Mechanistic biomarker analyses following administration of BT7480, a novel Nectin-4/CD137 Bicycle tumor-targeted immune cell agonist®, in a Phase 1/2 study in patients with advanced solid tumors

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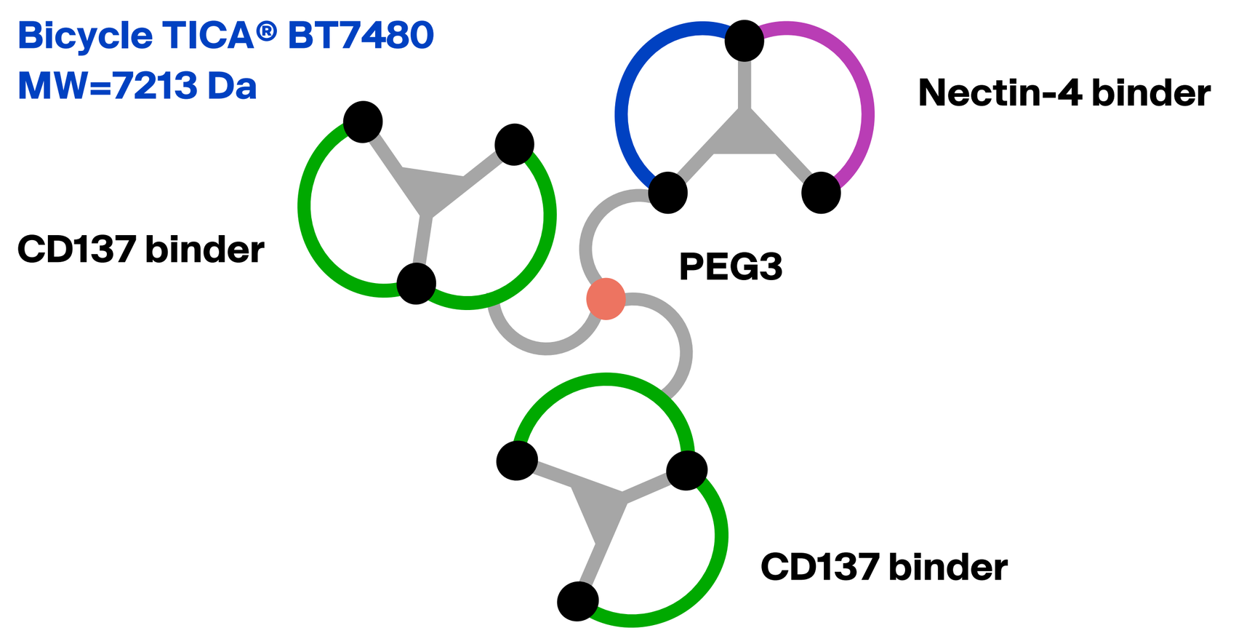
BACKGROUND

- Bicycle® molecules are an innovative therapeutic class in development that offers the manufacturing and pharmacokinetic properties of a small molecule with the high binding specificity of a biologic,[1–3] making them ideally suited for the targeted delivery of a range of payloads to solid tumors
- The Bicycle® molecule BT7480 is a novel, synthetic Bicycle tumor-targeted immune cell agonist® (Bicycle TICA®) comprising three bicyclic peptides, one targeting Nectin-4 and two targeting CD137, conjugated by a three-arm branched trimeric polyethylene glycol (PEG3) linker (Figure 1)[4,5]
  - Nectin-4 is overexpressed in many cancers, including lung, breast, esophageal, and head and neck cancers and in urothelial carcinoma (UC)[6–9]
  - CD137 is a member of the tumor necrosis factor (TNF) receptor superfamily; on ligation, CD137 provides costimulatory signals to immune cells, resulting in T-cell proliferation, anti-apoptosis, cytokine secretion, chromatin remodeling, and promotion of mitochondrial fitness; it is expressed on activated immune cells and is highly expressed in tumors[10–13]
  - Nectin-4 and CD137 co-ligation is hypothesized to cause tumor-localized CD137 agonism (based on preclinical findings)[4]
- An ongoing Phase 1/2 study (NCT05163041) is evaluating BT7480 ± nivolumab in patients with advanced solid tumors associated with Nectin-4 expression, with initial results demonstrating that BT7480 monotherapy is generally well tolerated with preliminary antitumor activity[14]
- Here we present a comprehensive clinical biomarker analysis of patients from the dose escalation part of the Phase 1/2 study who were treated with BT7480 monotherapy, to explore the downstream immune effects of CD137 agonism and to assess the translation of preclinical pharmacodynamics to patients

FIGURE 1. BT7480 STRUCTURE[4]



METHODS

- Adults with advanced solid tumors associated with Nectin-4 expression who were refractory to/ineligible for standard therapy were included in this open-label dose-escalation study; patients with prior CD137-targeted therapy were excluded
- BT7480 was administered as an intravenous infusion, starting at 0.002 mg/kg weekly; patients were enrolled sequentially to increasing doses using a 3 + 3 design to a dose of 3.5 mg/kg weekly
- Safety was the primary endpoint, with efficacy, pharmacokinetics, and CD137 target engagement in peripheral blood as secondary endpoints; additional biomarker analyses were exploratory endpoints
- A comprehensive biomarker strategy was used to inform dose selection of BT7480 and to provide proof-of-mechanism (Table 1)
  - Tumor tissue (archival or fresh) was collected at baseline for retrospective assessment of Nectin-4 and CD137 expression and measurement of immune cell infiltration
  - Peripheral blood samples were collected pre- and post-dosing to evaluate biomarkers associated with pharmacological activity and target engagement
- Immunohistochemistry (IHC) and multiplex immunofluorescence (mIF) were used to assess Nectin-4 and CD137 expression[4,15–16]; mIF was also used to assess immune cell infiltration in tumor tissue. Flow cytometry was used to determine CD137 receptor occupancy[17] and levels of activated CD4+ T cells, Olink® was used for profiling of soluble CD137 (sCD137) and CXCL9, and NanoString was used for mRNA profiling of peripheral blood samples

TABLE 1. BIOMARKER MONITORING STRATEGY

Tumor Biomarkers	Peripheral Biomarkers
▶ Nectin-4 protein expression	▶ CD137 receptor occupancy
▶ CD137 protein expression and immune cell infiltration	▶ Immune cell activation (transcriptomic and proteomic)
▶ Immune activation profiling and gene expression signatures[a]	▶ Cytokines and soluble mechanistic proteins
▶ Immune cell/tumor spatial proteomics[a]	▶ Antidrug antibody incidence monitoring[a]
	▶ Pharmacogenomics, circulating tumor DNA (ctDNA)[a]

[a]Data for these biomarkers will be presented at a later date.

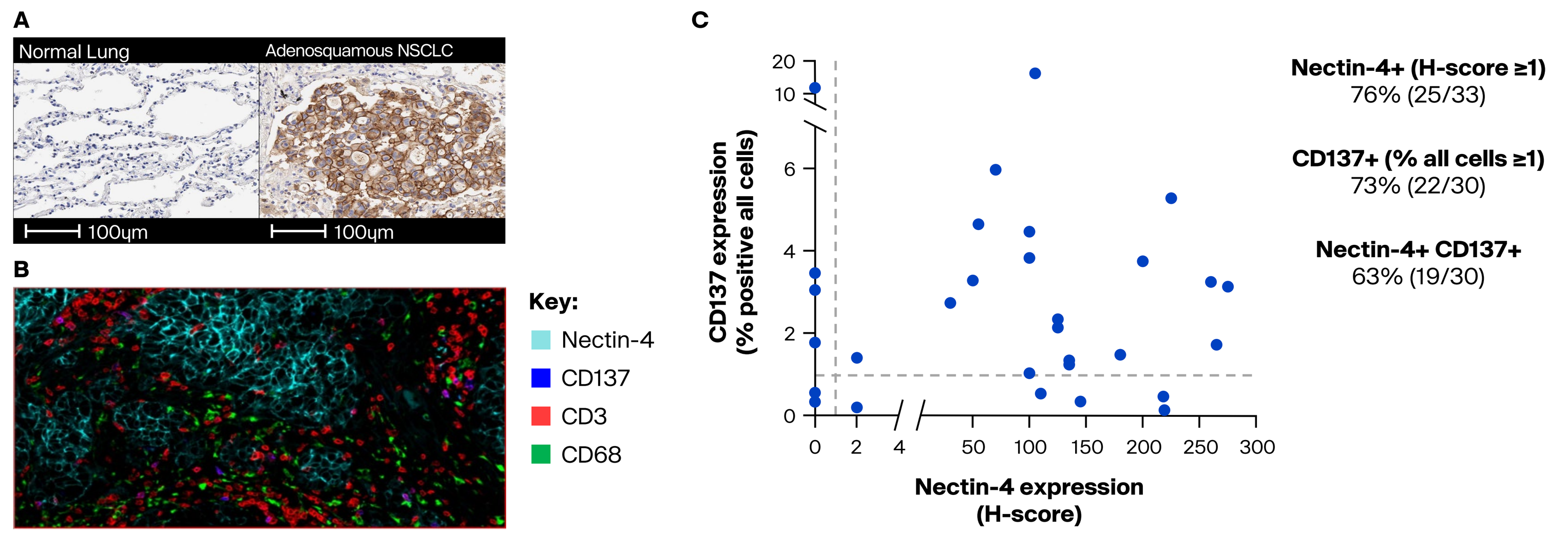
RESULTS

- As of April 29, 2024, 40 patients had received BT7480; evaluable patients per assay by tumor type are reported in Table 2
- Most patients were Nectin-4+ and CD137+ at baseline (Figure 2)
- In this small sample size, varying levels of Nectin-4 expression, CD137 expression, and immune cell infiltrate were seen across tumor types at baseline. Nectin-4 expression was highest in breast cancer, cervical cancer, UC, and non-small cell lung cancer (NSCLC); CD137 expression and immune cell infiltrate were lowest in patients with UC (Figure 3)

TABLE 2. EVALUABLE PATIENTS PER ASSAY BY TUMOR TYPE

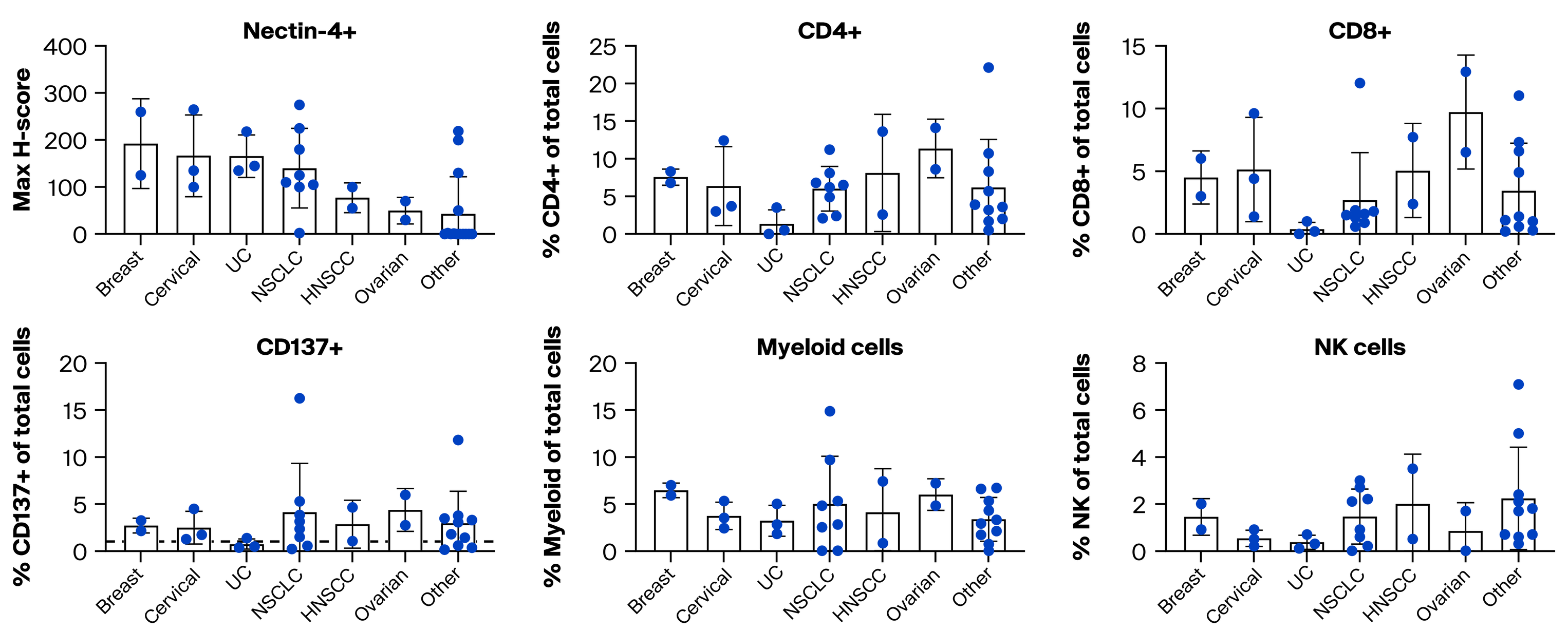
Primary Cancer Type, n (%)	Nectin-4 IHC n=33	CD137/immune markers n=30
NSCLC	8 (24)	8 (27)
Cervical	3 (9)	3 (10)
UC (transitional cell)	3 (9)	3 (10)
Breast	2 (6)	2 (7)
HNSCC	2 (6)	2 (7)
Ovarian	2 (6)	2 (7)
Other	13 (39)	10 (33)
Colorectal	5 (15)	2 (7)
Bladder (adenocarcinoma)	1 (3)	1 (3)
Esophageal	1 (3)	1 (3)
Hepatocellular	1 (3)	1 (3)
Intrahepatic bile duct cholangiocarcinoma	1 (3)	1 (3)
Pancreatic	1 (3)	1 (3)
Peripheral nerve sheath tumor	1 (3)	1 (3)
Squamous cell carcinoma of the anus	1 (3)	1 (3)
Thyroid	1 (3)	1 (3)

FIGURE 2. TUMOR BIOMARKER EXPRESSION



A) Nectin-4 staining of human lung (left) and adenosquamous NSCLC tumor (right) by IHC[15]; B) CD137, CD3, CD68, and Nectin-4 staining of human TNBC tumor by mIF[4,16]; C) Proportion of tumor cells co-expressing Nectin-4 and CD137 for each baseline tumor sample. Nectin-4 positivity was determined by IHC as ≥1 maximum H-score (highest of cytoplasmic or membrane staining score); CD137 positivity was determined by mIF as ≥1% positive of total cells in tumor area; dotted lines represent thresholds for determining positivity.

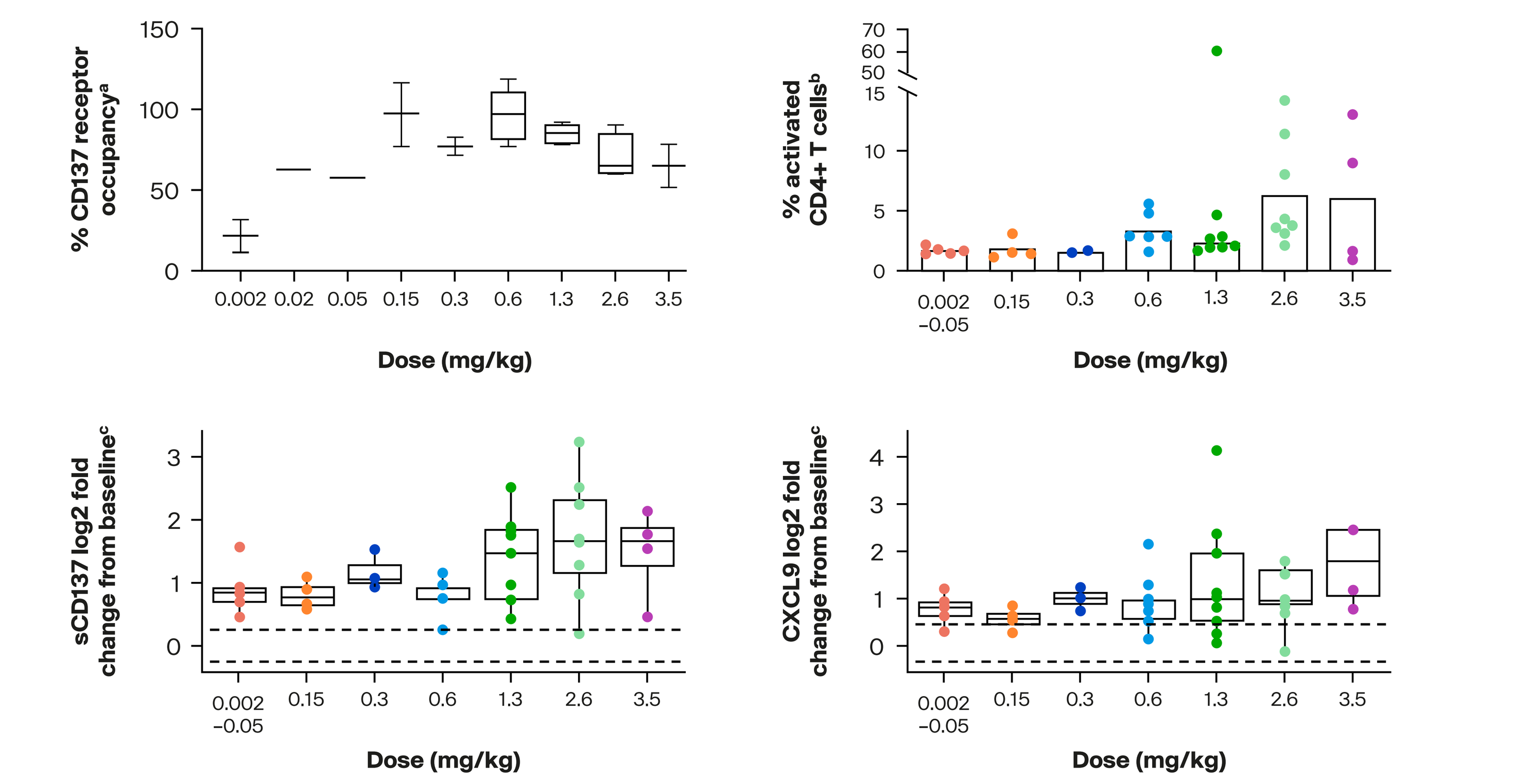
FIGURE 3. BIOMARKER EXPRESSION AND IMMUNE CELL INFILTRATE BY TUMOR TYPE[a]



[a]"Other" category includes colorectal, esophageal, bladder (adenocarcinoma), intrahepatic bile duct cholangiocarcinoma, peripheral nerve sheath tumor, hepatocellular, thyroid, squamous cell carcinoma of the anus, and pancreatic cancers. Myeloid cells were defined as CD3-CD68+; NK cells were defined as CD3-CD56+.

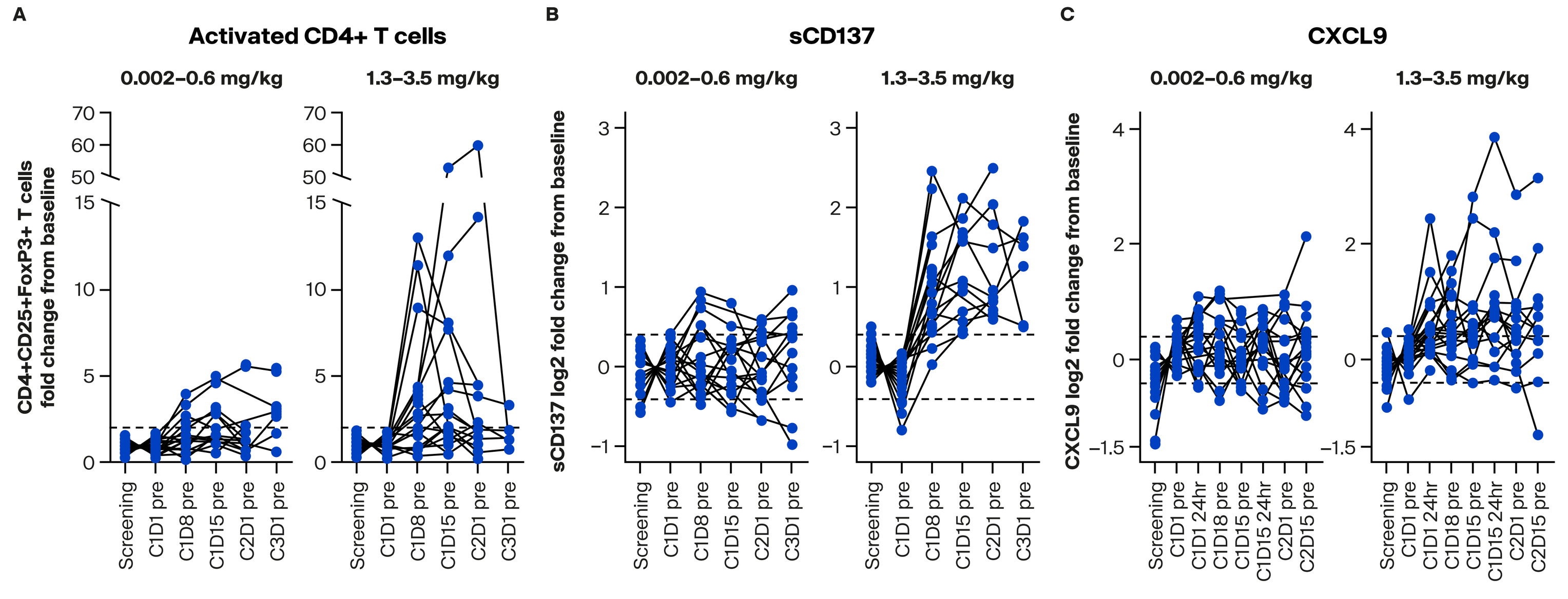
- Receptor occupancy of circulating CD137 was observed in a dose-dependent manner, with target saturation in peripheral blood at doses ≥0.15 mg/kg (Figure 4)
- Robust induction of activated CD4+ T cells, sCD137, and CXCL9 was observed through two cycles, with no hook effect observed at higher doses (Figure 4)
- Transient increases in immune activation markers occurred within the first two cycles at doses ≥1.3 mg/kg (Figure 5)

FIGURE 4. TARGET ENGAGEMENT AND INDUCTION OF IMMUNE CELL ACTIVATION SIGNALS IN PERIPHERAL BLOOD



[a]Measured at C1D1, 20 minutes post–end of infusion, divided by the baseline value. [b]Maximum value reported, through C2. [c]Maximum value of sCD137 and CXCL9 reported through C2D15. Each dot represents one patient; bars and horizontal lines represent the median; whiskers show the maximum and minimum values. Dashed lines = 1 standard deviation from baseline.

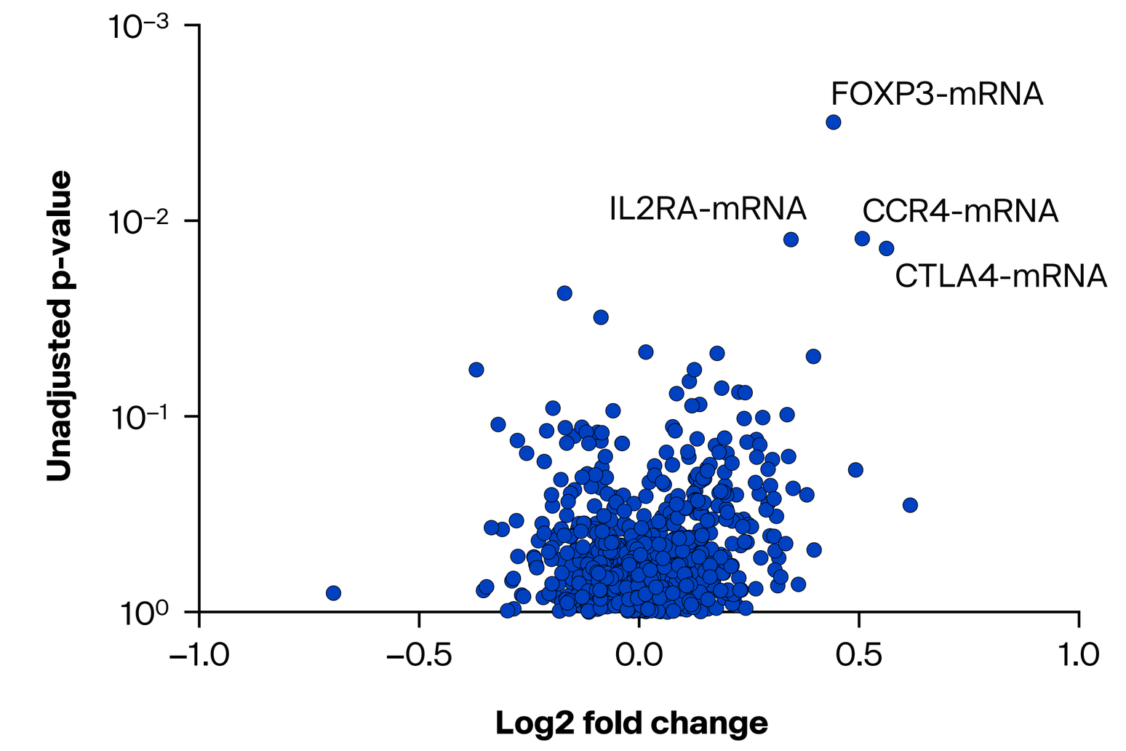
FIGURE 5. KINETICS OF IMMUNE CELL ACTIVATION SIGNALS IN PERIPHERAL BLOOD


A
Activated CD4+ T cells
B
sCD137
C
CXCL9
0.002–0.6 mg/kg
1.3–3.5 mg/kg
CD4+CD25+FoxP3+ T cells fold change from baseline
sCD137 log2 fold change from baseline
CXCL9 log2 fold change from baseline
Screening
C1D1 pre
C1D8 pre
C1D15 pre
C2D1 pre
C3D1 pre
C1D1 24hr
C1D18 pre
C1D15 24hr
C2D15 pre

A) CD4+CD25+FoxP3+ T cells were transiently elevated in patients' peripheral blood (evaluated by flow cytometry of PBMCs), with a greater magnitude of changes observed in dose levels of 1.3 mg/kg and above. Fold change calculated from average value of patient-matched baseline (screening and C1D1 pre-dose) samples; dashed line indicates fold change = 2. Increases in sCD137 (B) and CXCL9 (C) were observed in patient plasma measured by Olink® following treatment with BT7480, with a greater magnitude of changes observed in dose levels of 1.3 mg/kg and above. In B) and C), values are log2 fold change calculated from the average value from patient-matched baseline samples. Dashed lines indicate 1 standard deviation at baseline in log2 fold change among patients with both baseline samples.

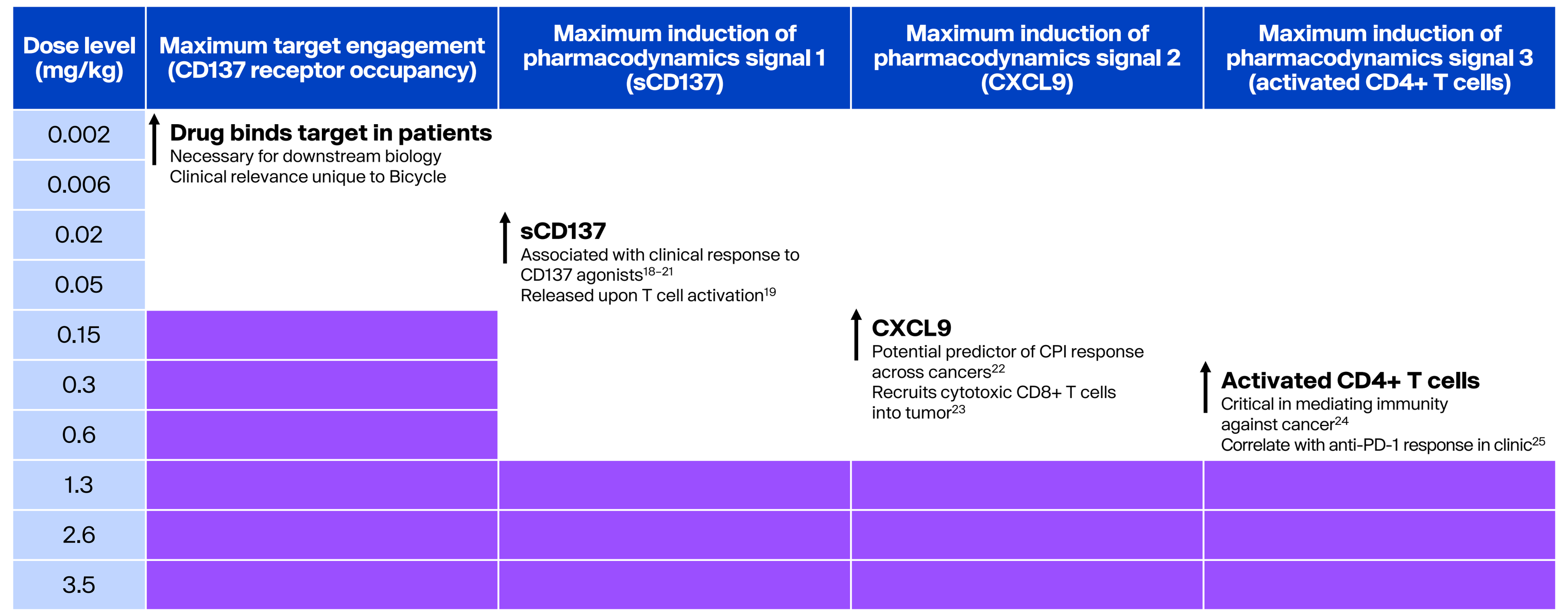
- RNA profiling of circulating immune cells demonstrated that administration of BT7480 led to immune cell reprogramming, promoting the expression of immune activation genes over baseline pretreatment samples (including *FOXP3, IL2RA, CCR4,* and *CTLA4* genes) (Figure 6)
- BT7480-induced pharmacodynamic changes reached a biological plateau in peripheral blood at doses of 1.3–3.5 mg/kg weekly (Figure 7)

FIGURE 6. INDUCTION OF GENES RELATED TO T-CELL ACTIVATION IN RESPONSE TO BT7480



mRNA isolated from patient peripheral blood was analyzed using the NanoString PanCancer IO 360™ panel; for each gene, the volcano plot shows log2 fold change of C2D1 post-dose samples vs C1D1 pre-dose samples for all evaluable patients (N=39).

FIGURE 7. BLOOD-BASED PHARMACODYNAMIC BIOMARKERS INFORM BT7480 DOSE SELECTION AND MECHANISM OF ACTION


Dose level (mg/kg)
Maximum target engagement (CD137 receptor occupancy)
Maximum induction of pharmacodynamics signal 1 (sCD137)
Maximum induction of pharmacodynamics signal 2 (CXCL9)
Maximum induction of pharmacodynamics signal 3 (activated CD4+ T cells)
0.002
0.006
0.02
0.05
0.15
0.3
0.6
1.3
2.6
3.5
Drug binds target in patients
Necessary for downstream biology
Clinical relevance unique to Bicycle
sCD137
Associated with clinical response to CD137 agonists18–21
Released upon T cell activation19
CXCL9
Potential predictor of CPI response across cancers22
Recruits cytotoxic CD8+ T cells into tumor23
Activated CD4+ T cells
Critical in mediating immunity against cancer24
Correlate with anti-PD-1 response in clinic25

Purple boxes represent maximum signal of biomarkers detected.

CONCLUSIONS

- Key clinical biomarker findings were consistent with preclinical mechanism of action observations and offer new mechanistic insights not previously reported for other CD137 agonists in the clinic (Figure 7)
- These data collectively indicate that BT7480 is a pharmacologically active CD137 agonist that induces innate and adaptive immune cell activation, thus supporting clinical activity as monotherapy in patients with tumors associated with Nectin-4 expression; additional cohorts are planned to investigate BT7480 in combination with nivolumab

ABBREVIATIONS

C, cycle; CCR4, C-C chemokine receptor type 4; CD, cluster of differentiation; CPI, checkpoint inhibitor; ctDNA, circulating tumor DNA; CTLA4, cytotoxic T-lymphocyte associated protein 4; CXCL9, C-X-C motif chemokine ligand 9; D, day; FOXP3, Forkhead box protein P3; HNSCC, head and neck squamous cell carcinoma; IHC, immunohistochemistry; IL2RA, interleukin-2 receptor alpha; mIF, multiplex immunofluorescence; mRNA, messenger RNA; MW, molecular weight; NK, natural killer; NSCLC, non-small cell lung cancer; PBMCs, peripheral blood mononuclear cells; PD-1, programmed cell death protein-1; PEG3, trimeric polyethylene glycol; sCD137, soluble CD137; TICA, tumor-targeted immune cell agonist; TNBC, triple-negative breast cancer; TNF, tumor necrosis factor; UC, urothelial carcinoma.

ACKNOWLEDGEMENTS

The authors would like to thank the participating patients and their families, clinicians, and the BT7480-100 study investigators. This study was sponsored by BicycleTx Ltd. Writing assistance was provided by Tamara Fink, PhD, and Becky Bradley, PhD, part of Avalere Health Group Limited, funded by BicycleTx Ltd.

KPP reports the following relationships: Consulting or Advisory Role - Basilea; Bicycle; Turning Point; Research Funding (institution) - 3D Medicines; AbbVie; ADC; Amgen; Anheart; AstraZeneca; Bayer; Bicycle; Biontech; CytomX; Daiichi Sankyo; Debiopharm Group; F-star; Incyte; Jounce; Kezar Life Sciences; Lilly; Linnaeus; MabSpace Biosciences; Merck; Mersana; Mirati; Monte Rosa; Pfizer; PharmaMar; Regeneron; Revolution Medicines; Sensei Biotherapeutics; Storm; Syros; Tempest; Treadwell.

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Presented at the 39th Annual Meeting of the Society for Immunotherapy of Cancer (SITC), Houston, Texas, USA, November 6–10, 2024