BT7480, a novel fully synthetic tumor-targeted immune cell agonist (TICA™) induces tumor localized CD137 agonism

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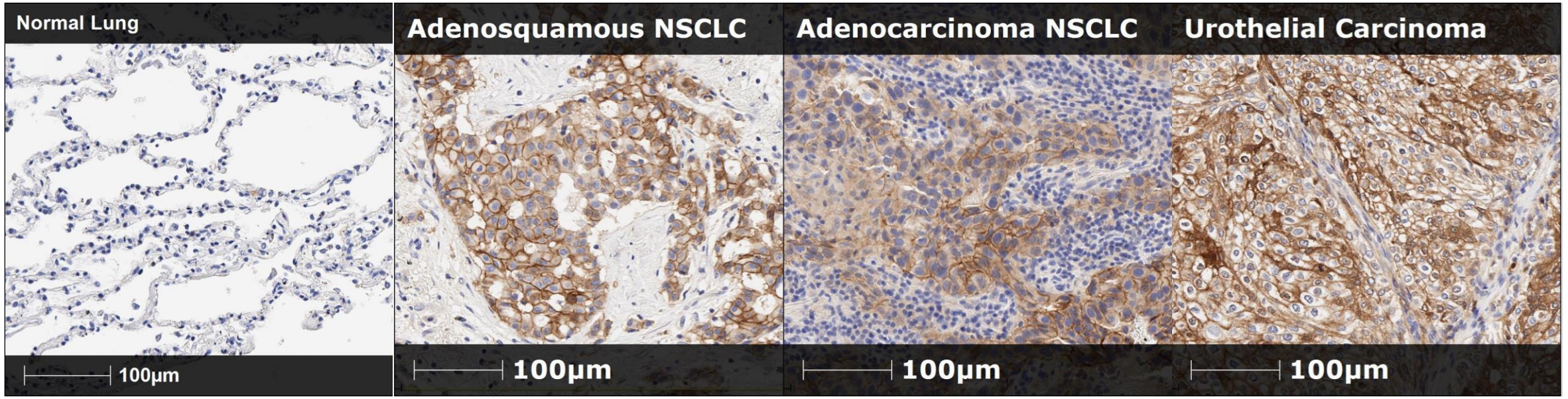
ABSTRACT

- CD137 (4-1BB/TNFRSF9) is a costimulatory molecule belonging to the TNF receptor superfamily that is expressed on activated T and NK cells.
- Despite compelling preclinical data, CD137 agonistic antibodies have been hampered by failure to delineate hepatotoxicity from efficacy in the clinic [1,2]. Next generation strategies are focused on bispecific approaches aimed at promoting target-mediated clustering of CD137 to limit systemic and liver toxicities [3,4].
- *Bicycles*® are fully synthetic, constrained bicyclic peptides that have high affinity and selectivity to their targets. We incorporated *Bicycle* binders specific for tumor antigens into multifunctional molecules with CD137 binding *Bicycles*. We termed these Tumor-targeted Immune Cell Agonists (TICAs). Unlike traditional biologic approaches, the small size (~4-8 kDa) and tunable pharmacokinetic (PK) parameters of *Bicycle* TICAs enable superior tumor penetration and allow exploration into the relationship between pulsatile dosing and CD137 activation while de-risking hepatoxicity concerns due to a differentiated renal elimination mechanism combined with a tumor-localized immune response.
- BT7480 is a TICA that activates CD137 by targeting the highly expressed tumor cell antigen Nectin-4 and demonstrates extremely potent CD137 agonism in primary human PBMC/tumor cell co-culture assays.
- Treatment of tumors expressing Nectin-4 with BT7480 in immune competent mouse models led to increased T cell infiltration and a cytotoxic gene signature.
- BT7480 induced complete regressions and resistance to re-challenge with intermittent dosing and the established immunologic memory was dependent on cytotoxic T cells.
- In non-human primates (NHPs), BT7480 exhibited dose linear exposure and is well tolerated up to 10 mg/kg. Liver enzymes and cytokines were not significantly altered by BT7480 in these healthy NHPs.

INTRODUCTION



<u>Figure 1:</u> Nectin-4/PVRL4 is a cell adhesion molecule that is highly expressed in multiple tumor types, including healthy lung tissue, non-small cell lung cancer (NSCLC) and urothelial (bladder) cancer. Representative images using a proprietary Nectin-4 IHC assay demonstrate clear Nectin-4 tumor cell membrane staining, with minimal background staining in non-tumor tissue.

INTRODUCTION

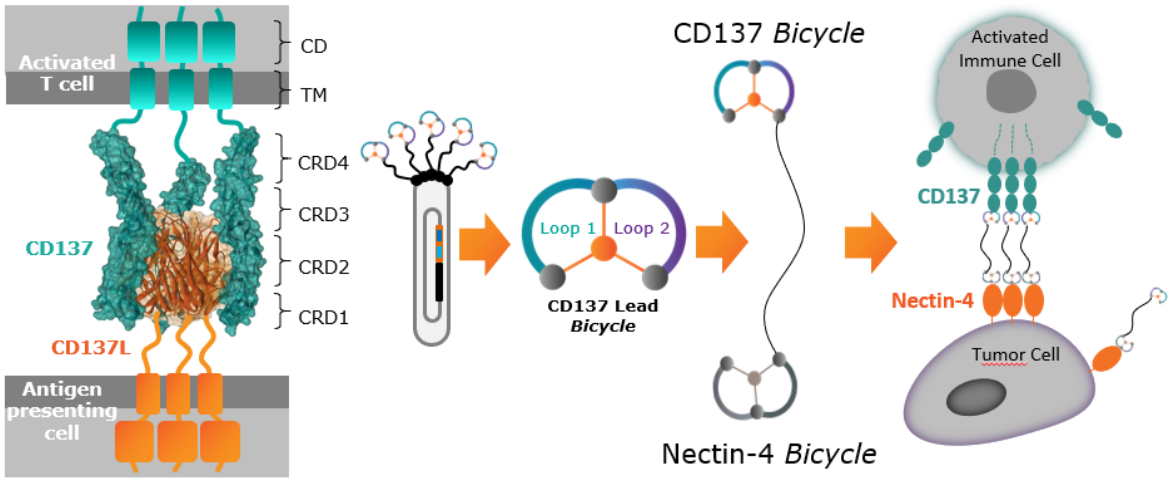


Figure 2: CD137 is a validated immunotherapy target that is expressed on T cells, NK cells, and other immune cells. CD137 requires trimerization for activation. Phage screening, affinity maturation, and chemical optimization resulted in the lead CD137-binding *Bicycle* that was then linked to a Nectin-4-binding *Bicycle*. Further chemical optimization yielded BT7480.

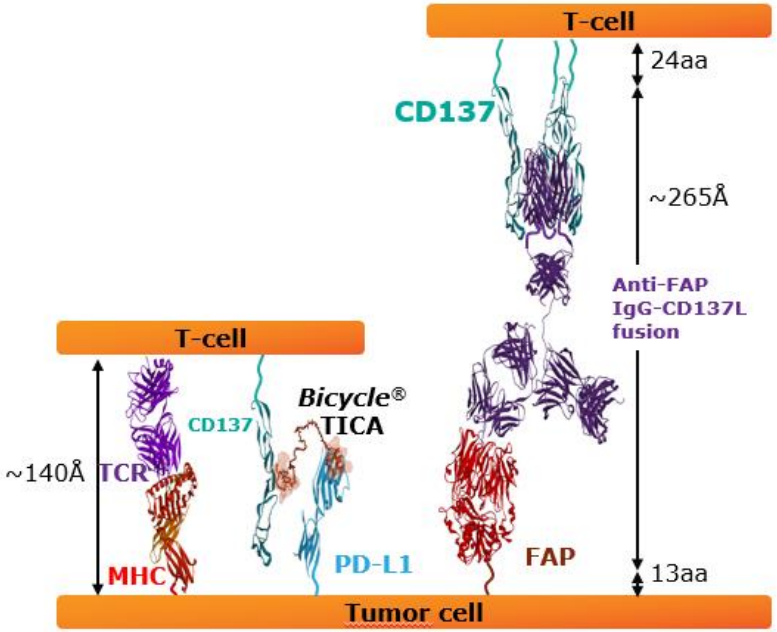


Figure 3: *Bicycle* TICAs enable optimum spacing compared to bulkier biologics. The typical immune cell receptor complex spacing formed through interaction of the TCR and MHC is 140Å. The representative *Bicycle* TICA illustrates the analogous spatial orientation compared to biologics bispecific molecules.

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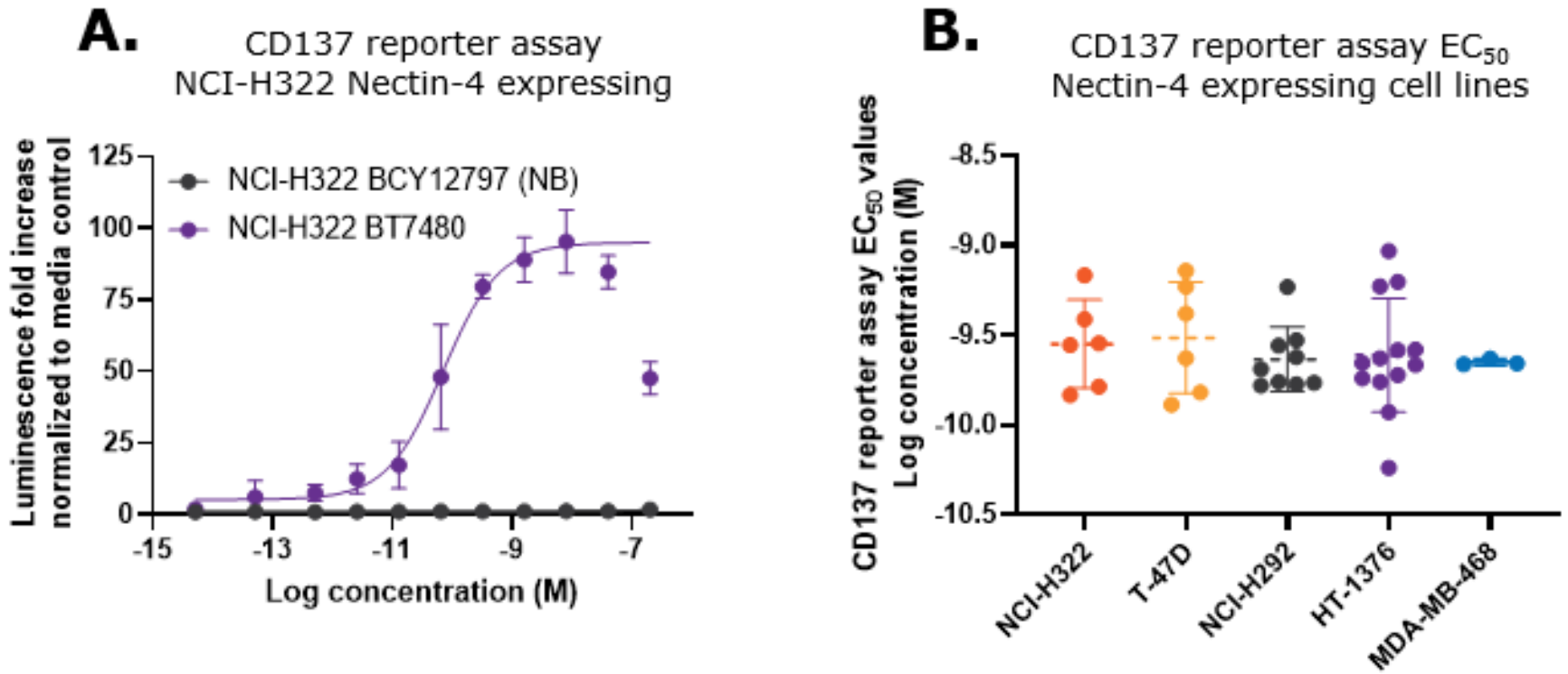
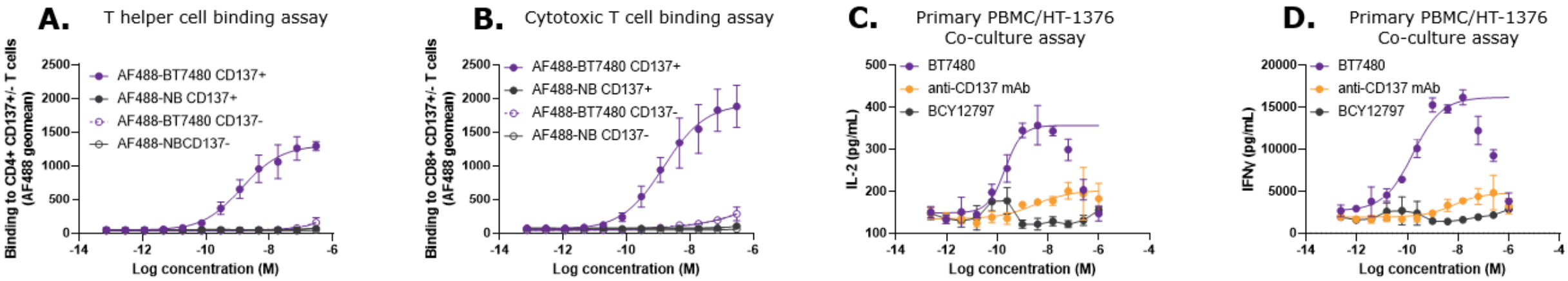
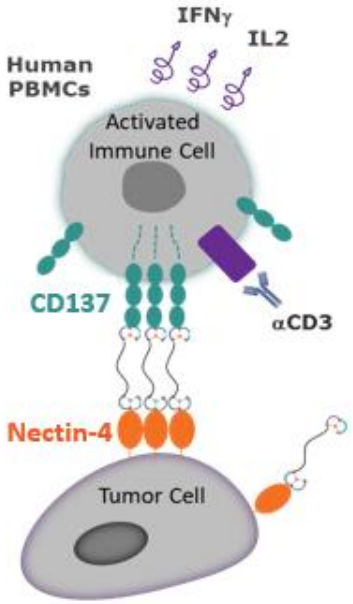
RESULTS



<u>Figure 4:</u> BT7480 a Nectin-4/CD137 TICA promoted a robust Nectin-4 dependent activity in a CD137 reporter assay. A) CD137-expressing Jurkat cells were co-cultured with Nectin-4 expressing cells and NFκB-driven luciferase production was monitored 6 hours post-treatment. BCY12797, a non-binding (NB) Nectin-4/CD137 *Bicycle* TICA represents a negative control and comparatively, BT7480 produced a robust response. B) The calculated EC_{50} values of BT7480 in co-culture with multiple Nectin-4 expressing cell lines including NCI-H322, T-47D, NCI-H292, HT-1376, and MDA-MB-468.

RESULTS





<u>Figure 5:</u> BT7480 bound specifically to CD137-expressing T cells and led to a Nectin-4-dependent increase in cytokine release in primary PBMC/tumor cell co-culture assays. A,B) BT7480 bound to primary T cells that express CD137. Human PBMCs were stimulated with anti-CD3 to induce CD137 expression. Alexa Fluor(AF)-488-tagged BT7480 binding to CD137+ T cells was monitored using flow cytometry. Binding to CD137-negative cells was not detected. AF488-(NB) is a non-binding analog of AF488-BT7480 and did not bind to T cells. C,D) BT7480 activity in a primary immune cell assay was Nectin-4 dependent. Human PBMCs were stimulated with anti-CD3 and co-cultured with human urothelial cancer cell line, HT1376 (n=3, +/-SD). BT7480 led to increased cytokine release in a co-culture assay using human PBMCs. A non-binding analog of BT7480, BCY12797, was inactive. The cytokine release induced by anti-CD137 antibody analog (Creative BioLabs) is shown for comparison.

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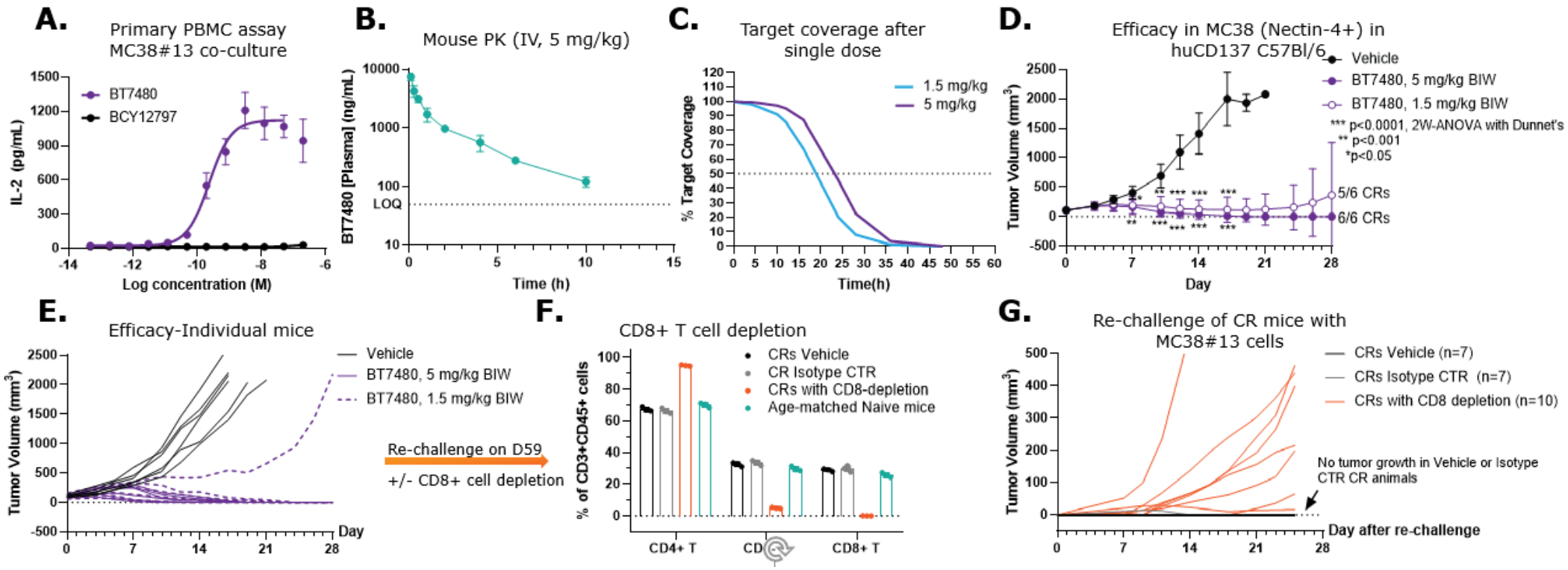

<u>Figure 6:</u> Intermittent dosing of BT7480 led to robust anti-tumor activity and resistance to re-challenge in a syngeneic mouse model. A) BT7480 activity in a human PBMC assay when in co-culture with MC38 cells that were engineered to express Nectin-4 (MC38#13). B) Plasma PK in CD1 mouse after a single IV dose of 5 mg/kg. C) With a 5 mg/kg dose, BT7480 can maintain at least 50% target coverage for ~25 hours. D) In syngeneic MC38#13 tumor bearing mice, BT7480 led to complete responses (CRs) in 11/12 mice dosed. E) Efficacy plots for individual animals from D. F) CD8+ T cells in CR mice were depleted prior to re-challenge, as indicated by flow cytometry. G) Complete responder (CR) mice (with or w/o CD8 depletion) were re-implanted with MC38#13 cells. All tumors were rejected in animals w/o CD8 depletion, indicating an established memory response. Memory response was dependent on the presence of CD8+ T cells since most tumors (8/10) grew in CD8 depleted mice.

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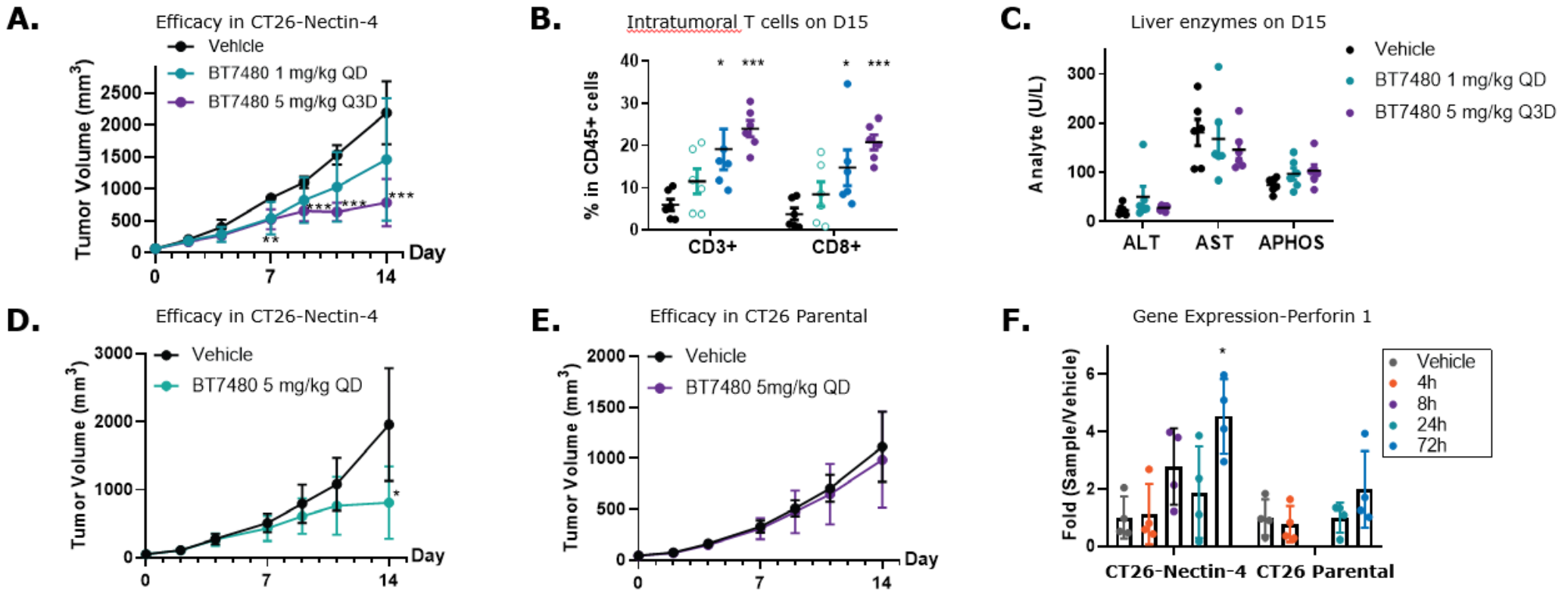
RESULTS



<u>Figure 7:</u> BT7480 led to increased tumor-infiltrating CD8+ T cells, and Nectin-4-dependent efficacy and gene expression changes. A) Intermittent IP dosing of BT7480 produced anti-tumor activity in a syngeneic mouse model carrying a CT26-Nectin-4-expressing tumor. B) Immune cell populations in the tumor were measured by flow cytometry at the end of study (day 15). C) Liver enzymes were measured on day 15; no significant changes were observed. D) and E) In a dual flank study, CT26 parental and CT26-Nectin-4-expressing tumors were implanted into the <u>same</u> mouse. Anti-tumor activity was observed in the tumor expressing Nectin-4 (D), but not in the parental tumor (E). F) Increased expression of a cytotoxic gene signature including the pore forming cytolytic gene, *Perforin 1*, was observed in response to BT7480 specifically in the tumor expressing Nectin-4. Gene expression in tumors was measured by Nanostring (n=4, +/-SD). (A, D, E) *p<0.05, **p<0.01, ***p<0.001 2way ANOVA with Dunnett's post test. (B, F) *p<0.05, ***p<0.001 t-test.

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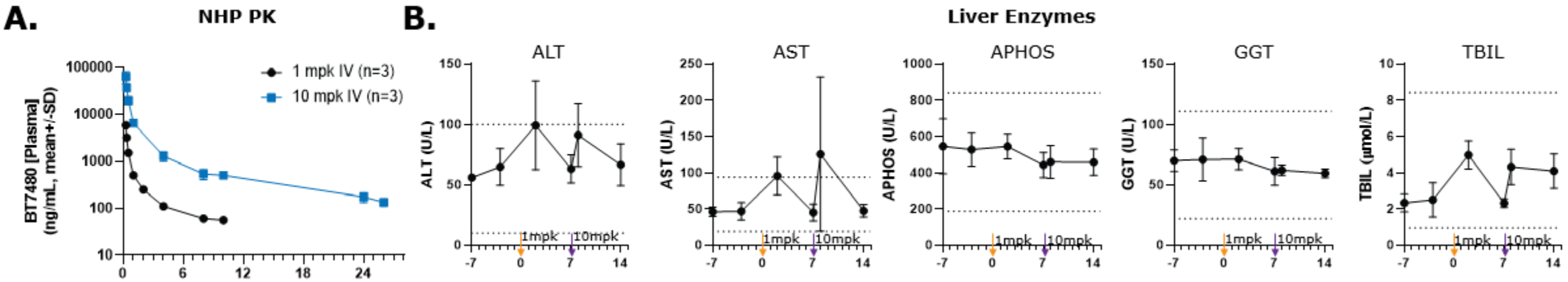

<u>Figure 8:</u> BT7480 exhibited dose linear exposure and is well tolerated in NHPs up to 10 mg/kg. A) Animals (n=3 +/- SD) were dosed 1 mg/kg IV on Day 0 and 10 mg/kg on Day 7. Exposures at 10 mg/kg are higher than those predicted to be required for a human efficacious dose. B) Clinical chemistry panel indicated that liver enzymes were generally well within the normal range (indicated by the dotted horizontal lines). The AST level in 1 out of 3 animals rose just above the normal range after the 10 mg/kg dose was administered and quickly recovered. Circulating cytokines were also monitored at 1h and 24h post-dose and were not significantly elevated in response to BT7480.

SUMMARY

- BT7480 is a Nectin-4/CD137 TICA that represents a new generation of chemically synthetic tumor antigen targeted CD137 agonists.
- BT7480 led to highly potent Nectin-4 dependent activity in vitro and in vivo, including complete responses and anti-tumor immunity in preclinical syngeneic mouse models.
- BT7480 was well tolerated in NHP at exposures above that of the predicted efficacious dose in humans. Further IND-enabling safety studies are ongoing.

References: [1] Segal et al, Clin Cancer Res 23(8): 1929-36 (2017); [2] Chester et al, Blood 131(1): 49-57 (2018);

[3] Hinner et al, Clin Cancer Res 25(19): 5878-89 (2019); [4] Claus et al, Sci Transl Med 11(496):5989 (2019)