%)
AST increased	0	0	0	0	4 (18%)	0	3 (38%)	0	1 (17%)	8 (8%)
Keratitis	0	1 (11%)	1 (11%)	0	2 (9%)	2 (22%)	1 (12%)	0	2 (33%)	9 (9%)
ALT increased	0	0	0	0	2 (9%)	0	2 (25%)	0	1 (17%)	5 (5%)
Anemia	1 (5%)	0	0	1 (8%)	3 (14%)	0	0	0	0	5 (5%)
Neuropathy ^a	0	1 (11%)	1 (11%)	1 (8%)	0	1 (11%)	0	0	1 (17%)	5 (5%)
Nausea	0	0	0	1 (8%)	1 (4%)	1 (11%)	1 (12%)	0	0	4 (4%)
Hyponatremia	0	0	2 (22%)	0	0	1 (11%)	0	0	0	3 (3%)
Fatigue	0	1 (11%)	0	0	0	0	1 (12%)	0	0	2 (2%)

Note: Data are n (%). Adverse events were graded by the investigators according to CTCAE version 4.03 and coded with the Medical Dictionary for Regulatory Activities version 16.1.

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; CTCAE, Common Terminology Criteria for Adverse Events; IRR, infusion-related reaction; SAE, serious adverse event; TEAE, treatment-emergent adverse event; TRAE, treatment-related adverse event.

^aNeuropathy was either peripheral neuropathy or peripheral sensory neuropathy.

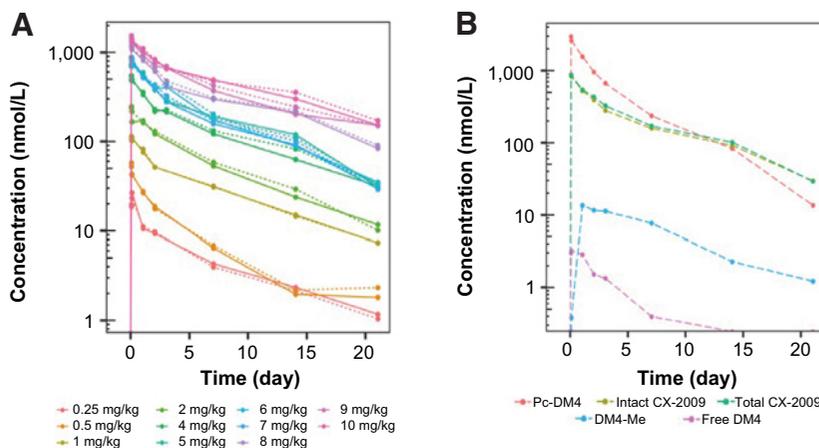
CX-2009 activity

Figure 3A and B shows plots of change in tumor burden in evaluable patients treated with CX-2009 at doses ≥4 mg/kg (no responses were seen in the 10 patients treated at doses of ≤2 mg/kg).

Supplementary Table S4 summarizes response assessment for all patients and for two subgroups of patients with breast cancer. In the HR⁺/HER2⁻ breast cancer subset (n = 22), 2 patients (9%) had confirmed PRs, and 10 patients (45%) had a best response of SD.

Figure 2.

A, Preliminary dose 1 intact CX-2009 (solid lines) and total CX-2009 (dashed lines) median plasma concentrations (nmol/L) versus time (days) following administration of up to 10 mg/kg CX-2009 every 3 weeks. **B,** Preliminary dose 1 Probody-conjugated-DM4 (Pc-DM4), intact CX-2009, total CX-2009, DM4-Me, and free DM4 median plasma concentrations (nmol/L) versus time (days) following administration of 7 mg/kg CX-2009 every 3 weeks. DM4, N-succinimidyl 4-(2-pyridyldithio) butanoate-N2'-deacetyl-N2'-(4-mercapto-4-methyl-1-oxopentyl)-maytansine; DM4-Me, S-methyl DM4; Pc, Probody-conjugated.



Notably, one of the patients with a confirmed PR had persistent bone metastases and received seven prior treatment regimens before experiencing durable response of almost 1 year, and complete disappearance of liver lesions with CX-2009. Of six reported unconfirmed PRs, three were in patients with TNBC ($n = 10$); one in a patient who had not responded to two prior treatments (Supplementary Fig. S2), two in patients with ovarian cancer, and one in a patient with HNSCC. The CBR16 and CBR24 rates in the HR⁺/HER2⁻ breast cancer subset were 36% and 23%, respectively, and were 40% and 40% for the TNBC subset. **Figure 3C** shows the course of tumor reduction by dose in response-evaluable patients with breast cancer treated at doses ≥ 4 mg/kg. Supplementary Table S5 summarizes the data on all 8 patients with confirmed and unconfirmed PRs.

Assessment of intratumoral CX-2009 and CD166 in on-treatment biopsies

A total of 27 on-treatment frozen biopsies were collected; of those, five were not evaluable due to very small size (<0.5 mg) and/or very low total protein yield (<50 μ g) upon lysis. The concentration of intact/masked and activated/unmasked CX-2009 was measured in evaluable biopsies to assess the extent to which tumor-associated proteases were able to cleave the substrate linker and to release the peptide mask from CX-2009. **Figure 4A** shows that activated CX-2009 was quantifiable in 18 samples (including in six of seven biopsies from patients with breast cancer) and demonstrates that the amount of measured intratumoral activated CX-2009 was variable and could differ by several fold even within the same dose group. In some of these cases, the extent of CX-2009 activation measured in the sample may be underestimated because of low tumor content in the biopsy, target-mediated drug disposition, or other factors. A comparison of activated CX-2009 in the tumor versus the intratumoral concentration of CD166 measured in the same sample (**Fig. 4B**) showed a significant correlation ($r^2 = 0.59$, $P = 0.00345$). This may arise from the greater retention of activated CX-2009 in high-CD166 tumors, or from alternative mechanisms. See Supplementary Table S6 for a complete data listing of results from analyses performed on on-treatment frozen biopsies.

Discussion

The goal of this phase I study was to evaluate the safety and preliminary antitumor activity of CX-2009, a first-in-class PDC targeting CD166, a previously undruggable target. Antibody–drug conjugates (ADC) are engineered to bind to a specific target antigen found on the surface of tumor cells, which upon internalization, are intended to provide targeted anticancer activity via their toxic payload. However, off-tumor on-target toxicity associated with traditional ADCs preclude safe administration when targeting transmembrane proteins, such as CD166, that are widely expressed in both normal and tumor tissues. PDCs are a new class of ADCs that are designed to be proteolytically activated in the tumor microenvironment by tumor-associated proteases while remaining largely inactive in circulation. The safety and antitumor activity results combined with novel imaging and translational analyses from this study show proof of platform with CX-2009, a first-in-class Pb-Tx, and represents the first successful targeting of CD166 with an anticancer therapeutic agent.

Pharmacokinetic data showed that circulating CX-2009 is predominantly ($>90\%$) in the intact/masked form, and free DM4 and methylated DM4 circulate at low concentrations relative to total CX-2009 with a ratio of <0.04 for exposure and peak concentration. CX-2009 was dosed using strategy that incorporated an adjustment to patient's

ideal body weight. CX-2009 AIBW dosing demonstrated lower variability in peak CX-2009 concentrations and exposure compared with CX-2009 doses using actual body weight. In addition, a correlation between early high CX-2009 exposure and ocular toxicity was observed, which could be in part addressed by utilizing AIBW versus total body weight (11).

First-in-human immuno-PET imaging with [⁸⁹Zr]Zr-CX-2009 as well as clinical imaging of an ADC show selective uptake of CX-2009 in tumor tissues, but shielding of major nontumor organs, as detected and confirmed by Patlak analysis (8). Analysis of on-treatment biopsies show CX-2009 is activated/unmasked in the tumor microenvironment. These results are consistent with the expectation that CX-2009 would be minimally activated in normal tissues but would be significantly activated in tumor tissues where upregulated and dysregulated protease activity, a recognized hallmark of cancer, enables cleavage of the linker. Taken together with the observations above, this supports the premise that CX-2009 is behaving as designed and is activated primarily in the tumor microenvironment.

Overall, this study demonstrates an acceptable safety profile for CX-2009 consistent with that of other DM4 ADCs (12–14) and provides indirect evidence of tumor-associated protease activity and the utility of the Probody platform. CX-2009 was successfully dose escalated to biologically active dose levels with an acceptable therapeutic window. There were no AEs identified that would suggest off-tumor on-target engagement of CD166 in normal tissues. DM4 payload toxicities are well described and include ocular, hepatic, neuropathic, and GI (15). Indeed, ocular toxicities, in the absence of mandatory prophylaxis, were the most common toxicity to result in treatment discontinuation. Ocular, hepatic, and neuropathic adverse events were also observed, and this profile is consistent with DM4-conjugated ADCs. Prophylactic treatment with corticosteroid and vasoconstrictor eye drops and artificial tears resulted in a trend toward symptomatic improvement and a reduction in the incidence of treatment-related ocular AE. However, formal statistical analysis could not be performed because of the small number of patients in which mandatory ocular prophylaxis was implemented from the start and the lack of a comparator cohort without ocular prophylaxis.

CD166 expression has been associated with grade, stage, and invasive potential for some tumors, including breast cancer, but has not been characterized as a predictive factor with respect to treatment outcomes (16, 17). This study is the first to evaluate the predictive potential of CD166 to support treatment with CX-2009 by enrolling patients with tumor types predicted to have high levels of CD166 using an investigational IHC assay. Analysis of on-treatment biopsies showed a correlation of levels of intratumoral CD166 with those of activated CX-2009 in the same lysate, which supports the promise of targeting CD166 with CX-2009. This correlation could be due to tumors with higher CD166 levels retaining a proportionally higher amount of activated CX-2009 at equilibrium. Additional research should address concordance of CD166 expression between primary and metastatic tumors, as well as the optimal diagnostic method to reliably identify patients who would most benefit from CX-2009 treatment. Other factors besides CD166 expression such as features of the tumor microenvironment (e.g., protease abundance), morphologic features, and/or tumor sensitivity to DM4 might be needed to generate a predictive algorithm to CX-2009 treatment outcomes.

In conclusion, the results of this trial validate CD166 as a viable first-in-class therapeutic target in cancer. The Probody platform enables administration of a CD166-directed ADC at tolerable doses, resulting in PRs. The RP2D of 7 mg/kg every 3 weeks is supported by the observed activity and safety profiles, as well as by pharmacokinetic and

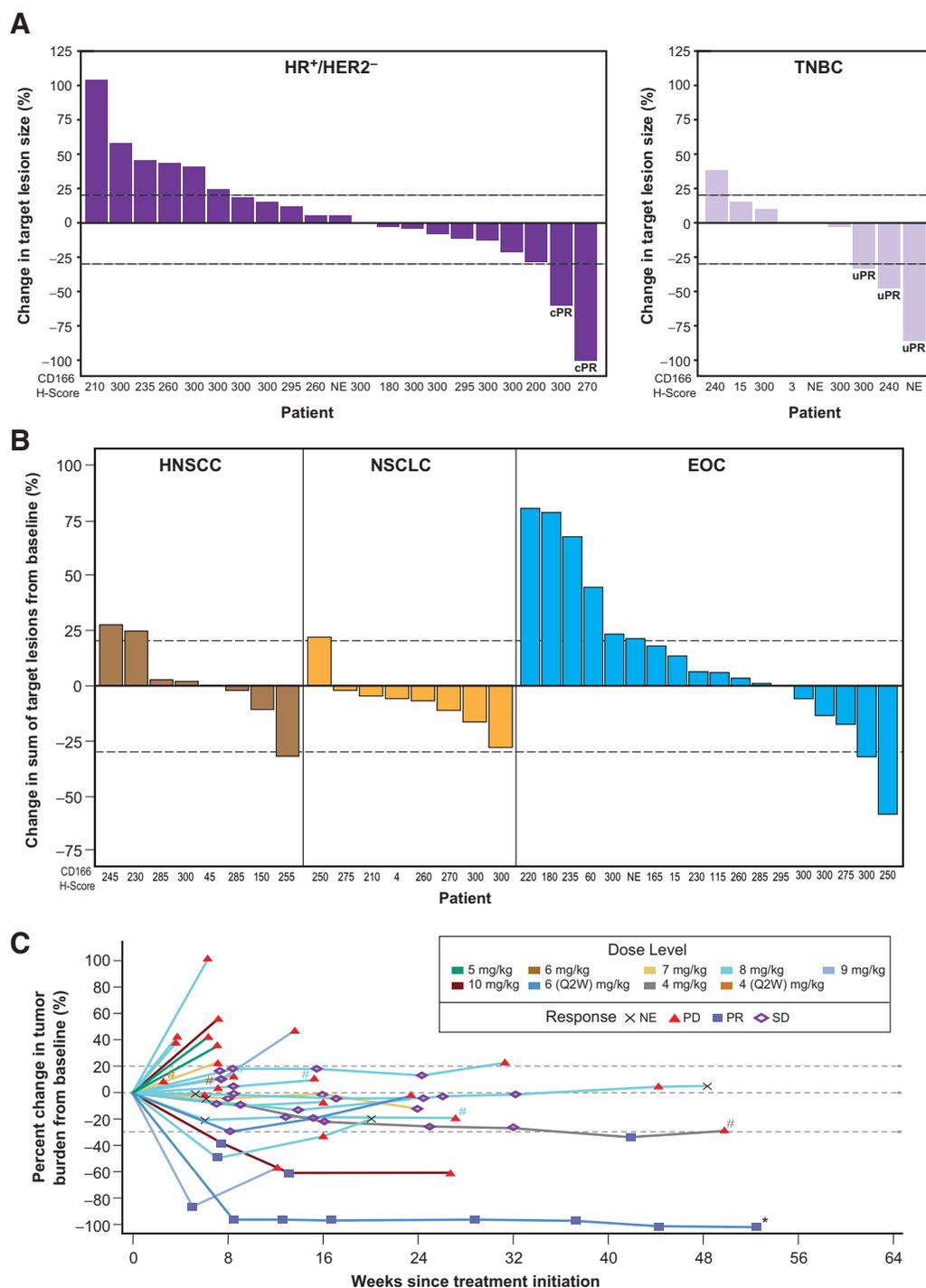


Figure 3. Waterfall plot of change in tumor burden in evaluable patients who received CX-2009 ≥ 4 mg/kg. **A**, Breast cancer and CD166 expression. Numbers along the x-axis represent a composite CD166 IHC score (H-score) for each patient’s archival/predose biopsy, with the highest potential score being 300 and the lowest being 0. See Supplementary IHC methods for a full description. Two of the 32 response-evaluable patients had new lesions, but no associated measurements to determine the percent change from baseline for the waterfall plot. cPR, confirmed partial response; uPR, unconfirmed partial response. **B**, HNSCC, head and neck squamous cell carcinoma; NSCLC, non-small cell lung cancer; EOC, epithelial ovarian carcinoma. **C**, Plot of tumor burden reduction by dose in patients with breast cancer treated with CX-2009 ≥ 4 mg/kg every 3 weeks. NE, not evaluable; PD, progressive disease; PR, partial response; SD, stable disease. *Patient started at 6 mg/kg every 2 weeks (Q2W), was dose reduced to 4 mg/kg Q2W due to keratitis. #Patient who, at the timepoint, had PD due to worsening nontarget lesion or presence of new lesions.

Downloaded from <http://aacrjournals.org/clinccancerres/article-pdf/28/10/2020/3120100/2020.pdf> by guest on 01 July 2022

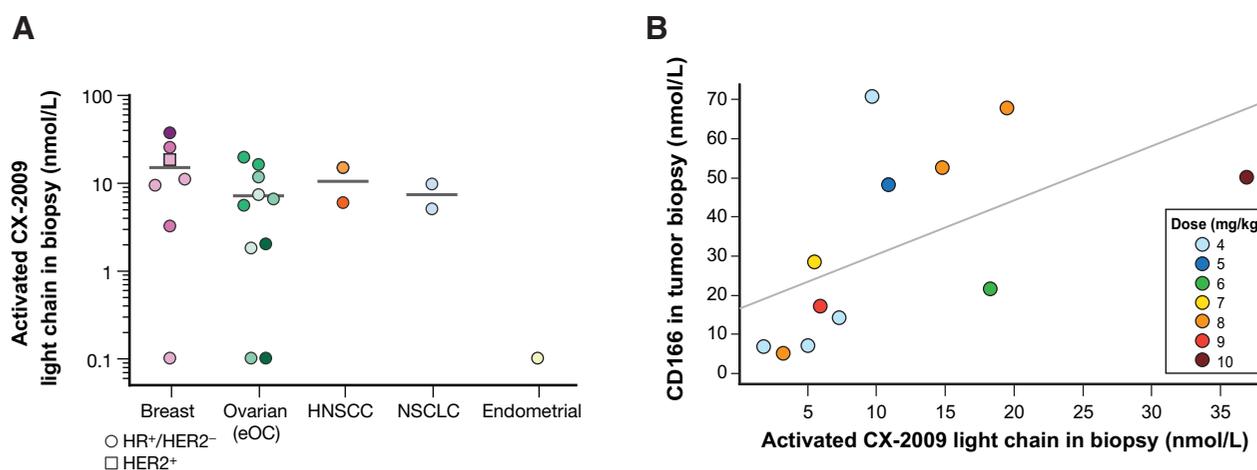


Figure 4.

Analysis of CX-2009 activation and CD166 levels in on-treatment biopsies. **A**, CX-2009 is activated in patient biopsies. On-treatment biopsies collected on CID4 were analyzed by CEI to determine the concentration of activated CX-2009. Values below the lower limit of quantification (LLOQ) are plotted as 0.1. The CX-2009 dose for the on-treatment biopsy patient subset ranged from 4–10 mg/kg. For a given indication, a darker color indicates a higher dose. For biopsies from patients with breast cancer, samples from HR⁺/HER2⁻ patients are represented as circles, and the single sample from a HER2⁺ patient is represented as a square. eOC, epithelial ovarian cancer. **B**, CD166 target levels correlate with activated CX-2009 in patient biopsies. CD166 in patient biopsy lysates was measured by CEI; the peak area measured for this assay focused on the molecular weight range expected for the glycosylated/membrane-associated form of CD166. Note that the activated CX-2009 values shown in **B** are a subset of the samples shown in **A**. CD166 was measured in all 22 evaluable samples, but 6 of the 18 samples with quantifiable activated CX-2009 were below the LLOQ for CD166; therefore, the total number of samples in which both analytes were quantifiable was 12.

nonclinical data (18, 19). A phase II trial is in progress (NCT04596150) to test the efficacy of single-agent CX-2009 in patients with HER⁺/HER2⁻ breast cancer and TNBC as well as to assess the efficacy of CX-2009 combined with CX-072 (a Probody therapeutic targeting PD-L1) in patients with TNBC.

Authors' Disclosures

V. Boni reports personal fees and non-financial support from CytomX during the conduct of the study as well as non-financial support from AbbVie, ACEO, Adaptimmune, Amcure, Amgen, AstraZeneca, BMS, CytomX, GSK, Genentech/Roche, H3, Incyte, Janssen, Kura, Lilly, Loxo, Nektar, MacroGenics, Menarini, Merck, Merus, Nanobiotix, Novartis, Pfizer, PharmaMar, Principia, Puma, Sanofi, Taiho, Tesaro, BeiGene, Transgene, Takeda, Inovio, MSD, PsiOxus, Seattle Genetics, Mersana, Daiichi, Astellas, ORCA, Boston Therapeutics, Dynavax, DebioPharm, Boehringer Ingelheim, Regeneron, Millennium, Synthron, Spectrum, Rigontec, and Zenith and personal fees from Puma Biotechnology, Ideaya Biosciences, Loxo Therapeutics, CytomX Therapeutics, Guidepoint, Oncoart, and Eli Lilly outside the submitted work. M.J. Fidler reports other support from Merck, Genentech, Alkermes, Rakuten, Amgen, Iovance, and Novartis; grants from Biodesix, Pfizer, Jazz, Silverback, and Daiichi; and personal fees from AstraZeneca during the conduct of the study. H.-T. Arkenau reports other support from Bicycle, BeiGene, Cellcentric, and Labgenius during the conduct of the study. A. Spira reports grants and personal fees from CytomX Therapeutics during the conduct of the study as well as personal fees from NEXT Oncology Virginia, Eli Lilly, AstraZeneca/MedImmune, Merck, and Bayer; grants and personal fees from Takeda, Amgen, Janssen Oncology, Novartis, Bristol-Myers Squibb, Incyte, Mirati Therapeutics, Gritstone Oncology, Jazz Pharmaceuticals, Janssen Research & Development, and Array BioPharma; and grants from LAM Therapeutics, Roche, AstraZeneca, Boehringer Ingelheim, Astellas Pharma, MedImmune, Newlink Genetics, AbbVie, Ignyta, Trovogene, MacroGenics, Astex Pharmaceuticals, Loxo, Arch Therapeutics, Gritstone, Plexikon, Daiichi Sankyo, ADCT, Rubius, and Synthekine outside the submitted work. F. Meric-Bernstam reports personal fees from AbbVie, Aduro BioTech Inc., Alkermes, AstraZeneca, DebioPharm, eFFECTOR Therapeutics, F. Hoffman-La Roche Ltd., Genentech Inc., IBM Watson, Infinity Pharmaceuticals, Jackson Laboratory, Kolon Life Science, Lengo Therapeutics, Origimed, PACT Pharma, Parexel International, Pfizer Inc., Samsung Bioepis, Seattle Genetics Inc., Tallac Therapeutics, Tyra Biosciences, Xencor, Zymeworks, Black Diamond, Biovica, Eisai, Immunomedics, Inflec-

tion Biosciences, Karyopharm Therapeutics, Loxo Oncology, Mersana Therapeutics, OnCusp Therapeutics, Puma Biotechnology Inc., Seattle Genetics, Silverback Therapeutics, Spectrum Pharmaceuticals, Zentalis, and Chugai Biopharmaceuticals and grants from Aileron Therapeutics, Inc., AstraZeneca, Bayer Healthcare Pharmaceutical, Calithera Biosciences Inc., Curis Inc., CytomX Therapeutics Inc., Daiichi Sankyo Co. Ltd., Debiopharm International, eFFECTOR Therapeutics, Genentech Inc., Guardant Health Inc., Klus Pharma, Takeda Pharmaceutical, Novartis, Puma Biotechnology Inc., and Taiho Pharmaceutical Co. outside the submitted work. N. Uboha reports other support from Taiho, Incyte, Astellas, QED, Pfizer, and AstraZeneca and grants from Ipsen, Eli Lilly, and EMD Serono outside the submitted work. R.E. Sanborn reports other support from CytomX during the conduct of the study as well as other support from AstraZeneca, Amgen, EMD Serono, Blueprint Medicines, Daiichi Sankyo, Lilly, Janssen Oncology, MacroGenics, Sanofi Aventis, Regeneron, Mirati Therapeutics, and Merck outside the submitted work. R.F. Sweis reports grants from CytomX during the conduct of the study as well as grants and personal fees from Astellas, AstraZeneca, BMS, Eisai, Janssen, Mirati, Pfizer, Genentech/Roche, and Novartis; personal fees from EMD Serono, Exelixis, Seattle Genetics, Aveo, Editas, and Aduro Biotech; and grants from AbbVie, Bayer, Eli Lilly, Immunocore, Ascendis Pharma, Merck, Moderna, QED Therapeutics, and Epivax Oncology outside the submitted work. P. LoRusso reports personal fees from AbbVie, Agios, Five Prime, GenMab, Halozyme, Roche-Genentech, Genentech, CytomX, Takeda, SOTIO, Cybrexa, Agenus, Tyme, IQVIA, TRIGR, Pfizer, ImmunoMet, Black Diamond, Glaxo-Smith Kline, QED Therapeutics, AstraZeneca, EMD Serono, Shattuck, Astellas, Salarius, Silverback, MacroGenics, Kyowa Kirin, Kineta, Zentalis, Molecular Templates, ABL Bio, SK Life, STCube, Bayer, I-MAB, Seagen, imCheck, Relay Therapeutics, and Stemline outside the submitted work. M. Nagasaka reports personal fees from AstraZeneca, Caris Life Sciences, Daiichi-Sankyo, Takeda, Novartis, EMD Serono, Blueprint Medicines, Janssen, Pfizer, Lilly, Genentech, and Mirati and personal fees and other support from An Heart outside the submitted work. S. Jalal reports other support from Astex Pharmaceuticals, Tesaro Pharmaceuticals, AstraZeneca, and Adaptimmune during the conduct of the study. J.J. Harding reports grants and personal fees from BMS, CytomX, Eli Lilly, and Zymeworks; personal fees from Eisai, Exelixis, Imvax, Merck, Adaptimmune, QED, HCC Connect, Research to Practice, MORE Health, and Genoscience; and grants from Novartis, Pfizer, Yiviva, Incyte, and Loxo outside the submitted work. S.K. Kim reports consultancy for CytomX, Seattle Genetics, ImmunoGen, AbbVie, and Zymeworks. D.J. Vugts reports grants from CytomX during the conduct of the study; in addition, D.J. Vugts has a patent for US 17/611872 pending. G.A.M.S. van Dongen reports grants from CytomX during the conduct of the study; in addition, G.A.M.S. van

Dongen has a patent for WO 2020/236679A1 issued to CytomX. C.W. Menke van der Houven van Oordt reports other support from CytomX during the conduct of the study. S. Wang reports personal fees from CytomX Therapeutics during the conduct of the study as well as personal fees from CytomX Therapeutics outside the submitted work. V. Paton is an employee of CytomX Therapeutics. A. Hannah reports personal fees from CytomX Therapeutics during the conduct of the study. J.F. Liu reports personal fees from AstraZeneca, Eisai, Clovis, Genentech/Roche, GSK, and Regeneron Pharmaceuticals outside the submitted work. No disclosures were reported by the other authors.

Authors' Contributions

V. Boni: Conceptualization, formal analysis, supervision, investigation, writing—original draft, writing—review and editing. **M.J. Fidler:** Investigation, writing—review and editing. **H.-T. Arkenau:** Investigation, writing—review and editing. **A. Spira:** Investigation, writing—review and editing. **F. Meric-Bernstam:** Investigation, writing—review and editing. **N. Uboha:** Investigation, writing—review and editing. **R.E. Sanborn:** Investigation, writing—review and editing. **R.F. Sweis:** Investigation, writing—review and editing. **P. LoRusso:** Investigation, writing—review and editing. **M. Nagasaka:** Investigation, writing—review and editing. **J. Garcia-Corbacho:** Investigation, writing—review and editing. **S. Jalal:** Investigation, writing—review and editing. **J.J. Harding:** Investigation, writing—review and editing. **S.K. Kim:** Investigation, writing—review and editing. **I.H.C. Miedema:** Investigation, writing—review and editing. **D.J. Vugts:** Investigation, writing—review and editing. **M.C. Huisman:** Investigation, writing—review and editing. **G.J.C. Zwezerijnen:** Investigation, writing—review and editing. **G.A.M.S. van Dongen:** Investigation, writing—review and editing. **C.W. Menke van der Houven van Oordt:** Investigation, writing—review and editing. **S. Wang:** Validation, investigation, writing—original draft, writing—review and editing. **T. Dang:** Data curation,

writing—review and editing. **I.A. Zein:** Data curation, writing—review and editing. **O. Vasiljeva:** Conceptualization, data curation, validation, investigation, writing—original draft, writing—review and editing. **S.K. Lyman:** Conceptualization, data curation, formal analysis, validation, investigation, writing—original draft, writing—review and editing. **V. Paton:** Formal analysis, supervision, writing—original draft, project administration, writing—review and editing. **A. Hannah:** Conceptualization, supervision, validation, writing—original draft, writing—review and editing. **J.F. Liu:** Investigation, writing—review and editing.

Acknowledgments

We are grateful to the patients, families, and staff who made this study possible. We thank Michael Hussey of International Drug Development Institute, Cambridge, MA, and Claire Sherman of CytomX, South San Francisco, CA, for providing biostatistical support. We also thank former employees of CytomX Therapeutics for their contributions: Luc R. Desnoyers and W. Michael Kavanaugh provided intellectual input to the CX-2009 program, including (LRD) the overall architecture of the biomarker strategy and supervision of all translational activities. Mark Stroh analyzed pharmacokinetic data and created the plots seen in Fig. 2. Under author direction, Mark Phillips and Monica Nicosia of Phillips Gilmore Oncology Communications, Inc, Philadelphia, PA provided medical editing and writing assistance, which was funded by CytomX. PROBODY is a registered U.S. trademark of CytomX Therapeutics, Inc.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received October 12, 2021; revised December 11, 2021; accepted February 7, 2022; published first February 11, 2022.

References

- Desnoyers LR, Vasiljeva O, Richardson JH, Yang A, Menendez EEM, Liang TW, et al. Tumor-specific activation of an EGFR-targeting probody enhances therapeutic index. *Sci Transl Med* 2013;5:207ra144.
- Polu KR, Lowman HB. Probody therapeutics for targeting antibodies to diseased tissue. *Expert Opin Biol Ther* 2014;14:1049–53.
- Deu E, Verdoes M, Bogyo M. New approaches for dissecting protease functions to improve probe development and drug discovery. *Nat Struct Mol Biol* 2012;19:9–16.
- Weidle UH, Eggle D, Klostermann S, Swart GWM. ALCAM/CD166: cancer-related issues. *Cancer Genomics Proteomics* 2010;7:231–43.
- Bouchard H, Viskov C, Garcia-Echeverria C. Antibody-drug conjugates—a new wave of cancer drugs. *Bioorg Med Chem Lett* 2014;24:5357–63.
- Guo W, Wang SJ, Yang S, Lynn H, Ji Y. A Bayesian interval dose-finding design addressing Ockham's razor: mTPI-2. *Contemp Clin Trials* 2017;58:23–33.
- Ji Y, Wang SJ. Modified toxicity probability interval design: a safer and more reliable method than the 3 + 3 design for practical phase I trials. *J Clin Oncol* 2013;31:1785–91.
- Jauw YWS, O'Donoghue JA, Zijlstra JM, Hoekstra OS, Menke-van der Houven van Oordt CW, Morschhauser F, et al. (89)Zr-Immuno-PET: toward a noninvasive clinical tool to measure target engagement of therapeutic antibodies in vivo. *J Nucl Med* 2019;60:1825–32.
- Chomet M, Schreurs M, Nguyen M, Howng B, Villanueva R, Krimm M, et al. The tumor targeting performance of anti-CD166 Probody drug conjugate CX-2009 and its parental derivatives as monitored by (89)Zr-immuno-PET in xenograft bearing mice. *Theranostics* 2020;10:5815–28.
- Merkul E, Sijbrandi NJ, Muns JA, Aydin I, Adamzek K, Houthoff H-J, et al. First platinum(II)-based metal-organic linker technology (Lx[®]) for a plug-and-play development of antibody-drug conjugates (ADCs). *Expert Opin Drug Deliv* 2019;16:783–93.
- Ponte JF, Running KL, Kirby M, Chan J, James J, O'Leary JJ, et al. Development of modified dosing approaches to achieve specific pharmacokinetic (PK) objectives in the first-in-human phase I clinical trial of IMGN853, a folate receptor α -targeting antibody drug conjugate [abstract]. In: Proceedings of the 105th Annual Meeting of the American Association for Cancer Research; 2014 Apr 5–9; San Diego, CA. Philadelphia (PA): AACR; *Cancer Res* 2014;74(19 Suppl): Abstract nr4641.
- Hassan R, Blumenschein GR, Moore KN, Santin AD, Kindler HL, Nemunaitis JJ, et al. First-in-human, multicenter, phase I dose-escalation and expansion study of anti-mesothelin antibody-drug conjugate anetumab ravtansine in advanced or metastatic solid tumors. *J Clin Oncol* 2020;38:1824–35.
- Moore KN, Borghaei H, O'Malley DM, Jeong W, Seward SM, Bauer TM, et al. Phase 1 dose-escalation study of mirvetuximab soravtansine (IMGN853), a folate receptor α -targeting antibody-drug conjugate, in patients with solid tumors. *Cancer* 2017;123:3080–7.
- Matulonis UA, Birrer MJ, O'Malley DM, Moore KN, Konner J, Gilbert L, et al. Evaluation of prophylactic corticosteroid eye drop use in the management of corneal abnormalities induced by the antibody-drug conjugate mirvetuximab soravtansine. *Clin Cancer Res* 2019;25:1727–36.
- Donaghy H. Effects of antibody, drug and linker on the preclinical and clinical toxicities of antibody-drug conjugates. *MAbs* 2016;8:659–71.
- Ihnen M, Köhler N, Kersten JF, Milde-Langosch K, Beck K, Höller S, et al. Expression levels of activated leukocyte cell adhesion molecule (ALCAM/CD166) in primary breast carcinoma and distant breast cancer metastases. *Dis Markers* 2010;28:71–8.
- Ihnen M, Müller V, Wirtz RM, Schröder C, Krenkel S, Witzel I, et al. Predictive impact of activated leukocyte cell adhesion molecule (ALCAM/CD166) in breast cancer. *Breast Cancer Res Treat* 2008;112:419–27.
- Stroh M, Li R, Lu H, Wada R, Richardson JH, Frye JW, et al. Preliminary clinical pharmacokinetics and dose-response to support a phase II dose selection for CX-2009: a masked Probody drug conjugate to CD166. *J Clin Oncol* 38: 15s, 2020 (suppl; abstr 3599).
- Stroh M, Sagert J, Burke JM, Apgar JF, Lin L, Millard BL, et al. Quantitative systems pharmacology model of a masked, tumor-activated antibody. *CPT Pharmacometrics Syst Pharmacol* 2019;8:676–84.