SGN-BB228 is a first-in-class CD228-targeted costimulatory Antibody Anticalin® bispecific delivering potent and conditional 4-1BB costimulation to tumor-specific T cells

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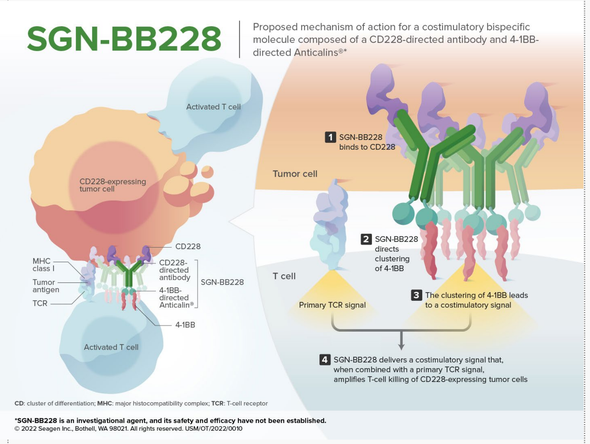
Background

- SGN-BB228, a first-in-class, investigational, CD228 x 4-1BB Mabcalin™ bispecific (antibody Anticalin® fusion) 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, and potentially expanding the therapeutic window for 4-1BB agonism.

Proposed Mechanism of Action



The proposed mechanism of action (MOA) for SGN-BB228 is CD228-conditional clustering of 4-1BB on antigen-experienced tumor-specific T cells, resulting in enhanced activation and cellular cytotoxicity.

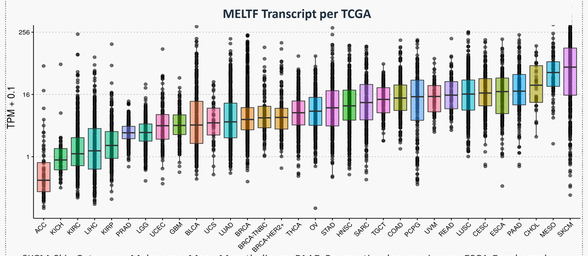
Disclosures

Disclosure of Potential Conflicts of Interest:

Authors [1] (Seagen) are employees and have equity interests in Seagen, Inc.
Authors [2] (Pieris) hold ownership interest (including patents) in Pieris Pharmaceuticals.

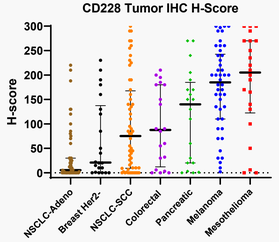
Results

CD228 is a tumor-associated antigen with enriched expression in multiple solid tumor types



SKCM-Skin Cutaneous Melanoma, Meso-Mesothelioma, PAAD-Pancreatic adenocarcinoma, ESCA-Esophageal carcinoma; CESC-Cervical carcinoma, LUSC-Lung squamous cell carcinoma, COAD-Colon adenocarcinoma

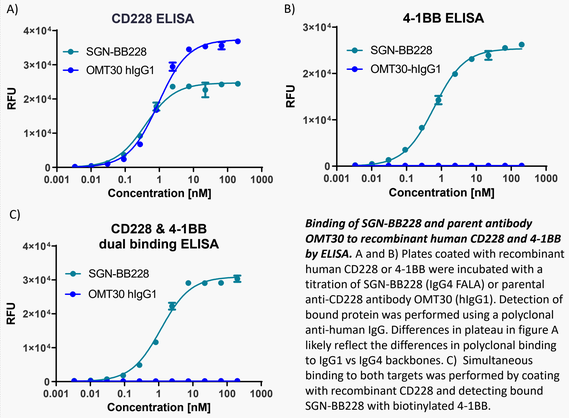
CD228 expression is found on multiple solid tumor types



Membrane H-Score quantification of IHC on tumor samples by tumor type. Immunohistochemistry was performed on fixed human tumor samples. Each point represents a unique tumor sample. H-Score was determined by pathologist review and calculated by multiplying an ordinal value 1-3 describing surface staining intensity (1=low, 2=med, 3=hi) by the % of tumor cells expressing CD228. A maximum H-score is 300. Bars indicate median H-score with interquartile range.

SGN-BB228 binds with high affinity to CD228 and 4-1BB

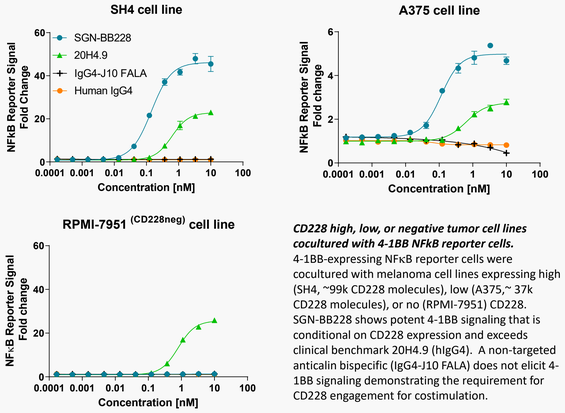
Binding to recombinant human CD228 and 4-1BB by ELISA



Binding of SGN-BB228 and parent antibody OMT30 to recombinant human CD228 and 4-1BB by ELISA. A and B) Plates coated with recombinant human CD228 or 4-1BB were incubated with a titration of SGN-BB228 (IgG4 FALA) or parental anti-CD228 antibody OMT30 (hIgG1). Detection of bound protein was performed using a polyclonal anti-human IgG. Differences in plateau in figure A likely reflect the differences in polyclonal binding to IgG1 vs IgG4 backbones. C) Simultaneous binding to both targets was performed by coating with recombinant CD228 and detecting bound SGN-BB228 with biotinylated 4-1BB.

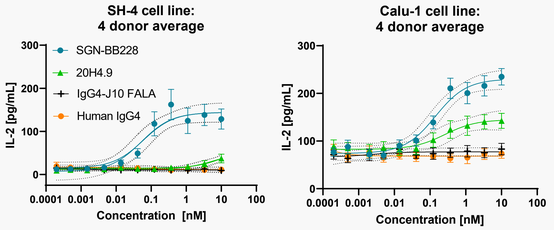
SGN-BB228 provides robust and CD228-conditional 4-1BB costimulation across a variety of assays

SGN-BB228 induced 4-1BB signaling is CD228-dependent



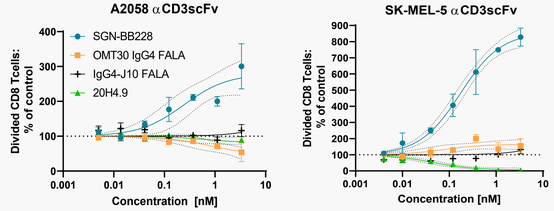
CD228 high, low, or negative tumor cell lines cocultured with 4-1BB NFkB reporter cells. 4-1BB-expressing NFκB reporter cells were cocultured with melanoma cell lines expressing high (SH4, ~99k CD228 molecules), low (A375,~ 37k CD228 molecules), or no (RPMI-7951) CD228. SGN-BB228 shows potent 4-1BB signaling that is conditional on CD228 expression and exceeds clinical benchmark 20H4.9 (hIgG4). A non-targeted anticalin bispecific (IgG4-J10 FALA) does not elicit 4-1BB signaling demonstrating the requirement for CD228 engagement for costimulation.

SGN-BB228 provides robust costimulation to primary T cells receiving a TCR signal



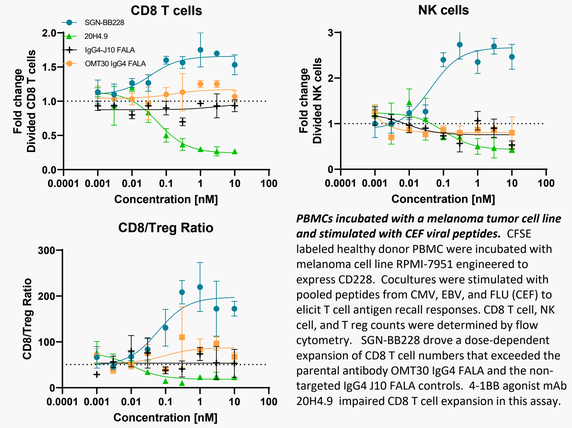
Purified T cells cocultured with CD228-expressing tumor cells on anti-CD3 coated plates. CD228-expressing melanoma (SH4) and lung cancer (Calu-1, ~164k CD228 molecules) cell lines were cocultured with enriched healthy donor T cells in plates coated with anti-CD3 to drive TCR signaling. IL-2 was measured from culture supernatant as a readout of T cell activation. SGN-BB228 drove a dose-dependent increase in IL-2 production from T cells that exceeded mAb 20H4.9 and a non-targeted 4-1BB bispecific fusion protein, IgG4-J10 FALA.

SGN-BB228 costimulates T cells engaged with tumor cells via direct TCR interactions



PBMC cocultured with CD228-expressing tumor cells engineered to express an anti-CD3scFv. CD228-expressing melanoma cell lines SKMEL-5 (~156k CD228 molecules) and A2058 (~51k CD228 molecules) were engineered to express a cell membrane-associated scFv fragment specific for CD3 to provide tumor cell-directed TCR stimulation. CFSE-labeled healthy donor PBMC were cocultured with anti-CD3scFv cell lines and CD8 T cell proliferation was measured by flow cytometry. SGN-BB228 drove dose-dependent amplification of proliferating CD8 T cell numbers that exceeded the parental antibody OMT30 IgG4 FALA and the non-targeted IgG4 J10 FALA controls. 4-1BB agonist mAb 20H4.9 failed to provide costimulation in this assay.

SGN-BB228 costimulates T cells in a viral antigen recall assay



PBMCs incubated with a melanoma tumor cell line and stimulated with CEF viral peptides. CFSE labeled healthy donor PBMC were incubated with melanoma cell line RPMI-7951 engineered to express CD228. Cocultures were stimulated with pooled peptides from CMV, EBV, and FLU (CEF) to elicit T cell antigen recall responses. CD8 T cell, NK cell, and T reg counts were determined by flow cytometry. SGN-BB228 drove a dose-dependent expansion of CD8 T cell numbers that exceeded the parental antibody OMT30 IgG4 FALA and the non-targeted IgG4 J10 FALA controls. 4-1BB agonist mAb 20H4.9 impaired CD8 T cell expansion in this assay.

Conclusions

- **SGN-BB228 is a first-in-class, investigational, CD228 X 4-1BB Antibody Anticalin® fusion bispecific with potent and CD228-conditional 4-1BB costimulatory activity with therapeutic potential in multiple solid tumor types.**
- **Across diverse primary T cell assays, SGN-BB228 displays potent and CD228-conditional costimulation that exceeds the clinical benchmark mAb 20H4.9.**
- **Altogether, these data support the evaluation of SGN-BB228 in the currently enrolling first-in-human phase 1 clinical study in melanoma and advanced solid tumors NCT05571839**

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