A First-in-Human Study of Cinrebafusp Alfa, a HER2/4-1BB Bispecific Molecule, in Patients with HER2-Positive Advanced Solid Malignancies

Sarina Piha-Paul[1], Shane A. Olwill[2], Erika Hamilton[3], Anthony Tolcher[4], Paula Pohlmann[5], Stephen V. Liu[5], Cornelia Wurzenberger[2], Laura-Carolin Hasenkamp[2], Eva-Maria Hansbauer[2], Rachna Shroff[6], Sara Hurvitz[7], Anuradha Krishnamurthy[8], Amita Patnaik[9], Noah Hahn[10], Raman Kumar[2], Manuela Duerr[2], Markus Zettl[2], Kayti Aviano[11], Louis Matis[11], Ingmar Bruns[11], and Geoffrey Ku[12]

ABSTRACT

Purpose: 4-1BB (CD137) is a costimulatory immune receptor expressed on activated T cells, activated B cells, NK cells, and tumor-infiltrating lymphocytes, making it a promising target for cancer immunotherapy. Cinrebafusp alfa, a monoclonal antibody-like bispecific protein targeting HER2 and 4-1BB, aims to localize 4-1BB activation to HER2-positive tumors. This study evaluated the safety, tolerability, and preliminary efficacy of cinrebafusp alfa in patients with previously treated HER2-positive malignancies.

Patients and Methods: This was a multicenter dose-escalation study involving patients with HER2-positive malignancies who received prior treatment. The study assessed the safety and efficacy of cinrebafusp alfa across various dose levels. Patients were assigned to different cohorts, and antitumor responses were evaluated. The study aimed to determine the MTD and to observe any clinical activity at different dose levels.

Results: Of 40 evaluable patients in the "active dose" efficacy cohorts, five showed an antitumor response, resulting in an overall response rate of 12.5% and a disease-control rate of 52.5%. Clinical activity was observed at the 8 and 18 mg/kg dose levels, with confirmed objective response rates of 28.6% and 25.0%, respectively. Cinrebafusp alfa was safe and tolerable, with grade ≤2 infusion-related reactions being the most frequent treatment-related adverse event. MTD was not reached during the study.

Conclusions: Cinrebafusp alfa demonstrates promising activity in patients with HER2-positive malignancies who have progressed on prior HER2-targeting regimens. Its acceptable safety profile suggests it could be a treatment option for patients not responding to existing HER2-directed therapies.

See related commentary by Eguren-Santamaría et al., p. 231

Introduction

The use of an immune checkpoint inhibitor (CPI) against T cell targets such as programmed cell death protein 1 (PD1), PDL1, and cytotoxic T lymphocyte–associated protein 4 (CTLA-4) has become a corner stone of cancer therapy in multiple indications, based on their ability to elicit durable clinical benefit (1, 2). Despite the emergence of CPIs, the majority of patients with cancer do not respond to or eventually become resistant to treatment (3). 4-1BB (CD137 and TNFRSF9) is a costimulatory receptor, expressed on both adaptive and innate immune cells including activated $CD8^+$ T cells and NK cells (4). Costimulation of 4-1BB increases T-cell effector function, such as cytokine release and cytotoxicity, as well as proliferation, survival, and memory formation (5, 6). 4-1BB agonism can modulate the metabolic reprograming of T cells and reinvigorate chronically exhausted $CD8^+$ tumor-infiltrating lymphocytes (7–10).

The potential of 4-1BB costimulation has been extensively studied in preclinical models; however, demonstrating clinical utility of 4-1BB agonists has thus far proven challenging. Agonistic 4-1BB mAbs, such as urelumab and utomilumab, were clinically tested, but they did not advance to later stage clinical trials (11–13). Urelumab showed antitumor activity in phase I/II studies but was limited by hepatotoxicity, whereas utomilumab, a less potent agonist, demonstrated limited activity (14–16). These clinical observations highlight the necessity to generate novel 4-1BB agonists, which can deliver potent pathway stimulation while avoiding systemic toxicity.

[1]Department of Investigational Cancer Therapeutics (A Phase I Clinical Trials Program), The University of Texas MD Anderson Cancer Center, Houston, Texas. [2]Department of Research and Development, Pieris Pharmaceuticals GmbH, Bavaria, Germany. [3]Department of Oncology, Sarah Cannon Research Institute/Tennessee Oncology, LLC, Nashville, Tennessee. [4]NEXT Oncology, San Antonio, Texas. [5]Department of Medicine, Georgetown University Lombardi Comprehensive Cancer Center, Washington, District of Columbia. [6]Department of Medicine, University of Arizona Cancer Center, Tucson, Arizona. [7]Department of Medicine, University of California Los Angeles Jonsson Comprehensive Cancer Center, Los Angeles, California. [8]University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania. [9]Department of Phase I Research, The START Center for Cancer Research, San Antonio, Texas. [10]Department of Oncology and Urology, Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, Maryland. [11]Department of Research and Development, Pieris Pharmaceuticals, Inc., Boston, Massachusetts. [12]Gastrointestinal Oncology Service, Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, New York.

Corresponding Author: Sarina Piha-Paul, Department of Investigational Cancer Therapeutics (A Phase I Clinical Trials Program), University of Texas MD Anderson Cancer Center, 1515 Holcombe Boulevard, Unit 455, Houston, TX 77030. E-mail: spihapau@mdanderson.org

Clin Cancer Res 2025;31:288–98

doi: 10.1158/1078-0432.CCR-24-1552

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

©2024 The Authors; Published by the American Association for Cancer Research

Translational Relevance

Although immunotherapy via checkpoint inhibition provides durable responses for a subset of patients, most subjects are refractory or become resistant to treatment. Preclinical data suggest activation of immune cells via 4-1BB agonism holds promise as an alternative therapeutic strategy in cancer; however, initial clinical efforts were hampered by lack of efficacy or severe hepatotoxicity. Herein, we describe the phase I clinical assessment of cinrebafusp alfa, a 4-1BB/HER2 bispecific, which was engineered to specifically activate 4-1BB in a tumor-dependent manner. Our data confirm immune cell activation via 4-1BB is safe and efficacious when restricted to the tumor microenvironment through bispecific targeting to cell surface molecules HER2/neu and 4-1BB. Dose-dependent expansion of tumor-infiltrating $CD8^+$ T cells and s4-1BB levels coupled with confirmed objective response highlight the potential utility of 4-1BB agonism.

The HER2 (also known as ERBB2) pathway has been found to be amplified, mutated, or overexpressed in a broad range of cancers including breast, gastric, non–small cell lung cancer, bladder, and colorectal cancer, often leading to a more aggressive disease (17–23). HER2-targeted therapies based on receptor blocking antibodies, antibody drug conjugates, and small-molecule kinase inhibitors greatly improved outcome for patients with breast, gastric, and lung cancers (24–33). To date, no approved HER2-targeted therapies that take advantage of the innate and adaptive immune system exist.

Cinrebafusp alfa (PRS-343) is a mAb-like bispecific protein targeting HER2 and 4-1BB. It is designed to conditionally activate T and NK cells in HER2-positive tumor tissue via the 4-1BB pathway. Cinrebafusp alfa is generated by genetic fusion of a variant of the HER2-targeting antibody trastuzumab with an anticalin protein specific for 4-1BB using a silenced IgG4 isotype to avoid Fc-mediated immune cell engagement (34). Although cinrebafusp alfa maintains the potential to inhibit HER2 signaling, its engineered isotype prevents any antibody- or complement-dependent cell-mediated cytotoxicity tumor killing. The mode of action of this bispecific agent is to promote 4-1BB agonism via dual binding to HER2-positive tumor cells and adjacent 4-1BB-positive immune cells. The HER2-dependent mode of action of 4-1BB receptor clustering and agonism is designed to activate immune cells specifically in the tumor microenvironment, thus avoiding liver toxicities observed with systemic 4-1BB agonists.

Preclinical studies showed that cinrebafusp alfa co-stimulates T-cell activation in a HER2-dependent manner in a panel of cell lines with variable levels of HER2 expression. Also, Cinrebafusp alfa led to tumor growth inhibition and a significant increase in tumor-localized $CD8^+$ T cells in a SK-OV-3 xenograft model (34).

Taken together, the preclinical data strongly support the concept that tumor-localized costimulatory immune cell activation by cinrebafusp alfa can lead to an antitumor immune response against HER2-expressing cells with reduced systemic toxicity. A phase I dose-escalation study (NCT03330561) was conducted to assess the safety, tolerability, pharmacokinetics (PK), pharmacodynamics, and preliminary clinical activity of cinrebafusp alfa in patients with HER2-positive advanced or metastatic solid tumors.

Patients and Methods

Study design and treatment

This was a multicenter, open-label, phase I dose-escalation study designed to determine the safety, MTD, and dosing schedule of cinrebafusp alfa in patients with HER2-positive advanced or metastatic solid tumors. Cinrebafusp alfa was administered by intravenous infusion over 2 hours at dose levels ranging from 0.0005 to 18 mg/kg. In the absence of disease progression and prohibitive toxicity, patients were allowed to continue treatment and were continually assessed for evidence of acute and cumulative toxicity. Several treatment schedules were evaluated: Day 1 of a 21-day cycle (Q3W), Days 1 and 15 of a 28-day cycle (Q2W), and Days 1, 8, and 15 of a 21-day cycle (QW).

The dose levels, patient numbers, and treatment schedules are described in Supplementary Table S1. Cohorts representing cinrebafusp alfa doses of 0.0005 to 0.05 mg/kg were conducted as single patient, accelerated titration cohorts with potential expansion to two patients in the case of a grade 2 treatment-related adverse event (TREA), and potential expansion to a 3+3 design in the case of dose-limiting toxicity (DLT); once the cinrebafusp alfa dose of 0.15 mg/kg was reached, the study was prospectively planned to automatically convert to a modified 3+3 design, regardless of the DLT status. Under the modified 3+3 design, at least 3 and up to 12 patients were initially enrolled in a given dose cohort, and observation of a DLT among any of these patients would prompt expansion to at least 6 evaluable patients. Next, to be evaluable for DLT, the patient was required to receive all planned doses of cinrebafusp alfa without modification in schedule or dose level and complete all safety assessments during Cycle 1.

Adverse events were graded according to the NCI Common Terminology Criteria for Adverse Events version 4.03 guidelines. Dose escalation was based on the incidence of dose-limiting toxicities during Cycle 1. The criteria for DLT were as follows: grade 4 neutropenia lasting >7 days; grade 4 thrombocytopenia >7 days or grade 3/4 thrombocytopenia with clinically significant bleeding; febrile neutropenia defined as a fever of ≥38.0°C with or without clinically or microbiologically documented infection with absolute neutrophil count (ANC) $<1.0 \times 10^9$/L; grade 3 hepatic toxicity; grade ≥3 nonhematologic, nonhepatic organ toxicity (alopecia, grade 3 nausea, vomiting or diarrhea that resolved within 3 days of treatment, grade 3 fatigue that resolved within 7 days, and grade 3 tumor pain were not considered DLT). Furthermore, MTD was defined as the highest dose level that did not lead to DLT in >33% of patients in the first cycle of treatment (an MTD was not reached in this study).

Patient population

Adult patients (aged ≥18 years) with HER2-positive advanced solid tumors for which standard treatment options were not available, were no longer effective, were not tolerated, or the patient refused standard therapy were eligible. Patients with breast, gastric, or gastroesophageal junction cancer must have received at least one prior HER2-directed therapy for advanced disease. HER2 positivity, defined as 3+ by IHC; 2+ by IHC with HER2 gene amplification; or by institutional guidelines, was established prior to study entry following the below criteria.

(a) Assessment of HER2 status in patients with breast cancer should follow the 2013 American Society of Clinical Oncology

Table 1. Patient demographics and baseline characteristics.

Characteristic	Patients (*n* = 78)
Age (years)	
Median (range)	62.5 (24–92)
Sex	
Female	46 (58.97%)
Male	32 (41.03%)
Cancer type (reported in ≥2 patients)	
Biliary tract	3 (3.85%)
Bladder	2 (2.56%)
Breast	16 (20.51%)
Colorectal	11 (14.10%)
Gall bladder	4 (5.13%)
Gastric/gastroesophageal	25 (32.1%)
Gynecological	7 (9.0%)
ECOG performance status at screening	
0	19 (24.36%)
1	58 (74.36%)
2	1 (1.28%)[a]
Prior systemic therapies	
Median prior regimens (range)	4 (1-11)
HER2-directed therapy, *n* (%)	53 (68%)

Values are given as *n* (%) of patients unless otherwise specified. Fifty-three patients received HER2-directed therapies in prior lines of treatment [these included trastuzumab (94%), pertuzumab (34%), trastuzumab emtansine (23%), trastuzumab deruxtecan (4%), zanidatamab (6%), other HER2 ADC (4%), and other anti-HER2 agents (8%)].
[a]Patient demonstrated ECOG status of 2 at screening but was reevaluated as ECOG status of 1 prior to receiving first treatment on Cycle 1 Day 1.

(ASCO)/College of American Pathologists criteria by Wolff and colleagues (35) as practicable.

(b) Assessment of HER2 status in patients with gastric and gastroesophageal junction adenocarcinoma should follow the criteria published by Rüschoff and colleagues (36) as practicable.

(c) Assessment of HER2 status in patients with non-breast/non-gastric cancers may follow local institutional criteria. These criteria should be made available to the Sponsor.

(d) All patients with breast and gastric/gastroesophageal junction cancers should receive HER2 testing performed using an FDA-approved test in a Clinical Laboratory Improvement Amendments (CLIA)-certified laboratory.

(e) Patients for whom the clinical pathology report includes only IHC as 3+ (does not refer to ISH) may enroll without written report of ISH-determined HER2 copy number, provided the investigational site confirms that archival tissue is available.

Patients were required to exhibit an Eastern Cooperative Oncology Group (ECOG) performance status ≤1 and measurable disease according to RECIST v1.1;. Women of child-bearing potential presented documentation of a negative pregnancy test. Treatment with cinrebafusp alfa did not begin until >4 weeks after the patient's last therapy or major surgical procedure. Acceptable hepatic, renal, hematologic, cardiac, and bone marrow function was required of all patients. Key exclusion criteria were uncontrolled CNS metastases, systemic steroid therapy or other form of immunosuppressive therapy within 7 days prior to the first infusion of cinrebafusp alfa, history of acute coronary syndromes, or ejection fraction drop below the lower limit of normal with trastuzumab and/or pertuzumab.

Clinical assessments

Radiologic and clinical tumor assessments were assessed according to the RECIST v1.1 [e.g., CT scan, MRI, and tumor markers (e.g., CEA, CA19.9, CA125, as applicable)]. Then, assessments were carried out at screening, after completion of every second cycle for the first 24 weeks of treatment and every 12 weeks thereafter, and at the end of treatment. Next, evidence of radiologic response [complete response (CR) or partial response (PR)] was confirmed by radiologic assessment at least 4 weeks after the initial observation. Stable disease (SD) was defined as per RECIST 1.1 criteria: Neither sufficient shrinkage to qualify for PR (at least 30% shrinkage) nor sufficient increase to qualify for progressive disease (PD; at least 20% increase), taking as reference the smallest sum diameters while on study. For confirmed SD responses, SD lasting for at least 12 weeks was used. Next, safety assessments were carried out based on clinical laboratory data, physical examinations, and reporting of adverse events; all patients who received at least one dose of cinrebafusp alfa were included in safety analyses. Cardiac function was monitored during screening as well as during the course of the study by left ventricular ejection fraction assessment at every cycle.

PK analyses

Venous blood samples for measurement of serum concentrations of cinrebafusp alfa were collected before infusion, 5 minutes after the end of infusion, and 1, 2, 4, 8, 24, 48, 72, and 168 hours after infusion on Cycle 1 Day 1, as well as Cycle 3 Day 1 for Q3W and Q2W dosing, and at the same timepoints on Cycle 1 Day 1 as well as Cycle 2 Day 1 for QW dosing. Additional samples were taken predose and 5 minutes after the end of infusion in other cycles on Day 1.

Cinrebafusp alfa serum concentrations were measured using an electrochemiluminescence assay. PK parameters, including AUC, Cmax, time to maximum dose concentration (Tmax), and terminal half-life ($t1/2$), were derived by noncompartmental analyses using Phoenix WinNonlin 8.3.4 (Pharsight Corporation).

Immunogenicity analyses

Serum samples were collected before treatment with cinrebafusp alfa on Days 1 and 15 of Cycle 1 and before treatment with cinrebafusp alfa, on Day 1 of Cycles 3 to 6, and at the end of treatment to evaluate development of antibodies directed against cinrebafusp alfa. Antidrug antibodies were measured using an electrochemiluminescence-based assay. A screening assay was followed by a confirmatory and a subsequent titer assay.

Biomarker analysis

Soluble 4-1BB was measured in serum samples at baseline and at the PK sampling timepoints during cycle 1 for all dose levels. Also, predose measurement of s4-1BB was measured across cycles 2 to 6 for subjects receiving 18 mg/kg cinrebafusp alfa. It was detected with a custom total immunoassay based on a fluorescent readout. Biopsies were taken at baseline and during cycle 2. Various tumor tissues were stained for IHC, manual pathology, and image analysis. Samples were H&E stained to assess the area and for CD8, PDL1, granzyme B, and CD56 positive cells. Then, fold changes for all analysis were calculated in reference to baseline values. To differentiate PDL1 and CD8 high/low patient populations, thresholds of 25% for PDL1 positivity and 250 $CD8^+$ cells/mm^2 were selected as cutoffs. Active and nonactive dose groups were analyzed with the unpaired Welch t test (one-tailed).

Table 2. TRAEs.

Event	All grades	Grade 3	Grade 4	Grade 5
Any TRAE	44 (56.4)	11 (14.1)	1 (1.3)	0 (0.0)
IRR	17 (21.8)	3 (3.8)	1 (1.3)	0 (0.0)
Fatigue	11 (14.1)	1 (1.3)	0 (0.0)	0 (0.0)
Nausea	9 (11.5)	0 (0.0)	0 (0.0)	0 (0.0)
Arthralgia	3 (3.8)	1 (1.3)	0 (0.0)	0 (0.0)
Flushing	2 (2.6)	1 (1.3)	0 (0.0)	0 (0.0)
Anemia	2 (2.6)	1 (1.3)	0 (0.0)	0 (0.0)
Paresthesia	2 (2.6)	1 (1.3)	0 (0.0)	0 (0.0)
Clostridium difficile colitis	1 (1.3)	1 (1.3)	0 (0.0)	0 (0.0)
Urinary tract infection	1 (1.3)	1 (1.3)	0 (0.0)	0 (0.0)
Lipase increased	1 (1.3)	1 (1.3)	0 (0.0)	0 (0.0)
Cardiac failure	1 (1.3)	1 (1.3)	0 (0.0)	0 (0.0)
Dysuria	1 (1.3)	1 (1.3)	0 (0.0)	0 (0.0)
Anxiety	1 (1.3)	1 (1.3)	0 (0.0)	0 (0.0)

Values are given as distinct *n* (%) of patients. Adverse events of all grades that affected more than 10% of patients or all adverse events being ≥ grade 3. $n = 78$.

Statistical considerations

This was a phase I study with primary objectives of evaluating safety, PK, and pharmacodynamics. All patients who received at least 1 infusion of cinrebafusp alfa were included in the safety analyses and determination of MTD. Descriptive analysis of antitumor activity, including response rate (CR or PR) and disease-control rate (CR or PR or SD), was summarized for those patients who received at least one dose of cinrebafusp alfa and received at least one post-baseline objective disease assessment. No formal statistical power calculations to determine sample sizes were carried out for this study. Researchers estimated that 1 to 3 patients would be enrolled per dose level in the accelerated titration portion of the study and 3 to 6 patients per dose level in the standard dose-escalation portion of the study, including at least six subjects treated at the MTD level.

For safety analyses, adverse events were graded by the treating physician based on Common Terminology Criteria for Adverse Events v 4.03. Safety data were included for all the patients who received at least one dose of cinrebafusp alfa.

Study oversight

This study was carried out in accordance with the Declaration of Helsinki and the principles of Good Clinical Practice. The protocol was approved by an Institutional Review Board at each hospital or site, and all patients provided written informed consent before any study procedures. The study was designed by the sponsor (Pieris Pharmaceuticals). Data were collected from the study sites and analyzed by representatives of the sponsor in conjunction with the investigators.

Data availability

The deidentified participant data and dataset generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Results

Demographics and patient characteristics

Seventy-eight patients with HER2-positive advanced solid tumors were enrolled in the dose-escalation part of the phase I PRS-343-PCS_04_16 study (NCT03330561) and received cinrebafusp alfa at escalating dose levels between 0.0005 and 18 mg/kg (Supplementary Table S1). Cinrebafusp alfa was administered every 3 weeks (Q3W) up to 8 mg/kg cohort. Also, dosing schedules of weekly (QW) and biweekly (Q2W) were explored at 8 mg/kg cohort. A Q2W dosing schedule was selected for 12 and 18 mg/kg cohorts. Additionally, to assess the effect of B-cell depletion on immunogenicity, an exploratory cohort of patients (8 mg/kg Q2W OB cohort) received a pretreatment with 1,000 mg obinutuzumab.

Patient demographics and baseline characteristics are presented in **Table 1**. The median age was 62.5 (range, 24–92), and most patients were female (59%).

The most common tumor types were gastric/gastroesophageal cancer ($n = 25$; 32.1%), breast cancer ($n = 16$; 20.5%), and colorectal cancer ($n = 11$; 14.1%). Seventy-four percent of patients demonstrated an ECOG performance status 1.

Patients were heavily pretreated having received a median of 4 (range up to 11) prior systemic treatment regimens. Fifty-three patients (68%) received prior HER2-directed systemic treatment. Representativeness of study participants is detailed in Supplementary Table S2.

Safety outcomes

For safety analyses, a total of 78 patients, having received at least one dose of study drug from all dose-escalation cohorts (0.0005–18 mg/kg) were considered. Treatment with cinrebafusp alfa was generally safe and tolerable. One DLT was reported in the study; 1 patient with breast cancer (1.2%) in the 12 mg/kg Q2W dose cohort experienced a grade 3 pleural effusion on study day 28, which led to drug discontinuation. At tested doses up to 18 mg/kg, no MTD was identified for the treatment of cinrebafusp alfa.

Overall, 44 patients (56.4%) experienced a TRAE; of these, 11 patients (14.1%) experienced grade 3 TRAEs, and 1 patient (1.3%) experienced a grade 4 TRAE (**Table 2**). No grade 5 TRAEs considered related to study drug occurred. The most frequently reported TRAEs were infusion-related reaction (IRR) in 17 patients (21.8%), fatigue in 11 patients (14.1%), and nausea in 9 patients (11.5%). Most of these events were grade ≤2; 4 patients (5.1%) experienced grade ≥3 IRRs, and 1 patient (1.3%) experienced grade 3 fatigue. One patient in the 5 mg/kg dose cohort experienced grade 4 IRR. The event started on study day 65 and resolved within 1 day. Also, a single patient in the 18 mg/kg Q2W ($n = 9$) dose level experienced a grade 3 cardiac failure on study day 308, that was assessed as possibly related to study drug. The patient recovered from the event on study day 312 (5 days after onset) but was withdrawn from the study. Other grade 3 toxicities were uncommon and are reported in **Table 2**. All events resolved, except for one event of grade 3 fatigue (0.015 mg/kg cohort), one event of grade 3 anemia (0.5 mg/kg cohort), and one event of grade 3 paresthesia (8 mg/kg OB cohort), which were ongoing at study end.

PK

Serum cinrebafusp alfa concentrations were low or below the limit of quantitation up to the 0.05 mg/kg dose level and were progressively measurable for longer periods of time at higher dose levels. Starting at 2.5 mg/kg dose level, serum concentrations were measurable throughout the dosing interval in several patients.

Cinrebafusp alfa PK after IV infusions were characterized by maximum concentrations attained at or around the end of the infusion, followed by a mono-phasic log-linear decline until the lower limit of quantitation was reached (**Fig. 1**). The geometric mean plasma half-life varied between 3 days at lower doses and 4 to 5 days

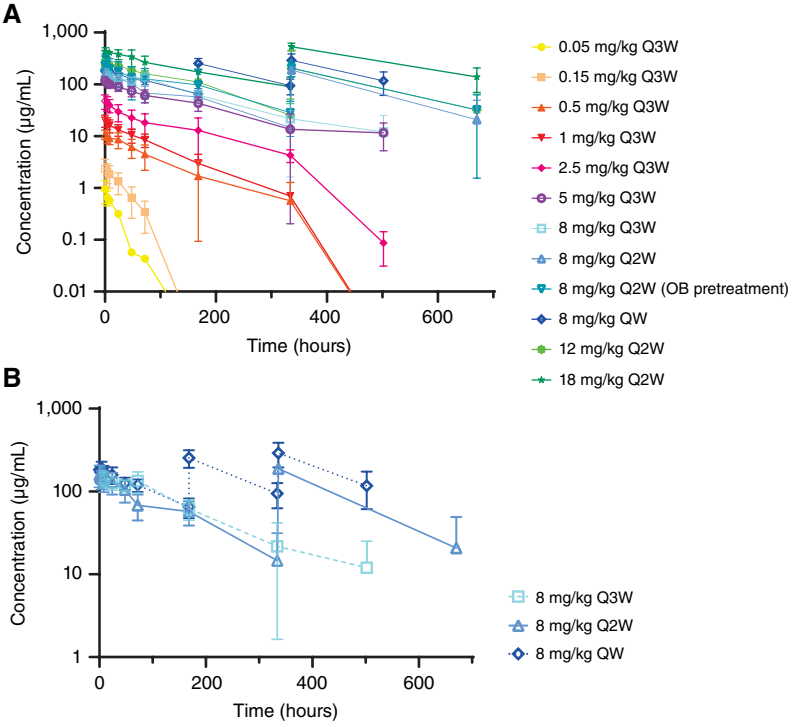

Figure 1.
PK of cinrebafusp alfa in patients with HER2-positive cancer. **A,** Mean plasma concentrations of cinrebafusp alfa during the first cycle of treatment. **B,** Impact of dosing frequency was evaluated at 8 mg/kg dose level.

in the dose levels of 8 to 18 mg/kg (**Fig. 1A**). Assessment of different dosing regimens at 8 mg/kg level led to selection of Q2W dosing for higher cohorts (**Fig. 1B**). Next, some accumulation was observed in the Q2W dosing regimen, especially in patients dosed at 18 mg/kg, and in the QW regimen, consistent with the observed half-life.

Some variability in exposure in later cycles was observed at lower dose levels. In general, exposure can be influenced by target-mediated drug disposition (TMDD), which could be caused by HER2 as well as 4-1BB and/or antidrug antibody (ADA) formation. Overall, preexisting ADAs were detected in 5% of patients, whereas 32% of patients were ADA positive on Cycle 1, Day 15. A reduction in ADA incidence was observed during dose escalation with 4 out of 17 patients (23.5%) being ADA positive on Cycle 3 Day 1 in the dose levels of 8 mg/kg and above, versus 8 out of 11 patients (72.7%) in the dose cohorts below 8 mg/kg. Patients with ADAs generally showed reduced exposure in Cycle 3 compared with exposure in Cycle 1.

In parallel to dose escalation, an exploratory cohort with obinutuzumab pretreatment was initiated to assess the consequence of B-cell depletion on ADA frequency and exposure (37). Although the obinutuzumab schedule was safe and resulted in low immunogenicity, it was not deemed to be necessary and hence was limited to five patients.

Pharmacodynamics

Soluble 4-1BB (s4-1BB) concentrations were measured, as a biomarker for pathway activation, in patient serum for subjects on Q2W and Q3W schedule. When baseline levels of s4-1BB were compared with on-treatment values until day 15 ($n = 65$), a dose-dependent increase of the maximum fold change was observed starting at a dose of 0.15 mg/kg (**Fig. 2A**). Timing of maximal elevation of s4-1BB was observed between C1D1 and C1D8 for majority of subjects. Timing of onset differed in a subset of five patients (at 5 and 8 mg/kg doses), which showed the strongest increase of s4-1BB between C1D8 and C1D15. Then, when these apparent outlier patients were omitted from the analysis, the data trend was maintained. The average fold change increased from 1.5 in the low dose cohorts to 6.0 to 9.3 in the 8 to 18 mg/kg cohorts, reaching a plateau at higher dose levels. One patient in the 8 mg/kg cohort showed a 20-fold increase in cycle 1, whereas only one patient overall experienced a reduction in s4-1BB levels. The observed cinrebafusp alfa dependent increase of s4-1BB strongly suggested target engagement and an activation-induced release of 4-1BB. Furthermore, a longitudinal assessment of s4-1BB across six treatment cycles (165 days) in the 18 mg/kg cohort demonstrated a persistent increase in the biomarker indicative of a durable effect (Supplementary Fig. S1).

Peripheral cytokines were predominantly studied from a safety assessment perspective. However, cytokines such as IFNγ, IFNγ-induced protein 10 (IP10), and IL18 have been reported as serum biomarkers of T-cell activation upon CPI treatment (38). No dose-dependent increase of IFNγ, IP10, or IL18 was observed, suggesting no peripheral T-cell activation occurred and supporting that cinrebafusp alfa treatment leads to a tumor-localized activation of 4-1BB positive immune cells with minimal systemic effects (Supplementary Tables S3–S5 respectively).

IHC analysis comparing baseline with on-treatment paired tumor biopsies ($n = 35$) showed a cinrebafusp alfa induced dose-dependent increase of $CD8^+$ T cells in the tumor parenchyma and stroma, with more than 2.6-fold average induction in the highest dose cohort at 18 mg/kg (**Fig. 2B**). Outlier data from two subjects from the 2.5 and 8 mg/kg dose cohorts were omitted as they showed increases of >600- and >300-fold, respectively. Furthermore, for

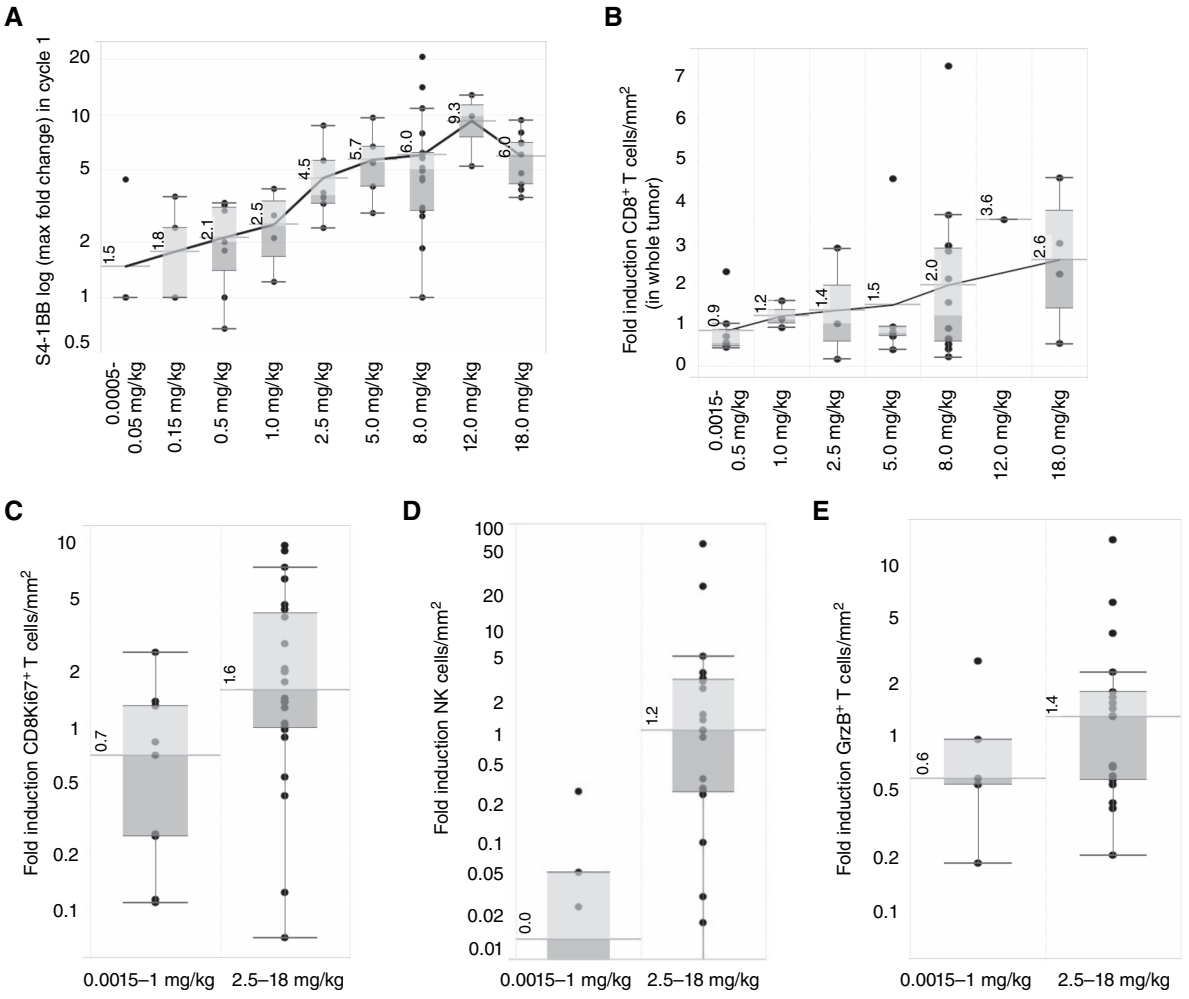

Figure 2.

Cinrebafusp alfa shows dose-dependent activity across key pharmacodynamic parameters. **A,** Fold change in s4-1BB was measured in serum during cycle 1 and compared with baseline values ($n = 65$), excluding outliers as described in the text. **B,** Fold change of $CD8^+$ T cells between baseline and on-treatment paired tumor biopsy samples ($n = 38$). **C–E,** Maximal fold change from baseline of the pharmacodynamic markers Ki67 ($n = 33$), CD56 (NK; $n = 25$), and granzyme B ($n = 23$) were measured in tumor tissue of nonactive and active dose cohorts. Treatment-induced increase of 1.6-fold was observed for Ki67 (**C**), which was significantly different from the nonactive dose cohorts ($P = 0.002$, unpaired Welch t test, one-tailed). Also, NK (CD56) and granzyme B increased from baseline in the active dose group (**D** and **E**), with $P = 0.051$ and 0.099, respectively, in comparison to the nonactive dose group.

these patients, the outlier values were likely impacted by low $CD8^+$ T-cell counts in the baseline samples.

Cohorts ranging from 2.5 to 18 mg/kg were defined as representing the active dose range based on predictions from preclinical studies (34) and emerging clinical PK and pharmacodynamic data (i.e., significant increase of soluble 4-1BB and $CD8^+$ T cells observed at these dose levels).

Grouped analyses of additional pharmacodynamic markers were performed for the nonactive and active dose cohorts. Treatment induced $CD8^+$ T-cell proliferation was assessed by Ki67 nuclear staining with a 1.6-fold increase in proliferation from baseline observed in the active dose cohorts (**Fig. 2C**), which was significantly different from that seen in the nonactive dose cohorts ($P = 0.002$; unpaired Welch's t test; one-tailed; Supplementary Fig. S2). Also, NK (CD56) cell numbers were seen to increase from baseline in the active dose group (**Fig. 2D**), and a similar trend was observed with granzyme B (**Fig. 2E**; $P = 0.051$ and 0.099, respectively, in comparison to nonactive dose group). Interestingly, although NK cell numbers were increased in both the tumor parenchyma and stroma region, we observed that Ki67 positive cells were more evident in stromal areas. Cells expressing granzyme B were increased in the region adjacent to tumor cells, possibly indicating their active killing of cancer cells. Next, a subset of responder patients was assessed for PDL1 status and plotted versus number of $CD8^+$ T cells (**Fig. 3**). Researchers observed that baseline levels of PDL1 and T cells were low in multiple responders, suggesting that the 4-1BB driven immune cell activation can exhibit meaningful impact in a broad patient population.

Antitumor activity outcomes

Fifty-nine patients enrolled across nonactive and active dose cohorts were considered evaluable for efficacy assessment based on

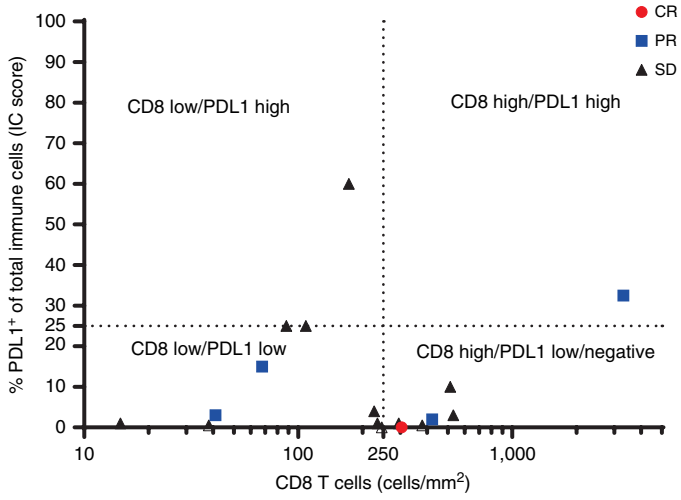

Figure 3.
Baseline assessment of PDL1 status (% of positive tumor-infiltrating immune cells, IC score) vs. number of $CD8^+$ T cells/mm^2 shows responder patients (CR, PR, or SD) represent various immunological phenotypes, including PDL1-low and PDL1-negative subjects ($n = 17$). Thresholds of 25% for PDL1 positivity and 250 $CD8^+$ cells/mm^2 were selected as cut-offs to differentiate PDL1- and CD8-high/low patient populations.

receipt of at least one dose of study drug and having a baseline and an on-treatment scan performed.

Nonactive dose cohorts (0.0005–1.0 mg/kg)

Nineteen patients were enrolled across dose cohorts ranging from 0.0005 to 1.0 mg/kg. These dose levels were included in the trial predominantly for safety reasons based on published experiences with 4-1BB agonists (14, 39). Based on translational studies and preclinical pharmacology, researchers anticipated that a lack of activity would be found in these cohorts. As anticipated, no responses occurred in these dose ranges, in which 12 (63%) patients demonstrated PD, and 7 (37%) patients demonstrated SD.

Active dose cohorts (2.5–18 mg/kg)

Forty patients were enrolled across dose cohorts ranging from 2.5 to 18 mg/kg. The dose range was defined as "active doses" based on preclinical analyses and qualified as clinical PK/biomarker data emerged. Five patients (12.5%) showed antitumor activity, with one patient achieving a confirmed CR, and four patients achieving a PR, three of which were confirmed. Interestingly, all responses occurred at either the 8 or 18 mg/kg Q2W dose cohorts (see **Fig. 4A**). Next, a total of 16 patients (40%) experienced SD (thereof six confirmed SD patients) as best overall response (BOR), so that the disease control rate (defined as SD or better) was 52.5%. When assessing individual cohorts, confirmed overall response rate was 28.6% in the 8 mg/kg Q2W ($n = 7$) cohort and 25.0% in the 18 mg/kg Q2W ($n = 8$) cohort.

The overall median duration of response, calculated as date of first documented response (CR or PR) until the first date of confirmed progression, was 284 days for the five responder patients (**Fig. 4B**).

Representative CT scans of two patients with PR as BOR to treatment with cinrebafusp alfa are presented in **Figs. 4C** and **D**. **Figure 4C** represents scans of a patient with cancer of unknown primary origin. This 81-year-old male received 1 prior line of treatment with gemcitabine resulting in a BOR of PR. Treatment with 18 mg/kg Q2W cinrebafusp alfa began approximately 1 month after progression from prior therapy. The patient achieved PR in overall target lesion size after the first on-treatment scan (study day 60) and a complete reduction of overall target lesion size at the second scan on study day 116. The patient stayed on treatment for 14 cycles (392 days). **Figure 4D** represents scans of a patient with stage 4 gastric adenocarcinoma. Also, this 80-year-old female received two prior lines of treatment including trastuzumab, pembrolizumab, oxaliplatin (first line), and nivolumab (second line), resulting in a BOR of SD in both lines. Treatment with 8 mg/kg Q2W cinrebafusp alfa began approximately 2 months after progression from prior therapy. The patient achieved PR in overall target lesion size after the second on-treatment scan (study day 80), which continued to reduce at subsequent on-treatment scans with highest overall target lesion size reduction of 63.7% on study day 127. The patient remained on treatment for seven cycles (196 study days).

Discussion

The modulation of immune cells via costimulation of 4-1BB holds great promise as an antitumor therapy. Initial efforts to harness the power of 4-1BB agonism with antibody-based approaches proved challenging due to hepatotoxicity (39). With the development of bispecific molecules, the possibility exists to activate receptors in a more targeted manner compared with conventional antibodies (40).

Cinrebafusp alfa is a monoclonal antibody-like bispecific protein which is designed to conditionally activate T and NK cells in HER2-positive tumor tissue via the 4-1BB pathway. In this first-in-human dose-escalation study of patients with HER2-expressing advanced solid tumors, cinrebafusp alfa was escalated up to a dose of 18 mg/kg without identifying an MTD. Next, in heavily pretreated patients, cinrebafusp alfa demonstrated encouraging monotherapy activity with an acceptable safety profile.

Cinrebafusp alfa was generally well-tolerated across all dose levels and schedules tested in the study population. IRRs were the most common TRAE; these were generally low grade (grade 1 or 2) and could be managed with standard supportive measures. Unlike the toxicity profile of urelumab (39), no critical transaminase elevations or hepatotoxicity was observed, consistent with cinrebafusp alfa tumor-localized activation of 4-1BB.

Cinrebafusp alfa PK profile showed a nonlinear elimination at low dose cohorts indicative of TMDD, which is consistent with experience of other HER2 targeted agents (41). Some accumulation was observed in the Q2W dosing regimen favoring this schedule

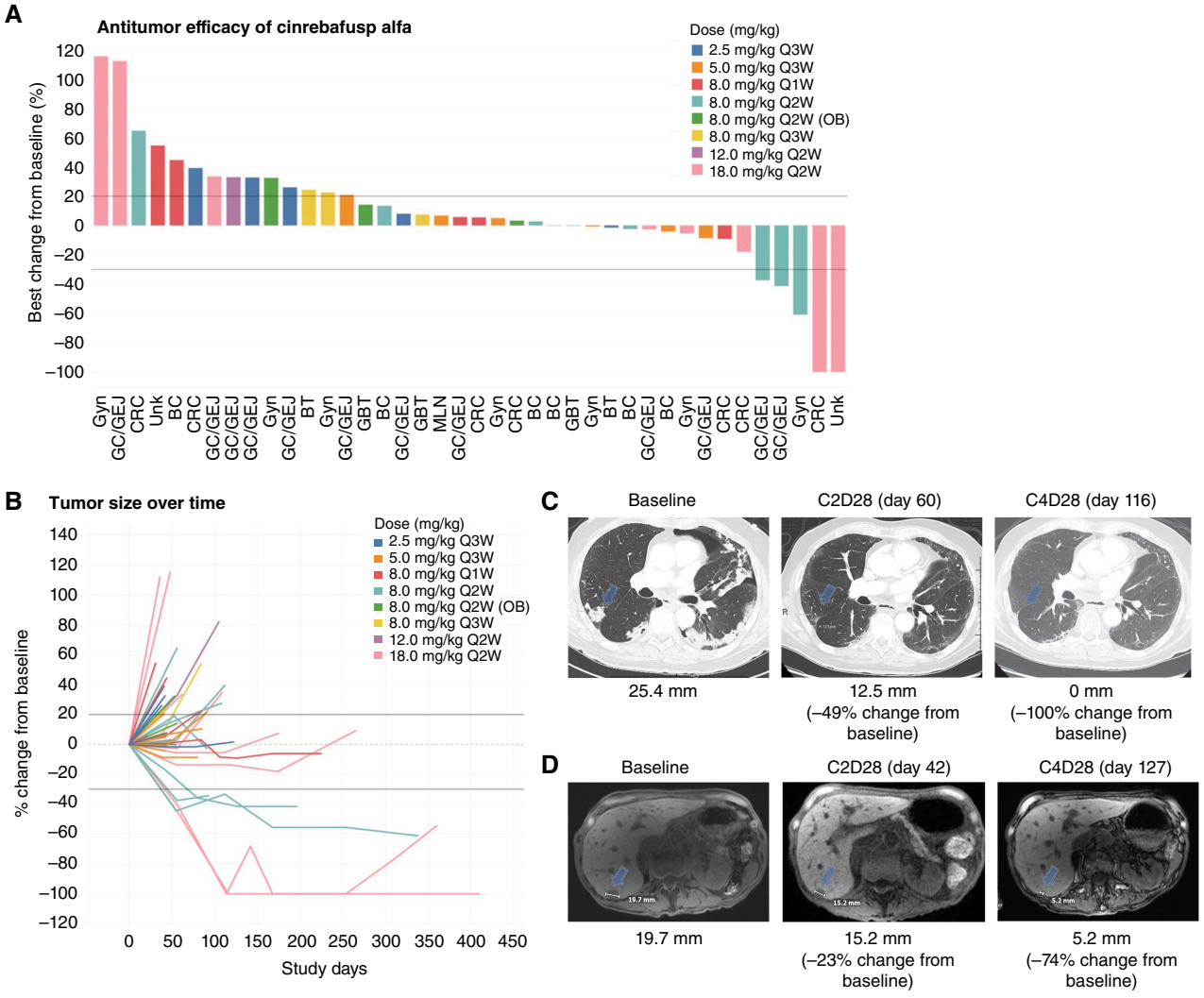

Figure 4.

Antitumor efficacy of cinrebafusp alfa. **A,** Waterfall plot of best relative change from baseline in tumor size. **B,** Spider plot outlines duration of response. Exemplar CT scans showing tumor reduction over time for patient with cancer of unknown primary origin treated with 18 mg/kg Q2W cinrebafusp alfa. **C,** A PR with 49% reduction in target lesion size was observed on study day 60, with a complete reduction of target lesion on study day 116. **D,** A second exemplar patient with BOR of PR had a 23% reduction in selected target lesion on study day 42% and 74% reduction achieved on study day 127. Represented cancers are BC, breast cancer; BT, bladder tumor; CRC, colorectal cancer; Duo, duodenal cancer; GBT, gallbladder tumor; GC/GEJ, gastric cancer/gastroesophageal cancer; Gyn, gynecological cancer/ovarian; MLN, melanoma; Unk, cancer of unknown primary.

over Q3W. ADAs to cinrebafusp alfa were predominantly observed in lower dose cohorts where, together with HER2 and/or 4-1BB-TMDD, they could exhibit an impact on drug exposure levels. The presence of ADAs was significantly reduced at 8 mg/kg and above (23.5%). Based on the current clinical data, ADAs did not impact efficacy, with three of the five responding patients testing positive during treatment. To increase our understanding of the agent, the investigation of cinrebafusp alfa in a phase 2 trial with a more homogeneous patient population is required.

The role of 4-1BB on immune cell activation has been well characterized both preclinically and clinically. Our preclinical work, together with that of other groups, showed that 4-1BB agonism impacts both the adaptive and innate immune system. We previously showed that cinrebafusp alfa leads to a tumor-localized increase of $CD8^+$ T cells in a mouse model (34). Analysis of baseline and on-treatment tumor tissue samples supported the anticipated mechanism of action of cinrebafusp alfa. Assessment of paired tumor biopsies confirmed a T-cell activation and dose-dependent increase in $CD8^+$ T cell numbers within the tumor. When further analyzed, subjects receiving >2 mg/kg demonstrated a higher fraction of $Ki67^+$ cells compared with those in the lower dose cohorts. Our clinical findings support previous studies proposing that 4-1BB agonism leads to phenotypic changes in T cells including increase in proliferation (Ki67), expansion of stem cells (TCF1), and increased cytotoxic behavior (granzyme B; refs. 42, 43). Also, costimulation of 4-1BB can impact activated NK cells, leading to expansion and increased functionality (44).

Our data show that cinrebafusp alfa treatment leads to increased NK cells in the tumor microenvironment. Recent clinical studies

with FAP-4-1BB (RO7122290) and PDL1-4-1BB (GEN1046) bispecifics also showed the ability of 4-1BB agonists to modulate T and NK cell populations (45, 46).

Also, we explored a serum-based biomarker assay to assess 4-1BB target engagement and pathway activation. We previously showed that 4-1BB agonism leads to a dose-dependent release of s4-1BB (47). Different mechanisms have been described that may drive such release of s4-1BB, including alternative splicing of 4-1BB transcripts (48, 49) or shedding from the cell surface by metalloproteases like ADAM10 or ADAM17 (50, 51). Additionally, characterization of s4-1BB as a biomarker by Glez-Vaz and colleagues showed that, beyond *de novo* s4-1BB biosynthesis and release, trapping of the biomarker in plasma may be aided when complexed with drug (52). Our *in vitro* data showed that s4-1BB release is strongly enhanced in the presence of a 4-1BB agonist. Next, serum samples from the phase 1 study indicated that s4-1BB correlates with the dose and agonistic activity of cinrebafusp alfa. Our serum-based findings were validated given that a similar dose-dependent trend was observed across the $CD8^+$ T cell and s4-1BB data sets.

Cinrebafusp alfa showed encouraging single agent activity in a heavily pretreated patient population across the active dose range. A disease-control rate of 52.5% was achieved in patients with a median of four prior lines of treatment. The responding population included patients with a variety of advanced tumor types. One patient with colorectal cancer achieved a confirmed CR, whereas a patient with cancer of unknown primary demonstrated 100% reduction in target lesion size. Also, PRs were achieved in one patient with gynecological cancer and two patients with gastric/gastroesophageal junction cancer, both of whom received 2 to 3 prior regimens, including anti-HER2 therapy (trastuzumab). Interestingly, both of these patients achieved a BOR of SD under trastuzumab treatment, whereas they achieved PR with cinrebafusp alfa treatment. Responses occurred at the 8 and 18 mg/kg dose levels. Based on clinical response and biomarker data, researchers anticipated that single agent doses of 8 mg/kg or above are required to achieve optimal 4-1BB pathway engagement and immune cell activation. Next, a phase 2 study of cinrebafusp alfa in combination with ramucirumab and paclitaxel in patients with HER2-positive gastric or gastroesophageal junction cancer was initiated to further explore its activity (NCT05190445). Similar to dosing regimens for other HER2 agents, a loading dose was employed in this study. Eighteen mg/kg Q2W was administered in cycle 1, to limit any impact of TMDD, followed by 8 mg/kg Q2W in subsequent cycles. Given the safety profile of cinrebafusp alfa, considering its utility in combination with several other agents is plausible. Beyond cytotoxic or antiangiogenic molecules, the assessment of cinrebafusp alfa in combination with other HER2-targeting agents including small molecules or ADC's are worthy of exploration given potential complimentary mechanisms of action.

In conclusion, the results from this phase I study provide clinical evidence that tumor targeted 4-1BB agonism is tolerable and exhibits antitumor activity in a heavily pretreated HER2-positive patient population. Based on its bispecific design, cinrebafusp alfa enhances immune cell activation while avoiding the hepatotoxicity associated with conventional 4-1BB antibodies. The differentiated mechanism of action of cinrebafusp alfa is corroborated by extensive biomarker data while encouraging single agent activity support its future clinical evaluation.

Authors' Disclosures

S. Piha-Paul reports other support from AbbVie, Inc., ABM Therapeutics, Inc., Acepodia, Inc., Alkermes, Aminex Therapeutics, BioMarin Pharmaceutical, Inc., Boehringer Ingelheim, Bristol Myers Squibb, Cerulean Pharma, Inc., Chugai Pharmaceutical Co., Ltd., Cullinan Oncology, Inc., Curis, Inc., Cyclacel Pharmaceuticals, Daiichi Sankyo, Inc., Eli Lilly, ENB Therapeutics, Epigenetix Inc., Five Prime Therapeutics, F-Star Beta, Limited, Gene Quantum Healthcare, Genmab A/S, Gilead Sciences, Inc., GlaxoSmithKline, Helix BioPharma Corp., Hengrui Pharmaceuticals, Co., Ltd., HiberCell, Inc., Immunomedics, Inc., Incyte Corp., Innovent Biologics, Co., Ltd., Jacobio Pharmaceuticals Co., Ltd., Jazz Pharmaceuticals, Jiangsu Simcere Pharmecutical Co., Ltd., Johnson & Johnson, Loxo Oncology, Inc., Lytix Biopharma AS, Medimmune, LLC, Medivation, Inc., Merck, Sharp and Dohme Corp., Nectin Therapeutics, Ltd., Novartis Pharmaceuticals, NRG Oncology, Nurix, OncoNano Medicine, Inc., Pieris Pharmaceuticals, Inc., Pfizer, Phanes Therapeutics, Principia Biopharma, Inc., ProfoundBio US Co., Puma Biotechnology, Inc., Purinomia Biotech, Inc., Rapt Therapeutics, Inc., Replimune, Roche/Blueprint, Seattle Genetics, Shasqi, Inc., Silverback Therapeutics, Strand Therapeutics, Inc., Synologic Therapeutics, Taiho Oncology, Tallac Therapeutics, Inc., Tesaro, Inc., Theradex Oncology, Toragen Therapeutics, Inc., TransThera Bio, Xencor, Inc., ZielBio, Inc., and F-Star Therapeutics, Limited and grants from NCI/NIH P30CA016672 - Core Grant (CCSG Shared Resources) outside the submitted work; in addition, S. Piha-Paul reports work as a consultant for CRC Oncology and Lilly USA, LLC. S.A. Olwill reports patents for WO2021089588A1 and WO2016177762A1 pending. E. Hamilton reports grants from Pieris Pharmaceuticals during the conduct of the study as well as grants from AbbVie, Acerta Pharma, ADC Therapeutics, Akesobio Australia, Amgen, Aravive, ArQule, Artios, AtlasMedx, BeiGene, Black Diamond, Bliss BioPharmaceuticals, Boehringer Ingelheim, Bristol Myers Squibb, Cascadian Therapeutics, Clovis, Compugen, Curis, Cytom X, Dana Farber Cancer Inst., Dantari, Deciphera, Duality Biologics, eFFECTOR Therapeutics, Eisai, Elucida Oncology, EMD Serono, Fochon Pharmaceuticals, FujiFilm, G1 Therapeutics, H3 Biomedicine, Harpoon, Hutchinson MediPharma, Immunogen, Immunomedics, Incyte, Infinity Pharmaceuticals, Inspirna, InvestisBio, Jacobio, Karyopharm, K-Group Beta, Kind Pharmaceuticals, Leap Therapetics, Loxo Oncology, Lycera, Mabspace Biosciences, Macrogenics, MedImmune, Merus, Millennium, Molecular Template, Myriad Genetic Laboratories, Nucana, OncoMed, Ocothyreon, ORIC Pharmaceuticals, Orinove, Orum Therapeutics, Pharma Mar, Pieris Pharmaceuticals, Pionyr Immunotherapeutics, Plexxikon, Prelude Therapeutics, Profound Bio, Radius Health, Regeneron, Rela Therapeutics, Repertoire Immune Medicine, Rgenix, Seagen, Sermonix Pharmaceuticals, Shattuck Labs, Silverback Therapeutics, StemCentRx, Sutro, Syndax, Syros, Taiho, TapImmune, Tesaro, Tolmar, Torque Therapeutics, Treadwell Therapeutics, Verastem, Zenith Epigenetics, and Zymeworks; grants and other support from Accutar Biotechnology, Arvinas, AstraZeneca, Daiichi Sankyo, Ellipses Pharma, Gilead Sciences, Lilly, Mersana, Novartis, Pfizer, Roche/Genentech, and Stemline Therapeutics; and other support from Circle Pharma, Entos, Fosun Pharma, Janssen, Jazz Pharmaceuticals, Jefferies LLC, Johnson and Johnson, Medical Pharma Services, Tempus Labs, Theratechnologies, Tubulis, Verascity Science, and Zentalis Pharmaceuticals outside the submitted work. A. Tolcher reports personal fees and other support from AbbVie, Inc., Aclaris Therapeutics, Affinia Therapeutics, Inc., Agenus, Inc., Asana Biosciences, Ascentage, Astex Pharmaceuticals, Axlmmune, Bayer, Blu Print Oncology, Coretag Therapeutics, Daiichi Sankyo, Inc., Exelixis, Inc., FibroGen, Gilde Healthcare Partners, HBM Partners, IDEA Pharma, Ikena Oncology, Immuneering, Immunomet Therapeutics, Inc., Impact Therapeutics US, Inc., Karma Oncology B.V., Kirilys Therapeutics, Inc., Lengo Therapeutics, Inc., Link Immunotherapeutics, Medicxi, Merck KGA, Mekanistic Therapeutics, Menarini Ricerche, Mersana, Nanobiotix, Nerviano Medical Sciences S.r.I. (NMS), Novo Nordisk Inc., Novo Ventures, Nurix Therapeutics, Ocellaris Pharma, Inc. & Eli Lilly, Partner Therapeutics, Pfizer Inc., Pierre Fabre, Praxia Precision Medicines, Pyramid Biosciences, Qualigen Therapeutics, Roche, Ryvu Therapeutics, Seattle Genetics, Singzyme Pte Ltd., SK Life Science, SOTIO Biotechnology Co., Senti Biosciences, Sun Pharma Advanced Research Company (SPARC), TheraTechnologies, Transcenta Therapeutics Inc., Transgene, Trillium Therapeutics Inc., Tubulis, Venus Oncology, Verastem Oncology, and Vida Ventures Advisors, LLC during the conduct of the study as well as personal fees and other support from AbbVie, Inc., Aclaris Therapeutics, Affinia Therapeutics, Inc., Agenus, Inc., Asana Biosciences, Ascentage, Astex Pharmaceuticals, Axlmmune, Bayer, Blu Print Oncology, Coretag Therapeutics, Daiichi Sankyo, Inc., Exelixis, Inc., FibroGen, Gilde Healthcare Partners, HBM Partners, IDEA Pharma, Ikena Oncology, Immuneering, Immunomet Therapeutics, Inc., Impact Therapeutics US, Inc., Karma Oncology B.V., Kirilys Therapeutics, Inc., Lengo Therapeutics, Inc., Link Immunotherapeutics, Medicxi, Merck KGA, Mekanistic Therapeutics, Menarini Ricerche, Mersana, Nanobiotix, Nerviano Medical Sciences S.r.I. (NMS), Novo Nordisk Inc., Novo Ventures, Nurix Therapeutics, Ocellaris Pharma, Inc. & Eli Lilly, Partner Therapeutics, Pfizer Inc., Pierre Fabre, Praxia Precision Medicines, Pyramid Biosciences, Qualigen Therapeutics, Roche, Ryvu Therapeutics, Seattle Genetics, Singzyme Pte Ltd., SK Life Science, SOTIO Biotechnology Co., Senti Biosciences, SPARC, TheraTechnologies, Transcenta Therapeutics Inc., Transgene, Trillium Therapeutics Inc., Tubulis, Venus Oncology, Verastem

Oncology, Vida Ventures Advisors, LLC, Cytel, Mythic Therapeutics, Adagene, Inc., Bioinvent, Boehringer Ingelheim International GmbH, Bright Peak Therapeutics, Cullinan Oncology, Elucida Oncology, EMD Serono/Merck KGaA, Hexagon Bio, Inc., Immunome, Jazz, Leerink, Mirati, NBE Therapeutics, Nested Therapeutics, Inc., Pheon Therapeutics, Pyxis Oncology, Roche, SPARC, Spirea Limited Inc., Tagworks Pharmaceuticals, Vincerx, VRise Therapeutics, Inc., Zentalis Pharmaceuticals, and ZielBio, Inc. outside the submitted work; in addition, A. Tolcher reports patents for Zentalis and Acentage Pharma issued. P. Pohlmann reports other support from Pieris Pharmaceuticals during the conduct of the study as well as personal fees from SABCS, OncLive, Frontiers in Oncology, and Pfizer and other support from Orum, Carisma, and SeaGen outside the submitted work; in addition, P. Pohlmann reports patents for US-9745377, US-8501417, US-8486413, and US-9023362 licensed to Immunonet. S.V. Liu reports grants and personal fees from AbbVie, AstraZeneca, Elevation Oncology, Genentech, Gilead, Merck, Merus, OSE Immunotherapeutics, and RAPT; personal fees from Amgen, Boehringer Ingelheim, Bristol Myers Squibb, Catalyst, Daiichi Sankyo, Guardant Health, Janssen, Jazz Pharmaceuticals, Mirati, Novartis, Pfizer, Regeneron, Revolution Medicines, Sanofi, Takeda, Yuhan, and Candel Therapeutics; and grants from Alkermes, Ellipses, Nuvalent, Puma, and Turning Point Therapeutics outside the submitted work. R. Shroff reports other support from AstraZeneca, Boehringer Ingelheim Phar, Clovis, Genentech, IMV Inc., Incyte, Merck, Servier, Zymeworks, Syros, Astellas, Natera, Hookipa Pharm., AbbVie, Duo Oncology, Ability Pharm., and Elevar Therapeutics; non-financial support from Bayer, Bristol Myers Squibb, Exelixis Pharm., Loxo, Novocure, NuCana, Pieris, Rafael Pharm., and SeaGen; and non-financial and other support from QED Therapeutics and Taiho outside the submitted work. S. Hurvitz reports grants and non-financial support from Pieris during the conduct of the study as well as grants from Ambrx, Amgen, Bayer, Celcuity, Daiichi Sankyo, Dantari, Gilead/ImmunoMedics, Eli Lilly/Loxo, Novartis, Orinove, Orum, Phoenix Molecular Designs, Puma, Radius, Sanofi, SeaGen, and Zymeworks/Jazz-and grants and other support from Arvinas, AstraZeneca, Genentech/Roche, G1 Therapeutics, OBI Pharma, and Pfizer outside the submitted work. N. Hahn reports grants from NCI P30CA006973, NCI R01CA235681, HTG Molecular Diagnostics, AstraZeneca, Bristol Myers Squibb, Genentech, Seattle Genetics, Pieris, Inovio, Principia Biopharma, Incyte, Loxo Oncology, Ikena Oncology, and Daiichi Sankyo during the conduct of the study as well as personal fees from AstraZeneca, Merck, BioGears, Seattle Genetics, Mirati, Incyte, Janssen, Pfizer, EMD Serono, Verity Pharmaceuticals, Huron Consulting, Guidepoint, Natera, Protara Therapeutics, Astellas Pharma, Tyra Biosciences, Medscape outside the submitted work; in addition, N. Hahn is a co-author on a provisional US patent for an immune-based biomarker to predict response to immunotherapy in patients with advanced bladder cancer. M. Zettl reports patents for WO2021089588A1 and US20230366884A1 pending. G. Ku reports grants and personal fees from Pieris during the conduct of the study as well as grants and personal fees from AstraZeneca, Bristol Myers Squibb, Daiichi Sankyo, I-MAB, Merck, Zymeworks, and Jazz and grants from Triumvira outside the submitted work. No disclosures were reported by the other authors.

Authors' Contributions

S. Piha-Paul: Conceptualization, investigation, methodology, writing–original draft, writing–review and editing. **S.A. Olwill:** Conceptualization, data curation, software, formal analysis, validation, methodology, writing–original draft, writing–review and editing. **E. Hamilton:** Investigation, writing–review and editing. **A. Tolcher:** Investigation, writing–review and editing. **P. Pohlmann:** Investigation, writing–review and editing. **S.V. Liu:** Investigation, writing–review and editing. **C. Wurzenberger:** Data curation, software, formal analysis, validation, methodology, writing–original draft, writing–review and editing. **L.-C. Hasenkamp:** Resources, supervision, writing–original draft, writing–review and editing. **E.-M. Hansbauer:** Data curation, software, formal analysis, validation, methodology, writing–original draft, writing–review and editing. **R. Shroff:** Investigation, writing–review and editing. **S. Hurvitz:** Conceptualization, investigation, writing–review and editing. **A. Krishnamurthy:** Investigation, writing–review and editing. **A. Patnaik:** Conceptualization, investigation, writing–review and editing. **N. Hahn:** Conceptualization, investigation, writing–review and editing. **R. Kumar:** Data curation, software, formal analysis, validation, methodology, writing–review and editing. **M. Duerr:** Resources, data curation, software, formal analysis, supervision, validation, methodology, writing–review and editing. **M. Zettl:** Data curation, software, formal analysis, validation, methodology, writing–review and editing. **K. Aviano:** Conceptualization, resources, supervision, writing–review and editing. **L. Matis:** Conceptualization, data curation, software, formal analysis, validation, methodology, writing–review and editing. **I. Bruns:** Conceptualization, resources, supervision, writing–review and editing. **G. Ku:** Conceptualization, investigation, writing–original draft, writing–review and editing.

Acknowledgments

The study was funded by Pieris Pharmaceuticals. We would like to thank the patients as well as their families and caregivers, for participating in this trial. We would like to thank Prabhu Rajagopalan for his assistance with analysis of pharmacokinetic data. We would like to thank the extended Pieris team who assisted in data interpretation, in particular Aizea Morales Kastresana, Corinna Schlosser, Richa Bharti, and Ralph Graeser, for their support with management of biomarker studies and initial data interpretation; Martha Leibbrandt who provided regulatory input; and Sebastian Hogl for his review and assistance in preparation of this manuscript.

Note

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

Received May 17, 2024; revised July 10, 2024; accepted September 3, 2024; published first September 5, 2024.

References

1. Nixon NA, Blais N, Ernst S, Kollmannsberger C, Bebb G, Butler M, et al. Current landscape of immunotherapy in the treatment of solid tumours, with future opportunities and challenges. Curr Oncol 2018; 25:e373–84.
2. Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, et al. Five-year survival and correlates among patients with advanced melanoma, renal cell carcinoma, or non–small cell lung cancer treated with nivolumab. JAMA Oncol 2019;5:1411–20.
3. Marei HE, Hasan A, Pozzoli G, Cenciarelli C. Cancer immunotherapy with immune checkpoint inhibitors (ICIs): potential, mechanisms of resistance, and strategies for reinvigorating T cell responsiveness when resistance is acquired. Cancer Cell Int 2023;23:64.
4. Kwon BS, Weissman SM. cDNA sequences of two inducible T-cell genes. Proc Natl Acad Sci U S A 1989;86:1963–7.
5. Hurtado JC, Kim YJ, Kwon BS. Signals through 4-1BB are costimulatory to previously activated splenic T cells and inhibit activation-induced cell death. J Immunol 1997;158:2600–9.
6. Lee H-W, Park S-J, Choi BK, Kim HH, Nam K-O, Kwon BS. 4-1BB promotes the survival of CD8$^+$ T lymphocytes by increasing expression of Bcl-x L and Bfl-1. J Immunol 2002;169:4882–8.
7. Teijeira A, Garasa S, Etxeberria I, Gato-Cañas M, Melero I, Delgoffe GM. Metabolic consequences of T-cell costimulation in anticancer immunity. Cancer Immunol Res 2019;7:1564–9.
8. Menk AV, Scharping NE, Rivadeneira DB, Calderon MJ, Watson MJ, Dunstane D, et al. 4-1BB costimulation induces T cell mitochondrial function and biogenesis enabling cancer immunotherapeutic responses. J Exp Med 2018;215:1091–100.
9. Leem G, Park J, Jeon M, Kim E-S, Kim SW, Lee YJ, et al. 4-1BB co-stimulation further enhances anti-PD-1-mediated reinvigoration of exhausted CD39$^+$ CD8 T cells from primary and metastatic sites of epithelial ovarian cancers. J Immunother Cancer 2020;8:e001650.
10. Melero I, Shuford WW, Newby SA, Aruffo A, Ledbetter JA, Hellström KE, et al. Monoclonal antibodies against the 4-1BB T-cell activation molecule eradicate established tumors. Nat Med 1997;3:682–5.
11. Segal NH, Logan TF, Hodi FS, McDermott D, Melero I, Hamid O, et al. Results from an integrated safety analysis of urelumab, an agonist anti-CD137 monoclonal antibody. Clin Cancer Res 2017;23:1929–36.
12. Fisher TS, Kamperschroer C, Oliphant T, Love VA, Lira PD, Doyonnas R, et al. Targeting of 4-1BB by monoclonal antibody PF-05082566 enhances T-cell function and promotes anti-tumor activity. Cancer Immunol Immunother 2012;61:1721–33.
13. Gopal AK, Levy R, Houot R, Patel SP, Popplewell L, Jacobson C, et al. First-in-human study of utomilumab, a 4-1BB/CD137 agonist, in combination with rituximab in patients with follicular and other CD20$^+$ non-hodgkin lymphomas. Clin Cancer Res 2020;26:2524–34.
14. Ascierto PA, Simeone E, Sznol M, Fu Y-X, Melero I. Clinical experiences with anti-CD137 and anti-PD1 therapeutic antibodies. Semin Oncol 2010;37:508–16.

15. Segal NH, He AR, Doi T, Levy R, Bhatia S, Pishvaian MJ, et al. Phase I study of single-agent utomilumab (PF-05082566), a 4-1BB/CD137 agonist, in patients with advanced cancer. Clin Cancer Res 2018;24:1816–23.
16. Li Y, Tan S, Zhang C, Chai Y, He M, Zhang CW-H, et al. Limited cross-linking of 4-1BB by 4-1BB ligand and the agonist monoclonal antibody utomilumab. Cell Rep 2018;25:909–20.e4.
17. Bougnoux P, Verrelle P, Chassagne J. [Intratumoral biological markers in breast cancers]. Rev Prat 1992;42:1935–42.
18. Dobianer K, Hruza C, Ehrlich G, Sevelda P, Czerwenka K, Soreq H, et al. HER-2 amplification but not butyrylcholinesterase multability reflects aggressiveness of European-originated ovarian tumors. Gynecol Oncol 1995;56:200–6.
19. Mazières J, Peters S, Lepage B, Cortot AB, Barlesi F, Beau-Faller M, et al. Lung cancer that harbors an HER2 mutation: epidemiologic characteristics and therapeutic perspectives. J Clin Oncol 2013;31:1997–2003.
20. Siena S, Sartore-Bianchi A, Marsoni S, Hurwitz HI, McCall SJ, Penault-Llorca F, et al. Targeting the human epidermal growth factor receptor 2 (HER2) oncogene in colorectal cancer. Ann Oncol 2018;29:1108–19.
21. Shigematsu H, Takahashi T, Nomura M, Majmudar K, Suzuki M, Lee H, et al. Somatic mutations of the HER2 kinase domain in lung adenocarcinomas. Cancer Res 2005;65:1642–6.
22. Schechter AL, Stern DF, Vaidyanathan L, Decker SJ, Drebin JA, Greene MI, et al. The neu oncogene: an erb-B-related gene encoding a 185,000-Mr tumour antigen. Nature 1984;312:513–6.
23. Heinmöller P, Gross C, Beyser K, Schmidtgen C, Maass G, Pedrocchi M, et al. HER2 status in non-small cell lung cancer: results from patient screening for enrollment to a phase II study of herceptin. Clin Cancer Res 2003;9:5238–43.
24. Verma S, Miles D, Gianni L, Krop IE, Welslau M, Baselga J, et al. Trastuzumab emtansine for HER2-positive advanced breast cancer. N Engl J Med 2012;367:1783–91.
25. Bang Y-J, Van Cutsem E, Feyereislova A, Chung HC, Shen L, Sawaki A, et al. Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): a phase 3, open-label, randomised controlled trial. Lancet 2010;376:687–97.
26. Modi S, Saura C, Yamashita T, Park YH, Kim S-B, Tamura K, et al. Trastuzumab deruxtecan in previously treated HER2-positive breast cancer. N Engl J Med 2020;382:610–21.
27. Van Cutsem E, di Bartolomeo M, Smyth E, Chau I, Park H, Siena S, et al. Trastuzumab deruxtecan in patients in the USA and Europe with HER2-positive advanced gastric or gastroesophageal junction cancer with disease progression on or after a trastuzumab-containing regimen (DESTINY-Gastric02): primary and updated analyses from a single-arm, phase 2 study. Lancet Oncol 2023;24:744–56.
28. Gradishar WJ, Moran MS, Abraham J, Aft R, Agnese D, Allison KH, et al. Breast cancer, version 3.2022, NCCN Clinical Practice Guidelines in Oncology. J Natl Compr Canc Netw 2022;20:691–722.
29. Ajani JA, D'Amico TA, Bentrem DJ, Chao J, Cooke D, Corvera C, et al. Gastric cancer, version 2.2022, NCCN Clinical Practice Guidelines in Oncology. J Natl Compr Canc Netw 2022;20:167–92.
30. Goto K, Goto Y, Kubo T, Ninomiya K, Kim S-W, Planchard D, et al. Trastuzumab deruxtecan in patients with HER2-mutant metastatic non-small-cell lung cancer: primary results from the randomized, phase II DESTINY-Lung02 trial. J Clin Oncol 2023;41:4852–63.
31. Tsurutani J, Iwata H, Krop I, Jänne PA, Doi T, Takahashi S, et al. Targeting her2 with trastuzumab deruxtecan: a dose-expansion, phase I study in multiple advanced solid tumors. Cancer Discov 2020;10:688–701.
32. Modi S, Park H, Murthy RK, Iwata H, Tamura K, Tsurutani J, et al. Antitumor activity and safety of trastuzumab deruxtecan in patients with HER2-low–expressing advanced breast cancer: results from a phase Ib study. J Clin Oncol 2020;38:1887–96.
33. Harding JJ, Fan J, Oh D-Y, Choi HJ, Kim JW, Chang H-M, et al. Zanidatamab for HER2-amplified, unresectable, locally advanced or metastatic biliary tract cancer (HERIZON-BTC-01): a multicentre, single-arm, phase 2b study. Lancet Oncol 2023;24:772–82.
34. Hinner MJ, Aiba RSB, Jaquin TJ, Berger S, Dürr MC, Schlosser C, et al. Tumor-localized costimulatory T-cell engagement by the 4-1BB/HER2 bispecific antibody-anticalin fusion PRS-343. Clin Cancer Res 2019;25:5878–89.
35. Wolff AC, Hammond MEH, Hicks DG, Dowsett M, McShane LM, Allison KH, et al. Recommendations for human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists Clinical Practice Guideline update. J Clin Oncol 2013;31:3997–4013.
36. Rüschoff J, Hanna W, Bilous M, Hofmann M, Osamura RY, Penault-Llorca F, et al. HER2 testing in gastric cancer: a practical approach. Mod Pathol 2012;25:637–50.
37. Peters S, Angevin E, Alonso-Gordoa T, Rohrberg K, Melero I, Mellado B, et al. Obinutuzumab pretreatment as a novel approach to mitigate formation of anti-drug antibodies against cergutuzumab amunaleukin in patients with solid tumors. Clin Cancer Res 2024;30:1630–41.
38. Herbst RS, Soria J-C, Kowanetz M, Fine GD, Hamid O, Gordon MS, et al. Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients. Nature 2014;515:563–7.
39. Segal NH, Logan TF, Hodi FS, McDermott D, Melero I, Hamid O, et al. Results from an integrated safety analysis of urelumab, an agonist anti-CD137 monoclonal antibody. Clin Cancer Res 2017;23:1929–36.
40. Klein C, Brinkmann U, Reichert JM, Kontermann RE. The present and future of bispecific antibodies for cancer therapy. Nat Rev Drug Discov 2024;23:301–19.
41. Krop IE, Beeram M, Modi S, Jones SF, Holden SN, Yu W, et al. Phase I study of trastuzumab-DM1, an HER2 antibody-drug conjugate, given every 3 Weeks to patients with HER2-positive metastatic breast cancer. J Clin Oncol 2010;28:2698–704.
42. Updegraff B, Urban J, Mutschler J, Szeto GL, O'Connor BP, Gardai SJ, et al. Abstract 5676: SGN-BB228, a CD228-directed costimulatory antibody anticalin® bispecific provides potent and conditional 4-1BB costimulation to T cells in vivo and in an in vitro model of T cell exhaustion. Cancer Res 2023;83:5676.
43. Pichler AC, Carrié N, Cuisinier M, Ghazali S, Voisin A, Axisa P-P, et al. TCR-independent CD137 (4-1BB) signaling promotes $CD8^+$-exhausted T cell proliferation and terminal differentiation. Immunity 2023;56:1631–48.e10.
44. Chester C, Sanmamed MF, Wang J, Melero I. Immunotherapy targeting 4-1BB: mechanistic rationale, clinical results, and future strategies. Blood 2018;131:49–57.
45. Muik A, Garralda E, Altintas I, Gieseke F, Geva R, Ben-Ami E, et al. Preclinical characterization and phase I trial results of a bispecific antibody targeting PD-L1 and 4-1BB (GEN1046) in patients with advanced refractory solid tumors. Cancer Discov 2022;12:1248–65.
46. Melero I, Tanos T, Bustamante M, Sanmamed MF, Calvo E, Moreno I, et al. A first-in-human study of the fibroblast activation protein-targeted, 4-1BB agonist RO7122290 in patients with advanced solid tumors. Sci Transl Med 2023;15:eabp9229.
47. Peper-Gabriel JK, Pavlidou M, Pattarini L, Morales-Kastresana A, Jaquin TJ, Gallou C, et al. The PD-L1/4-1BB bispecific antibody-anticalin fusion protein PRS-344/S095012 elicits strong T-cell stimulation in a tumor-localized manner. Clin Cancer Res 2022;28:3387–99.
48. Labiano S, Palazón A, Bolaños E, Azpilikueta A, Sánchez-Paulete AR, Morales-Kastresana A, et al. Hypoxia-induced soluble CD137 in malignant cells blocks CD137L-costimulation as an immune escape mechanism. Oncoimmunology 2015;5:e1062967.
49. Luu K, Shao Z, Schwarz H. The relevance of soluble CD137 in the regulation of immune responses and for immunotherapeutic intervention. J Leukoc Biol 2020;107:731–8.
50. Nielsen MA, Andersen T, Etzerodt A, Kragstrup TW, Rasmussen TK, Stengaard-Pedersen K, et al. A disintegrin and metalloprotease-17 and galectin-9 are important regulators of local 4-1BB activity and disease outcome in rheumatoid arthritis. Rheumatology (Oxford) 2016;55:1871–9.
51. Seidel J, Leitzke S, Ahrens B, Sperrhacke M, Bhakdi S, Reiss K. Role of ADAM10 and ADAM17 in regulating CD137 function. Int J Mol Sci 2021;22:2730.
52. Glez-Vaz J, Azpilikueta A, Olivera I, Cirella A, Teijeira A, Ochoa MC, et al. Soluble CD137 as a dynamic biomarker to monitor agonist CD137 immunotherapies. J Immunother Cancer 2022;10:e003532.