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

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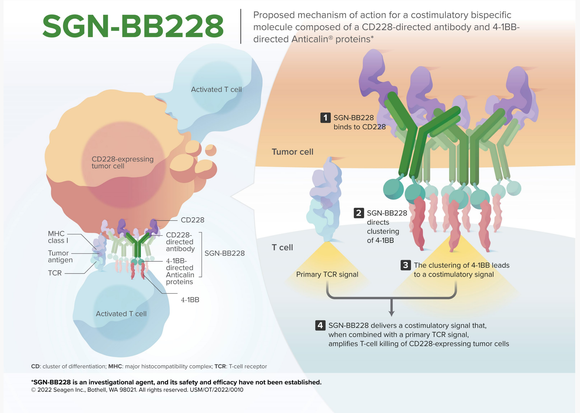
Background

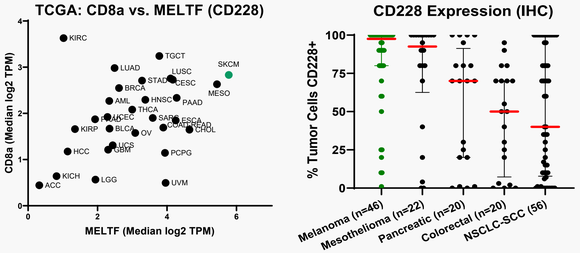
- SGN-BB228, a first-in-class, investigational, CD228 x 4-1BB costimulatory antibody Anticalin bispecific (Mabcalin™ protein) was created to overcome the safety and efficacy limitations of systemic anti-4-1BB antibodies.
- SGN-BB228 targets CD228 (melanotransferrin), a GPI-anchored membrane protein with prevalence and high expression across multiple tumor types but limited normal tissue expression.
- SGN-BB228 is designed to provide a potent costimulatory bridge between tumor-reactive cytotoxic T cells and CD228-expressing tumor cells, improving and constraining T cell-mediated cytotoxicity in tumors, potentially expanding the therapeutic window for 4-1BB agonism.
- SGN-BB228 is currently being evaluated in a first-in-human phase 1 study in melanoma and advanced solid tumors (NCT05571839).

Proposed Mechanism of Action



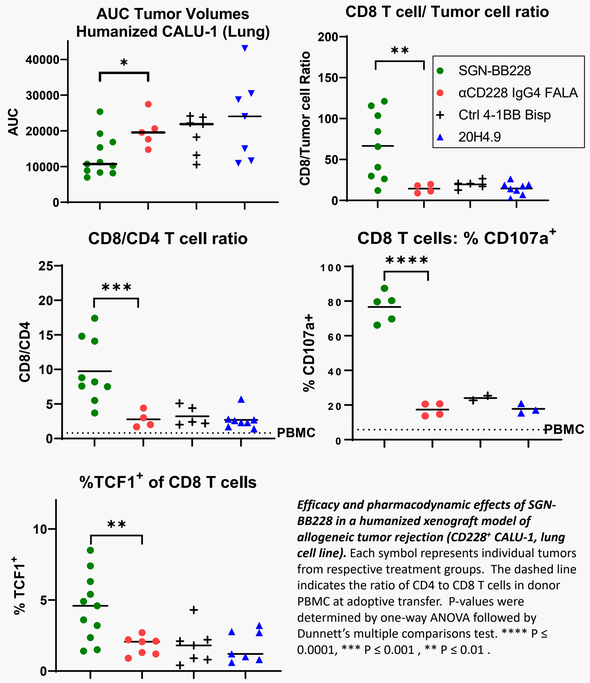
Results

CD228 is a tumor-associated antigen with expression in multiple solid tumor types with T cell involvement



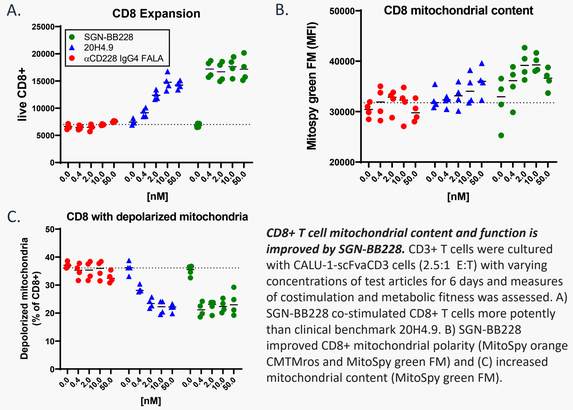
CD228 (MELTF) and CD8 transcript expression, taken from TCGA, and CD228 expression by IHC. Red bars indicate Median values with interquartile range.
(TCGA: SKCM, Skin cutaneous melanoma, Meso-Mesothelioma, PAAD-Pancreatic adenocarcinoma, LUSC-Lung squamous cell carcinoma, COAD-Colon adenocarcinoma)

SGN-BB228 improves cytotoxic T cell activity in an allogeneic humanized xenograft model



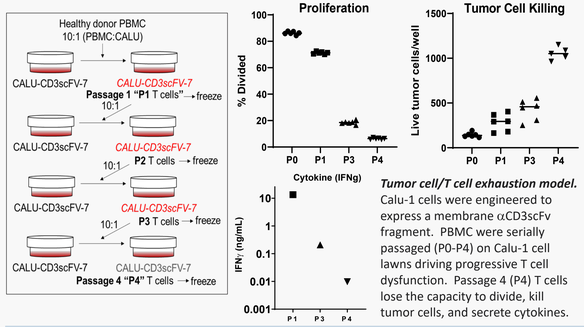
Efficacy and pharmacodynamic effects of SGN-BB228 in a humanized xenograft model of allogeneic tumor rejection (CD228+ CALU-1, lung cell line). Each symbol represents individual tumors from respective treatment groups. The dashed line indicates the ratio of CD4 to CD8 T cells in donor PBMC at adoptive transfer. P-values were determined by one-way ANOVA followed by Dunnett's multiple comparisons test. **** P ≤ 0.0001, *** P ≤ 0.001 , ** P ≤ 0.01 .

SGN-BB228 costimulation supports T cell activity by improving T cell mitochondrial content and quality



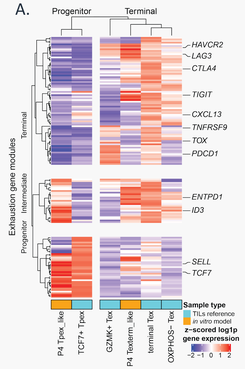
CD8+ T cell mitochondrial content and function is improved by SGN-BB228. CD3+ T cells were cultured with CALU-1-scFvaCD3 cells (2.5:1 E:T) with varying concentrations of test articles for 6 days and measures of costimulation and metabolic fitness was assessed. A) SGN-BB228 co-stimulated CD8+ T cells more potently than clinical benchmark 20H4.9. B) SGN-BB228 improved CD8+ mitochondrial polarity (MitoSpy orange CMTMros and MitoSpy green FM) and (C) increased mitochondrial content (MitoSpy green FM).

Serial passage of T cells on anti-CD3ScFv engineered tumor cells drives progressive functional exhaustion



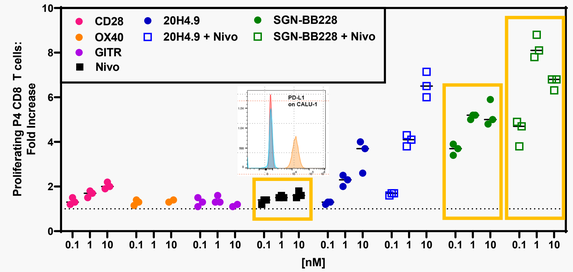
Tumor cell/T cell exhaustion model. Calu-1 cells were engineered to express a membrane αCD3scFv fragment. PBMC were serially passaged (P0-P4) on Calu-1 cell lawns driving progressive T cell dysfunction. Passage 4 (P4) T cells lose the capacity to divide, kill tumor cells, and secrete cytokines.

Single-cell RNAseq identified progenitor-like and terminal-like exhausted CD8+ T cell states in the P4 in vitro exhaustion model



scRNA sequencing of P4 exhausted T cells. A) CD8+ T cells from the P4 in vitro exhaustion model were analyzed by single-cell RNAseq and clustered into 2 states resembling progenitor (P4 Tpex_like) and terminally exhausted (P4 Texterm_like) CD8+ T cells. Average gene expression per state was scaled for each dataset, and then shown in the heatmap for our P4 cells or exhausted CD8+ T cell pan-cancer TILs atlas (1). We assessed the similarity of P4 Tpex_like and P4 Texterm_like to bona fide TIL CD8+ T cells by hierarchical clustering based on marker genes of exhausted T cell states. These results demonstrated P4 Tpex_like and P4 Texterm_like share significant overlap of gene signatures with bona fide exhausted TILs.

SGN-BB228 reinvigorates exhaustion model T cells as a single agent and in combination with PD-1 blockade



Exhausted T cell reinvigoration in vitro. P4 exhausted T cells were incubated with αCD3scFv Calu-1 cells in spheroid cocultures in the presence of a titration of costimulatory agonists (solid symbols). SGN-BB228 elicited robust T cell proliferation from P4 T cells that was superior to urelumab (20H4.9). P4 activity was further amplified by combining with the PD1-blocking mAb nivolumab. Inset histogram shows PD-L1 expression (orange) on CALU-1 cells compared to no-stain or isotype (red, blue)

Conclusions

- SGN-BB228 is a first-in-class, investigational, CD228 X 4-1BB antibody Anticalin bispecific (Mabcalin protein) with potent and CD228-conditional 4-1BB costimulatory activity with therapeutic potential in multiple solid tumor types.
- In in vivo and in vitro models, SGN-BB228 displays potent and CD228-conditional costimulation that exceeds the clinical 4-1BB benchmark 20H4.9.
- Consistent with the known effects of 4-1BB costimulation, SGN-BB228 robustly improved the metabolic capacity of cytotoxic T cells in vitro.
- In an in vitro model of T cell exhaustion, 4-1BB costimulation from SGN-BB228 restored CD8 T cell proliferation that combined with PD-1 blockade.
- Importantly, anti-PD-1 alone, as well as agonists of other costimulatory axes (CD28, OX40, GITR), failed to elicit proliferation from functionally exhausted CD8 T cells, highlighting the distinct therapeutic potential of tumor-targeted 4-1BB costimulation.
- Altogether, these data support the evaluation of SGN-BB228 in the currently enrolling first-in-human phase 1 study in melanoma and advanced solid tumors NCT05571839.

References

1) Zheng L et al. Pan-cancer single-cell landscape of tumor-infiltrating T cells. *Science*. 2021 Dec 17;374(6574):

Disclosures

Authors [1] (Seagen) are current or former employees and have equity interests in Seagen, Inc.
Authors [2] (Pieris) hold ownership interest (including patents) in Pieris Pharmaceuticals.
*Work conducted while at Seagen but no longer currently employed at Seagen

Poster No. 5676
AACR, Orlando, FL; April 14–23, 2023

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