Published OnlineFirst June 3, 2021; DOI: 10.1158/1078-0432.CCR-21-0194



# Phase I, First-in-Human Study of the Probody Therapeutic CX-2029 in Adults with Advanced Solid Tumor Malignancies

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## ABSTRACT

**Purpose:** PROCLAIM-CX-2029 is a phase I first-in-human study of CX-2029, a Probody–drug conjugate targeting CD71 (transferrin receptor 1) in adults with advanced solid tumors. Although the transferrin receptor is highly expressed across multiple tumor types, it has not been considered a target for antibody–drug conjugates (ADCs) due to its broad expression on normal cells. CX-2029 is a masked form of a proprietary anti-CD71 antibody conjugated to monomethyl auristatin E, designed to be unmasked in the tumor microenvironment by tumor-associated proteases, therefore limiting off-tumor toxicity and creating a therapeutic window for this previously undruggable target.

**Experimental Design:** This was a dose-escalation, multicenter trial to evaluate the safety, pharmacokinetics, pharmacodynamics, and antitumor activity of CX-2029. The primary endpoint was to determine the maximum tolerated dose (MTD) and cycle 1 dose-limiting toxicity (DLT). CX-2029 was administered i.v. every 3 weeks.

**Results:** Forty-five patients were enrolled in eight dose levels. No DLTs were reported in the dose escalation through 4 mg/kg. At 5 mg/kg, there were two DLTs (febrile neutropenia and pancytopenia). Following expansion of the 4 mg/kg dose to six patients, two additional DLTs were observed (infusion-related reaction and neutropenia/anemia). Both the 4 and 5 mg/kg doses were declared above the maximum tolerated dose. The recommended phase II dose is 3 mg/kg. The most common dose-dependent hematologic toxicities were anemia and neutropenia. Confirmed partial responses were observed in three patients, all with squamous histologies.

**Conclusions:** The Probody therapeutic platform enables targeting CD71, a previously undruggable ADC target, at tolerable doses associated with clinical activity.

## Introduction

Antibody–drug conjugates (ADCs) are monoclonal antibodies (mAbs) conjugated to cytotoxic small molecules through a cleavable linker. ADCs have shown their greatest clinical utility when targeting antigens expressed at high levels primarily on cancer cells, exemplified by trastuzumab emtansine for the treatment of HER2$^+$ breast cancer and brentuximab vedotin for cluster of differentiation (CD) 30-positive Hodgkin's disease and anaplastic large cell lymphoma (1, 2). Other known cell surface antigens highly expressed on cancer cells are potentially attractive as ADC targets, but many of these targets have not been pursued because of concerns regarding their broad expression in normal tissue.

One such target is CD71, also known as transferrin receptor 1 (TfR1). This antigen is highly expressed on rapidly proliferating cancer cells and is involved in the cellular uptake of iron (3). The high expression of CD71 allows tumor cells to meet the iron requirement for proliferation (4, 5). High expression of CD71 has been documented for many human cancers including cervical, breast, esophageal, pancreatic, and renal cancer (6–10). Upon internalization and trafficking to low pH endosomes where iron is released, CD71 rapidly recycles back to the cell surface and as such is a highly efficient delivery system. Furthermore, CD71 cannot be readily downregulated by cancer cells because they depend on transferrin-mediated iron uptake for their growth and proliferation. These features can be utilized to enable efficient delivery of cytotoxic payloads into cancer cells via CD71-based ADCs. However, the widespread expression of CD71 on many normal human tissues, including erythroid precursor cells and other cells with a high iron requirement, leads to on-target off-tumor toxicity and has precluded the development of CD71-directed ADCs to date.

Probody therapeutic candidates use a novel platform designed to improve the safety and tolerability profile of mAbs, ADCs, and other antibody-based therapies. These fully recombinant mAb prodrugs comprise an active antibody (or antibody fragment) component, a masking peptide, and a protease-cleavable substrate linker peptide. Probody therapeutic candidates remain largely intact as an inactive prodrug in circulation, and by exploiting the dysregulated protease activity in the tumor microenvironment (TME), the masking peptide linker is cleaved allowing antibody binding to the target antigen expressed on tumor (11, 12). The potential for improved safety and

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

**Previous Presentations:** These study results were presented in part at the American Society of Clinical Oncology (ASCO) Virtual Meeting, May 29, 2020; the American Society of Clinical Oncology (ASCO) Annual Meeting, May 31–June 4, 2019; Chicago, IL USA.

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Clin Cancer Res 2021;XX:XX–XX

**doi:** 10.1158/1078-0432.CCR-21-0194

Published OnlineFirst June 3, 2021; DOI: 10.1158/1078-0432.CCR-21-0194



## Translational Relevance

The Probody therapeutic platform is an innovative approach to target tumor antigens that are difficult to target due to safety concerns around expression in normal tissues with resulting on-target, off-tumor activity. Despite high levels of CD71 expression across a wide variety of tumor types, attempts to target this antigen have previously been unsuccessful due to safety concerns related to normal tissue expression. CX-2029 is a Probody–drug conjugate targeting CD71 (transferrin receptor 1) designed to be unmasked in the tumor microenvironment by tumor-associated proteases. In this article, we established the safety profile and a recommended dose for future study of CX-2029 as a novel cancer therapeutic targeting CD71, a previously undruggable antibody–drug conjugate target. Our findings demonstrate promising clinical activity at tolerable doses in patients with squamous histologies supporting the continued clinical development of CX-2029 in patients with advanced malignancies.

tolerability with Probody therapeutics may allow for dose optimization and more tolerable combination therapies, allowing us to drug the undruggable.

CX-2029 is a CD71-targeting Probody–drug conjugate (PDC) composed of a protease-activatable antibody prodrug targeting the TfR1 conjugated to the cytotoxin monomethyl auristatin E (MMAE) via a cysteine protease-cleavable dipeptide linker, valine–citrulline (vc), with a drug–antibody ratio of 2 (13). The antibody prodrug portion of CX-2029 is derived from a humanized mAb. In its intact prodrug form, both light chains of CX-2029 are modified at their N-terminus by recombinant inclusion of a 47-amino acid prodomain, which serves to mask the target-binding region of the antibody. Cleavage of the prodomain by proteases, commonly active in the TME, releases the mask and yields the activated form of CX-2029. The mechanism of action for CX-2029 involves binding of the activated PDC to its target antigen on the cell surface and as seen with other vcMMAE ADCs, receptor-mediated internalization, and lysosomal processing of the activated PDC, which releases the cytotoxin (14). Auristatin toxins including MMAE are microtubule-inhibiting agents that induce cell-cycle ($G_2$–M) arrest of dividing cells, resulting in cell death (15). MMAE-containing ADCs have been successfully developed in the treatment of Hodgkin lymphoma and anaplastic large cell lymphoma (brentuximab vedotin; refs. 1, 16), relapsed/refractory diffuse large B-cell lymphoma (polatuzumab vedotin-piiq; ref. 17), and advanced bladder cancer (enfortumab vedotin-ejfv; ref. 18).

In studies of multiple mouse cell line–derived and human patient–derived xenograft tumor models, one to two dose administrations every 2 weeks of CX-2029 between 3 and 6 mg/kg produced complete regression and durable responses. These models included lung, breast, ovarian, gastric, esophageal, colorectal, head and neck, diffuse large B-cell lymphoma, pancreatic, and prostate cancers, as well as multiple myeloma and mesothelioma (19). CX-2029 exhibited cross-reactivity with cynomolgus monkey CD71 and was tolerated in monkeys at doses of up to 6 mg/kg (the human equivalent, 2 mg/kg after adjusting for body surface area). In contrast, the unmasked CD71 ADC was lethal at low doses (2 mg/kg) in cynomolgus monkeys due to principally neutropenic sepsis but tolerated at 0.6 mg/kg. Whereas the human equivalent of a tolerable dose of the unmasked CD71 ADC (0.2 mg/kg after adjusting for body surface area) is well below its projected biologically active dose (2 mg/kg), the projected biologically active dose of CX-2029 in humans was similar to its recommended phase II dose (RP2D).

These preclinical results suggest that CX-2029 may offer significant potential as a novel therapeutic approach to harness the potential of CD71 as an ADC target and address currently unmet medical needs in patients across multiple cancer types. PROCLAIM-CX-2029 (NCT03543813), the first-in-human study of CX-2029, evaluated tolerability and preliminary antitumor efficacy of CX-2029 in patients with advanced, unresectable solid tumors. Here, we report safety and efficacy data with CX-2029 monotherapy during dose-escalation phase of the trial.

## Materials and Methods

### Study design

In this open-label study, adult patients with metastatic or locally advanced unresectable solid tumors without approved life-prolonging treatment options were recruited at 12 sites in the United States, Spain, and the United Kingdom. The primary objective of the dose-escalation phase was to determine an MTD and RP2D.

In the dose-escalation phase, patients received CX-2029 (0.1, 0.25, 0.5, 1, 2, 3, 4, or 5 mg/kg) i.v. over 90 minutes [later increased to 180 minutes to mitigate infusion-related reactions (IRR)] every 3 weeks. Due to the novelty of this target and unknown potential for toxicity, the starting dose of CX-2029 was 1/20th rather than the typical 1/6th of the highest nonseverely toxic dose in monkeys. Starting at 2 mg/kg (provisionally set by nonclinical data observed in the cynomolgus monkey), additional patients were enrolled to characterize the protease activity and measure the cleavage of CX-2029 in tumor biopsies and peripheral blood. The dose-limiting toxicity (DLT) assessment period was 21 days. Although the trial was planned to initiate with single-patient cohorts, the first dose level was expanded to three patients due to observation of a grade 3 IRR. Dose escalation thereafter followed standard 3+3 dose-escalation rules. A continual reassessment method was included in the protocol to begin at 3 mg/kg; however, as no cycle 1 DLTs were observed up to and including the 4 mg/kg dose level, this methodology was not implemented. The study incorporated a Safety Review Committee (SRC), which was composed of active CX-2029 study investigators and the sponsor. An independent Data Safety Monitoring Board reviewed data from the on-going trial at regular intervals of no less than every 6 months.

Adverse events (AEs) were evaluated from the start of treatment until 30 days after the final treatment and were coded using Medical Dictionary for Regulatory Activities terminology (version 22.0) and graded using the National Cancer Institute's Common Terminology Criteria for Adverse Events (version 5.0). Serum hematology and biochemistry were evaluated weekly. Physical examination, vital signs, and electrocardiograms were assessed at regular intervals throughout the study. Tumor biopsies were collected during the screening period (baseline) and 3 to 5 days after the first dose of CX-2029 (if the patient consented to this procedure).

### Population

Eligible candidates were aged ≥18 years with Eastern Cooperative Oncology Group (ECOG) performance status 0 or 1, with archival tissue or biopsy available for tissue analyses, adequate organ and bone marrow function, and an anticipated life expectancy of ≥3 months. Patients with brain metastasis were allowed if they were clinically stable. Patients in the dose-escalation phase were required to have metastatic or advanced unresectable solid tumor (measurable or nonmeasurable disease) for whom there was no approved therapy

Published OnlineFirst June 3, 2021; DOI: 10.1158/1078-0432.CCR-21-0194



with life-prolonging benefit or who were intolerant or not suitable for other therapy. CD71 positivity by immunohistochemistry (IHC) was not required for study entry; tumor tissue (archival or fresh biopsy during the screening period) was requested of all patients. Tumor types for patients enrolled in the mandatory on-study biopsy cohorts were limited to head and neck squamous cell carcinoma (HNSCC), non–small cell lung carcinoma (NSCLC, squamous cell histology only), or esophageal carcinoma (measurable disease by RECIST v1.1 was required). These additional patients with selected tumor types were enrolled into dose levels previously declared to be safe by the SRC and were limited to dose levels of 2 and 3 mg/kg.

Exclusion criteria included neuropathy grade >1; significant cardiac disease; HIV-related illness; hepatitis B or C; solid organ or bone marrow transplant; prior or current autoimmune disease; unresolved grade >1 acute toxicity from prior anticancer therapy; moderate or severe hepatic impairment (Child–Pugh B or C); and severe renal impairment (creatinine clearance < 30 mL/min). Patients were excluded if they demonstrated transfusion-dependent anemia requiring transfusion of at least one unit of packed red blood cells required every 90 days to maintain hemoglobin >10 g/dL over the past 12 months; use of iron chelators and patients with a history of clinically significant iron metabolism disorder (e.g., sickle cell anemia) were also excluded. This study was conducted in accordance with the Institutional Review Board or independent ethics committee–approved protocol, and International Conference on Harmonisation Good Clinical Practice Guidelines. All patients provided written informed consent.

### Outcomes

Safety and tolerability parameters included DLT, AEs, physical examination, triplicate electrocardiograms, vital signs, and laboratory assessments.

DLT was defined as grade 4 treatment-related AEs (TRAE; unless <7 days or without clinical sequelae) or grade 3 TRAEs (with exceptions including transient uncomplicated hematologic abnormalities, electrolyte abnormalities, gastrointestinal toxicity resolving < 72 hours, fatigue, or tumor flare) occurring during the first cycle of treatment (21 days). In addition, an IRR lasting greater than 6 hours was also considered a DLT. Events meeting DLT criteria occurring after the first cycle of treatment were categorized as "delayed DLT" and were also considered for the determination of the RP2D.

Primary endpoints were to evaluate the safety and tolerability of multiple doses of CX-2029 and to determine the MTD and DLTs. Secondary endpoints were overall response rate (ORR; defined according to RECIST v1.1) and duration of response (DOR; defined as the time from first documentation of objective response to first documented disease progression by RECIST v1.1 or death from any cause). Disease control was defined as a confirmed objective response by RECIST v1.1 or stable disease of at least 16-week (±7 days) duration. CT or MRI scans were performed to evaluate tumor response every 8 weeks for 12 months, and every 12 weeks thereafter until progressive disease.

### Pharmacokinetics

Blood samples were obtained at various time points before and after CX-2029 administration for pharmacokinetics (PKs) testing (Supplementary Methods). The following analytes were measured in human plasma via validated LC-MS/MS assays (Supplementary Methods): (1) Intact CX-2029 (referring to the masked prodrug form of CX-2029 ± MMAE); (ii) conjugated MMAE (pc-MMAE, Probody-conjugated MMAE); (iii) unconjugated MMAE (free MMAE); and (iv) Total CX-2029 (intact and activated forms of CX-2029 ± MMAE). CD71 was assessed by a single central pathology laboratory by IGC. CD71 expression was defined by overall tumor staining using a proprietary antibody. Given the rapid recycling of this cell surface receptor, both membranous and overall/cytoplasmic expressions were characterized. High CD71 expression was defined as IHC staining of ≥50% tumor cells at 2+/3+ intensity for overall staining; low expression was defined as 1+ (Supplementary Methods).

### Statistical analyses

Response-evaluable population (REP) was defined as all patients in the safety analysis population who have adequate disease assessments with measurable disease by RECIST at both baseline and at least one postbaseline assessment. The REP was used for efficacy analyses related to objective response, including ORR, time to response, and DOR. Time to tumor response and DOR were summarized for patients with a confirmed response.

## Results

### Patient population

Patients were enrolled in the dose-escalation phase of the study between June 18, 2018, and March 12, 2020; data are reported as of August 14, 2020. A total of 45 patients were enrolled at the following dose levels: CX-2029 0.1 mg/kg ($n = 3$), 0.25 mg/kg ($n = 3$), 0.5 mg/kg ($n = 6$), 1 mg/kg ($n = 3$), 2 mg/kg ($n = 8$), 3 mg/kg ($n = 12$), 4 mg/kg ($n = 6$), and 5 mg/kg ($n = 4$), of these 45 patients, nine were entered into the mandatory biopsy cohorts of 2 mg/kg ($n = 3$) and 3 mg/kg ($n = 6$). Reasons for treatment discontinuation in the dose-escalation phase were disease progression [$n = 30$ (68%)], withdrawal by patient [$n = 6$ (13%)], symptomatic deterioration [$n = 3$ (7%)], AE [$n = 2$ (4%)], and investigator decision [$n = 1$ (2%)]. Baseline patient characteristics are presented in **Table 1**. The median age was 60 years, and there were more males than females (62% vs. 38%, respectively). The most common tumor types included NSCLC [adenocarcinoma ($n = 5$) and squamous cell carcinoma ($n = 4$)], HNSCC ($n = 8$), and colorectal cancer ($n = 7$). Patients had received a median of three prior to cancer regimens (range, 1–16). Most patients had an ECOG performance status of 1. CD71 expression was high in 38%, low in 46%, and unknown in 16% of patients (tissue unavailable).

### Safety

There were 45 patients dosed up to and including 5 mg/kg with CX-2029 in the dose-escalation phase of Study CTMX-M-2029-001. Following Data and Safety Monitoring Board input, dose level 0.5 mg/kg was expanded to six patients in order to assess the effects of premedications on the incidence and severity of IRRs. No DLTs were reported in the initial dose escalation through 4 mg/kg. At 5 mg/kg, there were two DLTs (febrile neutropenia and pancytopenia of greater than 7 days' duration). Following expansion of the 4 mg/kg dose level to six patients, two additional DLTs were observed (IRR lasting greater than 6 hours; grade 4 neutropenia with grade 3 anemia leading to delay of initiation of cycle 2). Both 4 and 5 mg/kg dose levels were declared above the MTD by the SRC. Dose levels of 2 and 3 mg/kg were subsequently expanded in order to better evaluate drug-related toxicity at these doses (to 8 and 12 patients, respectively). Following assessment of these two dose levels, the SRC declared the RP2D to be 3 mg/kg every 3 weeks, based on several safety parameters: there were no cycle 1 DLTs, no discontinuations due to toxicity, and the long-term

Published OnlineFirst June 3, 2021; DOI: 10.1158/1078-0432.CCR-21-0194

**Table 1.** Demographics and baseline characteristics—CX-2029 monotherapy (dose escalation parts A and B) safety analysis population.

| | Dose CX-2029 (mg/kg) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **0.1 (*N* = 3)** | **0.25 (*N* = 3)** | **0.5 (*N* = 6)** | **1 (*N* = 3)** | **2 (*N* = 8)** | **3 (*N* = 12)** | **4 (*N* = 6)** | **5 (*N* = 4)** | **All cohorts (*N* = 45)** |
| Median age at enrollment, years (min, max) | 45 (31, 72) | 52 (44, 53) | 59 (38, 67) | 48 (47, 49) | 67 (45, 74) | 66 (52, 75) | 67 (44, 75) | 64 (53, 73) | 60 (31, 75) |
| Sex, *n* (%) | | | | | | | | | |
| Male | 0 | 1 (33%) | 5 (83%) | 3 (100%) | 5 (63%) | 9 (75%) | 4 (67%) | 1 (25%) | 28 (62%) |
| Female | 3 (100%) | 2 (67%) | 1 (17%) | 0 | 3 (38%) | 3 (25%) | 2 (33%) | 3 (75%) | 17 (38%) |
| Race, *n* (%) | | | | | | | | | |
| Asian | 0 | 0 | 0 | 0 | 1 (13%) | 1 (8%) | 0 | 0 | 2 (4%) |
| Black or African American | 0 | 0 | 2 (33%) | 0 | 1 (13%) | 0 | 0 | 0 | 3 (7%) |
| White | 3 (100%) | 3 (100%) | 4 (67%) | 3 (100%) | 6 (75%) | 9 (75%) | 6 (100%) | 4 (100%) | 38 (84%) |
| Unknown | 0 | 0 | 0 | 0 | 0 | 2 (17%) | 0 | 0 | 2 (4%) |
| Number of prior cancer treatment regimens, median (min, max) | 3 (3, 5) | 4 (1, 8) | 6 (2, 7) | 4 (1, 7) | 4 (1, 5) | 3 (1, 7) | 4 (2, 6) | 3 (3, 16) | 3 (1, 16) |
| Median time since last prior cancer treatment end, weeks (min, max) | 6 (5, 17) | 4 (3, 126) | 6 (3, 16) | 6 (4, 49) | 9 (4, 53) | 7 (5, 52) | 10 (3, 81) | 8 (5, 10) | 7 (3, 126) |
| Cancer type, *n* (%) | | | | | | | | | |
| Adenoid cystic carcinoma of parotid gland | 0 | 0 | 1 (17%) | 0 | 0 | 0 | 1 (17%) | 0 | 2 (4%) |
| Colorectal cancer | 1 (33%) | 2 (67%) | 0 | 1 (33%) | 0 | 0 | 2 (33%) | 1 (25%) | 7 (16%) |
| Cutaneous melanoma | 0 | 0 | 0 | 0 | 0 | 1 (8%) | 0 | 0 | 1 (2%) |
| Endometrial cancer | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 (25%) | 1 (2%) |
| Head and neck squamous cell cancer | 0 | 0 | 0 | 0 | 3 (38%) | 5 (42%) | 0 | 0 | 8 (18%) |
| Hepatocellular carcinoma | 0 | 0 | 0 | 0 | 1 (13%) | 0 | 0 | 0 | 1 (2%) |
| Mesothelioma | 0 | 0 | 0 | 0 | 0 | 0 | 1 (17%) | 0 | 1 (2%) |
| NSCLC | 1 (33%) | 0 | 0 | 1 (33%) | 3 (38%) | 3 (25%) | 0 | 1 (25%) | 9 (20%) |
| Adenocarcinoma[a] | 1 | 0 | 0 | 0 | 3 | 1 | 0 | 0 | 5 |
| Squamous[a] | 0 | 0 | 0 | 1 | 0 | 2 | 0 | 1 | 4 |
| Ocular melanoma | 0 | 0 | 1 (17%) | 0 | 0 | 0 | 0 | 0 | 1 (2%) |
| Oncocytic carcinoma of parotid gland | 0 | 0 | 1 (17%) | 0 | 0 | 0 | 0 | 0 | 1 (2%) |
| Ovarian cancer | 1 (33%) | 0 | 0 | 0 | 0 | 0 | 0 | 1 (25%) | 2 (4%) |
| Pancreatic cancer | 0 | 0 | 0 | 0 | 0 | 1 (8.3%) | 0 | 0 | 1 (2%) |
| Perivascular epithelioid cell tumor | 0 | 0 | 0 | 0 | 1 (13%) | 0 | 0 | 0 | 1 (2%) |
| Prostate cancer | 0 | 0 | 1 (17%) | 0 | 0 | 1 (8.3%) | 1 (17%) | 0 | 3 (7%) |
| Soft-tissue sarcoma | 0 | 1 (33%) | 2 (33%) | 0 | 0 | 1 (8.3%) | 0 | 0 | 4 (9%) |
| Thymoma or thymic cancers | 0 | 0 | 0 | 1 (33%) | 0 | 0 | 0 | 0 | 1 (2%) |
| Thyroid carcinoma | 0 | 0 | 0 | 0 | 0 | 0 | 1 (17%) | 0 | 1 (2%) |
| CD71 (overall) status at baseline, *n* (%) | | | | | | | | | |
| High | 3 (100%) | 2 (67%) | 1 (17%) | 1 (33%) | 2 (25%) | 5 (42%) | 2 (33%) | 1 (25%) | 17 (38%) |
| Low | 0 | 0 | 5 (83%) | 2 (67%) | 5 (63%) | 3 (25%) | 3 (50%) | 3 (75%) | 21 (47%) |
| Unknown | 0 | 1 (33%) | 0 | 0 | 1 (13%) | 4 (33%) | 1 (17%) | 0 | 7 (16%) |

Note: Percentages are based on the Safety Analysis Population.

[a]Lung cancer histology was not initially captured in the database but was verified as part of a *post hoc* analysis.

Published OnlineFirst June 3, 2021; DOI: 10.1158/1078-0432.CCR-21-0194



**Table 2.** Treatment-emergent adverse events occurring in ≥10% of patients.

| | Dose CX-2029 (mg/kg) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Preferred term, *n* (%)** | **0.1 (*N* = 3)** | **0.25 (*N* = 3)** | **0.5 (*N* = 6)** | **1 (*N* = 3)** | **2 (*N* = 8)** | **3 (*N* = 12)** | **4 (*N* = 6)** | **5 (*N* = 4)** | **All cohorts (*N* = 45)** |
| Patients with at least one treatment-emergent adverse event | 3 (100) | 3 (100) | 6 (100) | 3 (100) | 8 (100) | 12 (100) | 6 (100) | 4 (100) | 45 (100) |
| IRR | 2 (67) | 3 (100) | 5 (83) | 3 (100) | 8 (100) | 9 (75) | 6 (100) | 3 (75) | 39 (87) |
| Anemia | 0 | 0 | 2 (33) | 2 (67) | 7 (88) | 11 (92) | 5 (84) | 3 (75) | 30 (67) |
| Nausea | 0 | 2 (67) | 3 (50) | 0 | 2 (25) | 5 (42) | 3 (50) | 1 (25) | 16 (36) |
| Decreased appetite | 0 | 0 | 0 | 1 (33) | 0 | 6 (50) | 3 (50) | 2 (50) | 12 (27) |
| Fatigue | 0 | 0 | 1 (17) | 1 (33) | 2 (25) | 4 (33) | 2 (33) | 2 (50) | 12 (27) |
| Constipation | 1 (33) | 2 (67) | 1 (17) | 0 | 1 (13) | 2 (17) | 1 (17) | 0 | 8 (18) |
| Neutropenia | 0 | 0 | 2 (33) | 0 | 0 | 3 (25) | 1 (17) | 2 (50) | 8 (18) |
| Vomiting | 1 (33) | 0 | 1 (17) | 0 | 0 | 4 (33) | 1 (17) | 1 (25) | 8 (18) |
| Dyspnea | 0 | 1 (33) | 1 (17) | 0 | 1 (13) | 3 (25) | 0 | 1 (25) | 7 (16) |
| Headache | 2 (67) | 0 | 1 (17) | 0 | 1 (13) | 0 | 1 (17) | 2 (50) | 7 (16) |
| Dizziness | 0 | 0 | 0 | 1 (33) | 0 | 3 (25) | 0 | 2 (50) | 6 (13) |
| White blood cell count decreased | 0 | 0 | 0 | 0 | 0 | 2 (17) | 2 (33) | 2 (50) | 6 (13) |
| Aspartate aminotransferase increased | 0 | 0 | 0 | 0 | 2 (25) | 2 (17) | 0 | 1 (25) | 5 (11) |
| Asthenia | 0 | 0 | 0 | 0 | 0 | 4 (33) | 0 | 1 (25) | 5 (11) |
| Back pain | 1 (33) | 0 | 0 | 0 | 1 (13) | 3 (25) | 0 | 0 | 5 (11) |
| Neutrophil count decreased | 0 | 0 | 0 | 0 | 0 | 1 (8) | 2 (33) | 2 (50) | 5 (11) |

tolerability of this dose appeared to be acceptable for chronic administration with supportive care for anemia. The median duration of CX-2029 treatment in all patients was 9 weeks.

No CX-2029 treatment–related deaths were reported. Treatment-emergent AEs (TEAEs) occurring in ≥10% of patients are summarized in **Table 2**. The most common TEAEs were IRR (87%), anemia (67%), nausea (36%), decreased appetite (27%), and fatigue (27%). At 3 mg/kg of CX-2029, any grade TRAEs reported in ≥10% patients included IRRs (75%), anemia (75%), nausea (33%), vomiting (25%), neutropenia (25%), white blood cell count decreased (17%), hypoxia (17%), and fatigue (17%). IRRs, although the most common treatment-related toxicity, were most frequently grades 1 to 2 in severity, associated with the first or second infusion, and successfully treated in all but one patient with prolonging the time of infusion and administration of premedications (added by protocol amendment) including acetaminophen, antihistamines, corticosteroids, and meperidine.

The most frequently reported grade ≥3 TEAEs related to CX-2029 were anemia (51%), neutropenia (25%), and leukopenia (11%) as listed in **Table 3**. These events were most frequently reported at CX-2029 doses of 4 and 5 mg/kg. At 3 mg/kg of CX-2029, related grade ≥ 3 TEAEs were reported in 67% of patients and were predominantly hematologic events with related grade ≥3 TEAE of anemia reported in 58% and neutropenia in 33% of patients. Overall, TEAEs leading to CX-2029 discontinuation were infrequent: One patient treated at 0.25 mg/kg discontinued treatment due to pain related to progressive disease that was unrelated to CX-2029 and one patient treated at 0.1 mg/kg with metastatic involvement of the lung discontinued treatment due to hypoxia in the setting of an IRR and clinical disease progression.

Anemia was a dose-dependent toxicity, more frequently reported at CX-2029 doses of 2 mg/kg or higher. The most common delayed DLT (occurring cycle 2 and beyond) was grade 3 anemia, with occasional reports of grade 3 neutropenia and one grade 4 febrile neutropenia in the absence of growth factor support were also reported. Red cell transfusion was reported in 26 (58%) patients, with a median of two transfusions (range, 1–13), and a median time from first infusion to transfusion was 36 days (range, 15–59). At a starting dose of 3 mg/kg, drug-related anemia was reported in 11 of the 12 patients of which

**Table 3.** Grade ≥3 treatment-emergent adverse events related to CX-2029.

| | Dose CX-2029 (mg/kg) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Preferred term, *n* (%)** | **0.1 (*N* = 3)** | **0.25 (*N* = 3)** | **0.5 (*N* = 6)** | **1 (*N* = 3)** | **2 (*N* = 8)** | **3 (*N* = 12)** | **4 (*N* = 6)** | **5 (*N* = 4)** | **All cohorts (*N* = 45)** |
| Patients with at least one grade ≥3 treatment-emergent adverse event | 1 (33) | 0 | 2 (33) | 1 (33.3) | 6 (75) | 8 (67) | 5 (83) | 4 (100) | 27 (60) |
| Anemia | 0 | 0 | 0 | 1 (33%) | 6 (75) | 7 (58%) | 5 (83) | 4 (100) | 23 (51) |
| Neutropenia | 0 | 0 | 1 (17) | 0 | 0 | 4 (33%) | 3 (50) | 3 (75) | 11 (25) |
| Leukopenia | 0 | 0 | 0 | 0 | 0 | 1 (8%) | 2 (33) | 2 (50) | 5 (11) |
| IRR | 1 (33) | 0 | 0 | 0 | 1 (13) | 0 | 1 (17) | 0 | 3 (7) |
| Acute myocardial infarction | 0 | 0 | 1 (17) | 0 | 0 | 0 | 0 | 0 | 1 (2) |
| Hypoxia | 1 (33) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 (2) |
| Lymphopenia | 0 | 0 | 0 | 0 | 1 (13) | 0 | 0 | 0 | 1 (2) |
| Thrombocytopenia | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 (25) | 1 (2) |

Published OnlineFirst June 3, 2021; DOI: 10.1158/1078-0432.CCR-21-0194



seven patients experienced grade 3 anemia (there were no reports of grade 4–5 anemia). The median time to onset of anemia was 42 days but has occurred as early as 6 days following the first CX-2029 infusion. Anemia led to CX-2029 dose reduction and dose delay in three and four patients, respectively. No events of anemia led to discontinuation of CX-2029. At 3 mg/kg CX-2029, 10 of the 12 patients received at least one red cell transfusion. Of note, an analysis of hemoglobin and reticulocytes divides the dose range into three groups: at doses of 0.1 to 0.25 mg/kg, there is little change in either hemoglobin or reticulocytes; at doses of 0.5 to 2 mg/kg, there is a decrease in hemoglobin levels with a reactive increase in reticulocytes; and at doses of 3 to 5 mg/kg, there is a decrease in hemoglobin levels without a reactive increase in reticulocytes, suggesting an effect on erythropoiesis (Supplementary Fig. S1A–S1C). To date, two patients received darbepoetin with a resultant reduced frequency of packed red blood cell transfusions; continued investigation into the utility of darbepoetin or erythropoietin to treat anemia is planned.

### Pharmacokinetics

Preliminary single-dose CX-2029 PK data (**Fig. 1**) suggested no trends from dose proportionality following 0.25 to 5.0 mg/kg. Following 0.25 to 5.0 mg/kg, CX-2029 circulated predominantly as intact CX-2029 (>90%), whereas free MMAE circulated at no greater than 4.3% of total CX-2029. The median terminal half-life spanned 2.3 to 5.8 days. Paired biopsies were obtained in only five patients (dosed at 2–3 mg/kg). Intact/masked CX-2029 was detected in all five samples using a capillary immunoelectrophoresis assay; however, given the low doses administered, the amount of activated CX-2029 fell below the assay quantification limit. Work is ongoing to improve the understanding of the pharmacology of CX-2029, including studies aimed at understanding what tumor environment is optimal for activation of the Probody therapeutic.

### Antitumor activity

Among the 45 patients who were treated, 37 patients were in the REP (**Table 4**). Three of four patients with squamous NSCLC had stable disease (SD) or better including two confirmed partial responses (PRs) (3 mg/kg, $n = 1$; 5 mg/kg, $n = 1$), and seven of eight patients with HNSCC had SD or better including one confirmed PR at 3 mg/kg and one prolonged SD ongoing at approximately 25 weeks as of this data cutoff. In addition, one patient with ocular melanoma treated at 0.5 mg/kg had durable SD lasting up to 36 weeks. Of the three responders, CD71 expression was considered high in the patient with HNSCC and low/unknown in both patients with squamous NSCLC. All patients had received standard-of-care treatment for their malignancy. Clinical activity by target lesion reduction was observed at doses of 2 mg/kg and higher, all in patients with squamous cell histology. Among those treated at 3 mg/kg, disease control was observed in five of the 10 response-evaluable patients (50%; 95% confidence interval, 18.7%–81.3%). Best percent change in target lesion size from baseline over time, along with tumor type among the response-evaluable patients treated at doses ≥2 mg/kg, is presented in **Fig. 2A**. Among responders, decreases in tumor size from baseline were observed in the first 8 to 16 weeks (**Fig. 2B**). No responses were seen in patients treated at doses ≤1 mg/kg (**Fig. 2C**). CT scans of responding patients treated at 3 mg/kg are presented in Supplementary **Fig. S2A** and **S2B**. Of note, both patients had been dose reduced from 3 to 2 mg/kg, and

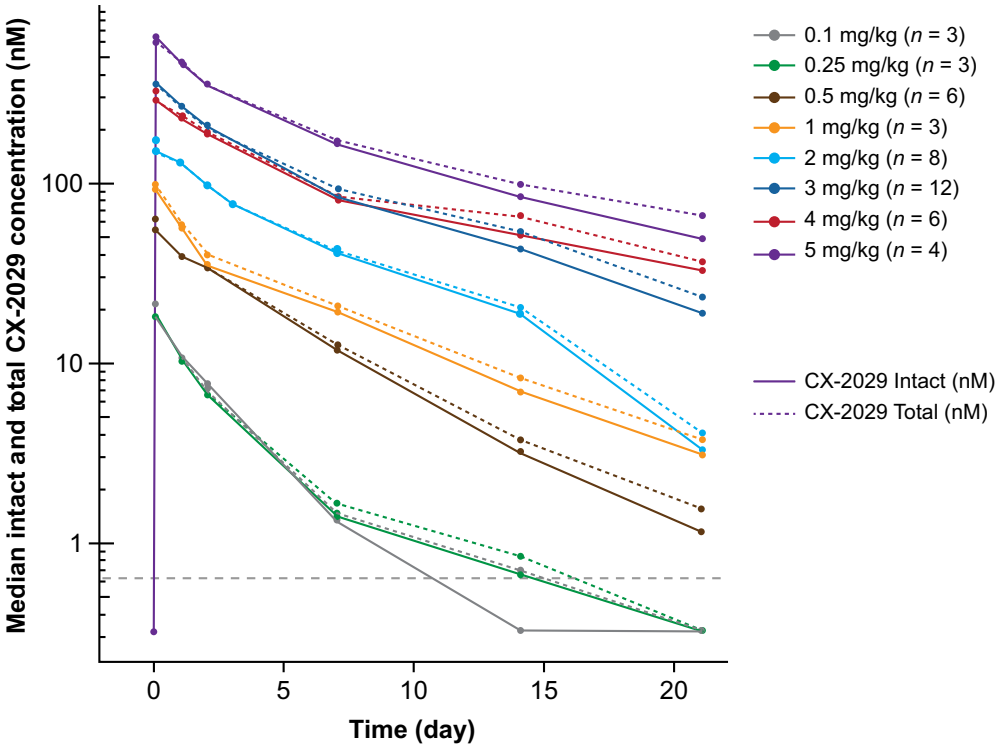


**Figure 1.**
Preliminary dose 1 intact and total CX-2029 median plasma concentrations versus time following administration of up to 5 mg/kg CX-2029.

Published OnlineFirst June 3, 2021; DOI: 10.1158/1078-0432.CCR-21-0194



**Table 4.** Antitumor activity—response-evaluable population.

| | Dose CX-2029 (mg/kg) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Category | 0.1 (*N* = 3) | 0.25 (*N* = 2) | 0.5 (*N* = 6) | 1 (*N* = 3) | 2 (*N* = 7) | 3 (*N* = 10) | 4 (*N* = 4) | 5 (*N* = 2) | All cohorts (*N* = 37) |
| Confirmed partial response, *n* (%) | | | | | | 2 (20.0) | | 1 (50.0) | 3 (8.1) |
| 95% CI | | | | | | 2.5–55.6 | | 1.3–98.7 | 1.7–21.9 |
| Stable disease, *n* (%) | 1 (33.3) | | 4 (66.7) | 1 (33.3) | 3 (42.9) | 4 (40.0) | | | 13 (35.1) |
| 95% CI | 0.8–90.6 | | 22.3–95.7 | 0.8–90.6 | 9.9–81.6 | 12.2–73.8 | | | 20.2–52.5 |
| Progressive disease, *n* (%) | 2 (66.7) | 2 (100.0) | 2 (33.3) | 2 (66.7) | 3 (42.9) | 4 (40.0) | 4 (100.0) | 1 (50.0) | 20 (54.1) |
| 95% CI | 9.4–99.2 | 15.8–100.0 | 4.3–77.7 | 9.4–99.2 | 9.9–81.6 | 12.2–73.8 | 39.8–100.0 | 1.3–98.7 | 36.9–70.5 |
| Not evaluable, *n* (%) | | | | | 1 (14.3) | | | | 1 (2.7) |
| 95% CI | | | | | 0.4–57.9 | | | | 0.1–14.2 |
| Disease control,[a] *n* (%) | | | 3 (50.0) | 1 (33.3) | 2 (28.6) | 5 (50.0) | | 1 (50.0) | 12 (32.4) |
| 95% CI | | | 11.8–88.2 | 0.8–90.6 | 3.7–71.0 | 18.7–81.3 | | 1.3–98.7 | 18.0–49.8 |

Abbreviation: CI, confidence interval.

[a]Disease control was defined as a confirmed objective response by RECIST v1.1 or stable disease of at least 16-week (±7 days) duration.

target lesions measurements remained either stable or continued to decrease at this dose.

## Discussion

CX-2029 is a first-in-class PDC targeting CD71 (TfR1), a previously undruggable, highly expressed cancer antigen involved in cellular iron transport that is known to be broadly expressed on normal cells (20). The dose-escalation portion of this first-in-human study provides preliminary evidence of the tolerability and clinical activity of CX-2029 monotherapy in previously treated patients with advanced or metastatic solid tumors.

A dose range of 2 to 4 mg/kg (equivalent to a human dose of approximately 1–2 mg/kg) had been predicted by monkey toxicology studies and nonclinical PK as likely to be biologically active. Dose escalation was uncomplicated up to 3 mg/kg. Although the SRC primarily used safety data to determine the choice of 3 mg/kg as the RP2D, the choice of this dose level was further supported by PK data that suggested that a typical patient receiving a dose of ≥2 mg/kg CX-2029 every 3 weeks would maintain a targeted drug level throughout the 3-week dosing interval, based on nonclinical data and systems pharmacology modeling (21).

Toxicity with traditional MMAE ADCs includes anemia, neutropenia, thrombocytopenia as well as MMAE-associated neuropathy. Although severe drug-related neuropathy was not reported in this study (observations of neuropathy were restricted to one patient treated at a dose of 1 mg/kg with one episode of grade 1 paraesthesias), the median duration of treatment may have precluded observation of this toxicity (this may also be due to a lower drug–antibody ratio for CX-2029: 2 compared with 3.5–4 for other approved ADCs with MMAE as a payload). MMAE-associated hematologic toxicity was however the primary toxicity observed upon treatment with CX-2029, occurring in a dose-dependent manner. CX-2029 IRRs were not dose dependent. They were mostly grades 1 and 2 in severity and resolved 2 to 3 hours after initiation of supportive care measures.

Anemia related to CX-2029 is of interest. This was initially observed in the toxicology studies prior to this clinical study (decreases in red blood cell parameters, including RBC mass and reticulocytes, were observed at a dose of 6 mg/kg in cynomolgus monkeys, equivalent to a human dose of 2 mg/kg). The etiology of anemia remains under investigation and is likely multifactorial in nature: CD71 is expressed in erythroid precursors in the bone marrow (22); MMAE (the cytotoxic payload conjugated to the CD71-targeted antibody) is also associated with anemia (23). Investigation into the utility of darbepoetin or erythropoietin to treat anemia is ongoing. Early data on a dose-dependent lack of reticulocytosis may support a bone marrow component to the observed anemia.

Although this trial was not designed or powered to assess antitumor activity, encouraging preliminary clinical activity was observed at doses of 2 mg/kg and higher in heavily pretreated patients (all activity was observed in squamous histologies). Going forward, CX-2029 at 3 mg/kg will be studied in four tumor types based on activity observed in this phase I study, nonclinical CD71 expression analysis, and xenograft studies. The propensity of responses among squamous cancers is of interest and is under further investigation. The association between CD71 expression and efficacy has not yet been fully elucidated, and work is ongoing regarding CD71 parameters (IHC; gene amplification) versus tumor regression.

As with all phase I dose-escalation studies, limitations include that the study was open-label with a small sample size and heterogeneity of dose levels and tumor types. This makes correlating CD71 expression with efficacy and defining a cutoff prospectively for the expansion study challenging. Larger studies are needed to confirm activity and tolerability findings observed in this phase I trial and examine the predictive value of CD71 expression for clinical efficacy. In addition, as a membrane transporter with a rapid rate of internalization and recycling to the plasma membrane, the role of CD71 (whether by protein expression, gene amplification, or other) in the selection of patients is currently unknown. As a correlation between any CD71 parameter and biological activity of this agent in patients is unknown, the phase II dose-expansion cohorts will continue to enroll an unselected patient population with a focused effort on better understanding CD71 biology in different advanced malignancies.

## Conclusion

This first-in-human trial validates CD71 as a viable therapeutic cancer target. The Probody-therapeutic platform is the only platform that has been successfully applied to enable targeting CD71, a previously undruggable ADC target, at tolerable doses associated with clinical activity. CX-2029 will be evaluated in four phase II expansion cohorts in squamous NSCLC, HNSCC, esophageal and gastroesophageal junction cancers (adenocarcinoma and squamous), and diffuse

Published OnlineFirst June 3, 2021; DOI: 10.1158/1078-0432.CCR-21-0194



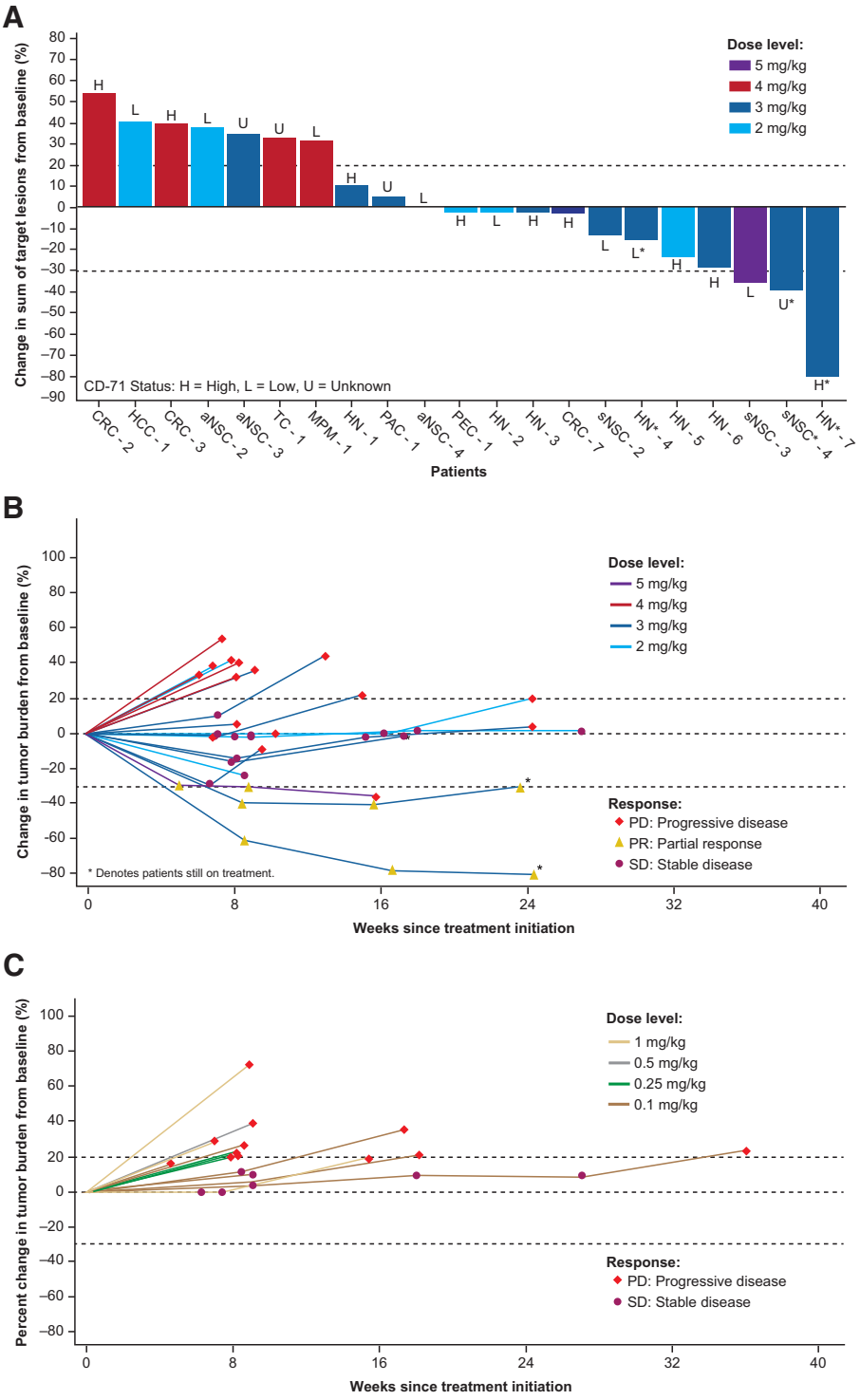


**Figure 2.**

Percent change in tumor burden from baseline for patients treated at 2 to 5 mg/kg (**A** and **B**), and patients treated at 0.1 to 1 mg/kg (**C**). *Denotes patients still on treatment. Abbreviations: CRC, colorectal cancer; HCC, hepatocellular carcinoma; HN, head and neck squamous cell carcinoma; MPM, malignant pleural mesothelioma; aNSC, non–small cell lung carcinoma (adenocarcinoma); sNSC, non–small cell lung carcinoma (squamous cell carcinoma); PAC, pancreatic cancer; PEC, perivascular epithelioid cell tumor; TC, thyroid carcinoma. CD-71 expression high is tentatively defined as IHC staining of ≥50% tumor cells at 2+/3+ intensity for overall staining. RC, CRC, and HCC are less/not sensitive to microtubule inhibitors (MTIs).

large B-cell lymphoma with work ongoing to evaluate CD71 parameters for optimal patient selection.

## Authors' Disclosures

M. Johnson reports other support from AbbVie, Achilles Therapeutics, AstraZeneca, Atreca, Boehringer Ingelheim, Calithera, Biosciences, EMD Serono, Genentech/Roche, GlaxoSmithKline, Gritstone Oncology, Guardant Health, Incyte, Janssen, Lilly, Loxo Oncology, Merck, Mirati Therapeutics, Novartis, Pfizer, Ribon Therapeutics, Sanofi, Association of Community Cancer Centers, Amgen, BMS, Cytomx, Daiichi, Editas, Eisai, G1 Therapeutics, Ideaya, and WindMIL outside the submitted work; in addition, M. Johnson reports a consulting/advisory role (spouse) and contract lobbyist role for Astellas, a contract lobbyist role for Otsuka Pharmaceuticals, and lodging/travel support from AstraZeneca, Roche/Genentech, Merck, Sanofi, Pfizer, AbbVie, and Incyte. A. El-Khoueiry reports personal fees and nonfinancial support from CytomX during the conduct of the study and personal fees and

nonfinancial support from BMS; grants and personal fees from AstraZeneca; and personal fees from Bayer, Merck, EISAI, Roche Genentech, Gilead, Pieris, QED, ABL Bio, Agenus, Exelixis, and EMD Serono outside the submitted work. N. Lakhani reports other support from CytomX during the conduct of the study. H. Mamdani reports grants from CytomX during the conduct of the study; personal fees from AstraZeneca and Zentalis; and nonfinancial support from AstraZeneca, Takeda, and Caris Life Sciences outside the submitted work. J. Rodon reports grants and personal fees from Novartis, Spectrum Pharmaceuticals, Inc., and Pfizer; personal fees from Eli Lilly, Orion Pharmaceuticals, Servier Pharmaceuticals, Peptomyc, Roche Pharmaceuticals, Ellipses Pharma, Certera, NovellusDx, and IONCTURA SA; personal fees and other support from Merck Sharp & Dohme, Kelun Pharmaceuticals/Klus Pharma, and Molecular Partners; grants, personal fees, and other support from Bayer; grants from Blueprint Medicines, Tocagen, Symphogen, BioAlta, GenMab, CytomX, Kelun-Biotech, Takeda-Millenium, and Ipsen; grants and other from GlaxoSmithKline; and other support from ESMO, Department of Defense, Louisiana State University, Huntsman Cancer Institute, Cancer Core Europe, Karolinska Cancer Institute, King Abdullah International Medical Research Center, WIN Consortium, Jansen, European Journal of Cancer, VHIO/Ministero De Empleo Y Serguridad Social, Chinese University of Hong Kong, SOLTI, and Elsevier outside the submitted work. R.E. Sanborn reports personal fees from Genentech/Roche and AstraZeneca outside the submitted work; travel to scientific meetings, AstraZeneca; panel expert, symposium presenter, AstraZeneca; educational presentation, Amgen. The Earle A. Chiles Research Institute received financial support due to our participation in the clinical trial. V. Boni reports other support from CytomX Therapeutics during the conduct of the study and personal fees from Puma Biotechnology, Ideaya Biosciences, Loxo Therapeutics, CytomX Therapeutics, Guidepoint, and Oncoart; and other support from AbbVie, ACEO, Adaptaimmune, 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, Incyte, Innovio, MSD, PsiOxus, Seattle Genetics, Mersana, GSK, Daiichi, Nektar, Astellas, ORCA, Boston Therapeutics, Dynavax, DebioPharm, Boehringer Ingelheim, Regeneron, Millenium, Synthon, Spectrum, Rigontec, and Zenith outside the submitted work. M. Stroh reports other support from CytomX outside the submitted work and BMS and Novartis covered his registration for a few conferences. A.L. Hannah reports other support from CytomX Therapeutics during the conduct of the study. H. Castro reports being a Cytomx employee. A. Spira reports grants from Cytomx during the conduct of the study and personal fees from Amgen, Mirati, AstraZeneca, Novartis, and BMS outside the submitted work. No disclosures were reported by the other authors.

## Data Sharing

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

## Role of the funding source

This study was designed by the sponsor in partnership with AbbVie and the study investigators. Data were collected by the sponsor; the sponsor collaborated with the study investigators in the analysis and interpretation of results. The study sponsor paid for writing and editorial support. All authors had full access to all data in the current analyses; the corresponding author had final responsibility for the decision to submit for publication.

## Authors' Contributions

**M. Johnson:** Conceptualization, data curation, formal analysis, validation, writing–original draft, writing–review and editing. **A. El-Khoueiry:** Data curation, writing–review and editing. **N. Hafez:** Data curation, writing–review and editing. **N. Lakhani:** Data curation, writing–review and editing. **H. Mamdani:** Data curation, writing–review and editing. **J. Rodon:** Data curation, writing–review and editing. **R.E. Sanborn:** Data curation, writing–review and editing. **J. Garcia-Corbacho:** Data curation, writing–review and editing. **V. Boni:** Data curation, writing–review and editing. **M. Stroh:** Conceptualization, data curation, formal analysis, validation, writing–original draft, writing–review and editing. **A.L. Hannah:** Conceptualization, formal analysis, supervision, methodology, writing–original draft, writing–review and editing. **S. Wang:** Data curation, validation, methodology, writing–original draft, writing–review and editing. **H. Castro:** Formal analysis, supervision, validation, writing–original draft, project administration, writing–review and editing. **A. Spira:** Data curation, writing–review and editing.

## Acknowledgments

The authors would like to acknowledge Cathrine Leonowens (generation of **Fig. 1**), Hong Lu, and Jennifer Richardson (bioanalytical support) for their contributions to this article. This study was funded by CytomX Therapeutics, Inc. Medical writing and editorial assistance were provided by Phillips Gilmore Oncology Communications, Inc. and were funded by CytomX Therapeutics, Inc. The authors thank the investigators, the patients, and their caregivers who participated in the study. PROBODY is a U.S.-registered trademark of CytomX Therapeutics, Inc.

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Received January 15, 2021; revised March 5, 2021; accepted April 2, 2021; published first June 3, 2021.

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# Phase I, First-in-Human Study of the Probody Therapeutic CX-2029 in Adults with Advanced Solid Tumor Malignancies

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*Clin Cancer Res* Published OnlineFirst June 3, 2021.