Utilizing PDX Models to Better Understand the Factors that Predict Response to SGN-CD228A, an Antibody-Drug Conjugate (ADC) for Solid Tumors

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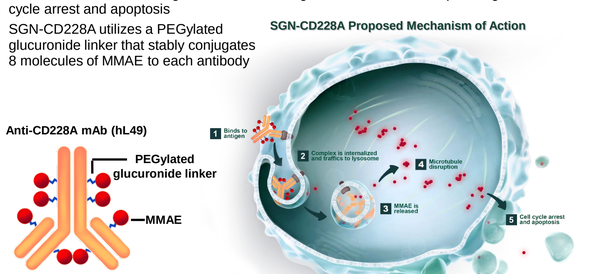
Background

SGN-CD228A is an Antibody-Drug Conjugate (ADC) Targeting CD228

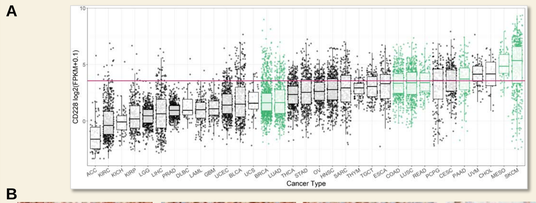
- CD228 (melanotransferrin, MELTF, MFI2) is an oncofetal GPI anchored protein that was first identified in melanoma
  - Low expression on normal tissues; overexpressed in many different types of solid tumors
  - May play a role in tumor migration and proliferation
  - 40% homology to transferrin and lactotransferrin
- SGN-CD228A is an investigational ADC that targets and kills CD228-expressing cells via cell cycle arrest and apoptosis
- SGN-CD228A utilizes a PEGylated glucuronide linker that stably conjugates 8 molecules of MMAE to each antibody



SGN-CD228A Proposed Mechanism of Action

SGN-CD228A is an investigational agent, and its safety and efficacy have not been established.

CD228 Has Broad Expression in Solid Tumors



A) The level of CD228 positivity by RNA was calculated from TCGA RNA-seq data using a threshold of RPKM expression derived by defining a % positive value identical to melanoma IHC tumor samples (red line). Indications highlighted in green will be included in the phase I trial. B) Full tumor sections were stained with a novel IHC optimized anti-CD228 monoclonal IHC antibody. The examples shown are representative of the mean staining for each subtype. Additionally, tumor microarrays (TMA) were stained for CD228 using a commercial rabbit polyclonal antibody (Sigma, HPA004880). Samples were scored as positive if they had any cells that had CD228 staining with ≥1+ intensity. For the tumor sections only, we also calculated the percent of positive cells with ≥1+ intensity and the average for each indication is reported in Table 1.

Table 1: Summary of CD228 expression

Indication	% tumors with staining (TMA)	% tumors with staining (Tumor sections)	Average % positive cells (Tumor sections)	% positive RNA	% tumors with staining (PDX samples)
Melanoma	76% (n=76)	100% (n=20)	82% (n=20)	76% (n=472)	100%, (n=9)
Mesothelioma	78% (n=49)	91% (n=22)	80% (n=22)	91% (n=87)	100%, (n=3)
Colorectal	48% (n=294)	85% (n=20)	45% (n=20)	54% (n=614)	ND
TNBC	56% (n=68)	77% (n=22)	36% (n=22)	64% (n=118)	72%, (n=18)
HER2-/HR+ Breast	43% (n=61)			6% (n=612)	ND
NSCLC-adeno	26% (n=97)	70% (n=10)	23% (n=10)	19% (n=530)	55%, (n=30)
NSCLC-squamous	64% (n=140)	80% (n=10)	40% (n=10)	53% (n=501)	78%, (n=9)
Pancreatic	88% (n=77)	90% (n=20)	56% (n=20)	62% (n=179)	ND

Experimental Methods

Study design using Patient-Derived Xenograft (PDX) Models

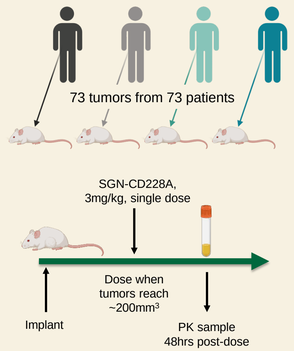


Table 2: Characteristics of PDX samples

Indication	Subtype	# of models
Melanoma	Cutaneous	9
Mesothelioma	Biphasic	2
	Epithelioid	1
Breast	Triple-negative	22
NSCLC	Adenocarcinoma	30
	Squamous	9

Tumors collected at end of study

- ½ frozen for LC-MS/MS & RNA seq
- ½ FFPE for IHC
- Studies were generally ended when tumors reached ~1500mm^3

PDX models were selected to include a wide range of CD228 expression.73 different PDX models were included in the study. Tumors were implanted and mice were randomized when they reached approximately 200mm^3. For each model, the number of control and treated animals ranged from 1-4 depending on the model. SGN-CD228A was given as a single dose at 3mg/kg. Tumors were collected at the end of the study for IHC, proteomics, and RNA sequencing.

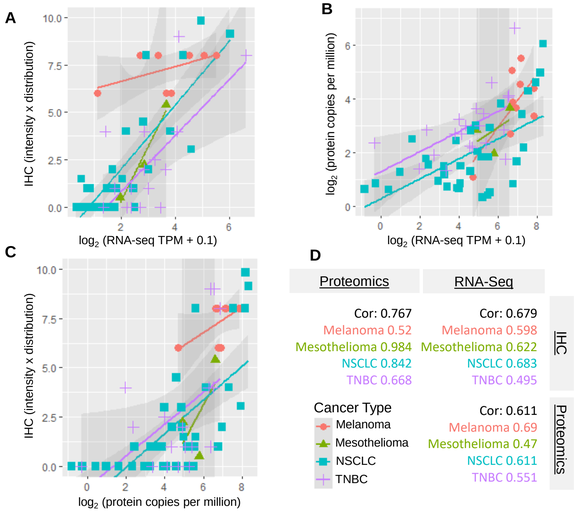
CD228 Expression in PDX Samples

CD228 expression by RNA, proteomics, and IHC correlates well between the techniques

IHC Score
CD228 IHC samples were scored for intensity (0-3+) and distribution of positive tumor cells (1=1-25%, 2=26-50%, 3=51-75%, and 4=76-100%). IHC score = intensity x distribution

RNA-seq
Frozen TNBC tumors were subject to RNA sequencing. For other tumor types, we relied on historical data. Data is plotted as transcripts per million.

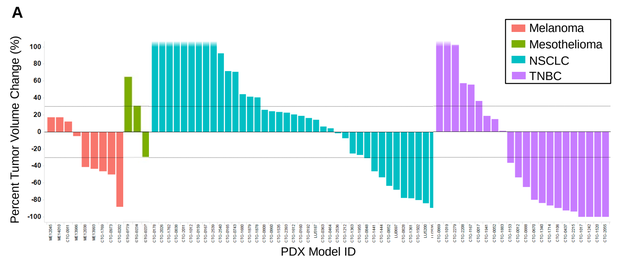
Proteomics
Frozen tumors were lysed, digested with trypsin and analyzed using a data independent acquisition scheme. Data is plotted as protein copies per million.



SGN-CD228A is Active in Multiple Tumor Types

SGN-CD228A was highly active in TNBC and Melanoma

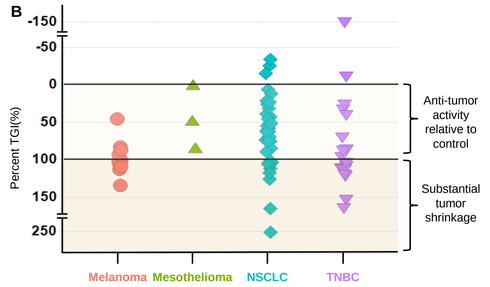
- Overall response rate using RECIST criteria (greater than 30% tumor shrinkage) was 38%
- TNBC (ORR, 59%) and melanoma (ORR, 56%) were the most sensitive to SGN-CD228A



A)The waterfall plot depicts the average of the percent tumor volume change for each model in which T_x is the smallest tumor volume achieved or for growing tumors the first measurement post dose and T_i is the initial tumor volume at dosing **(100 * (T_x-T_i)/T_i)**. B) Percent tumor growth inhibition (TGI) is determined by normalizing the growth of treated **(T)** and control **(C)** animals, **f** denotes the final/end of study tumor volume. **(100 x [1-(T_f-T_i)/(C_f-C_i)])**

Table 3: Overall best response to SGN-CD228A

RECIST criteria	Overall (n=73)	TNBC (n=22)	NSCLC (n=39)	Melanoma (n=9)
ORR (<-30%)	38%	59%	26%	56%
SD (<30%)	32%	14%	36%	44%
PD (>30%)	30%	27%	38%	0%



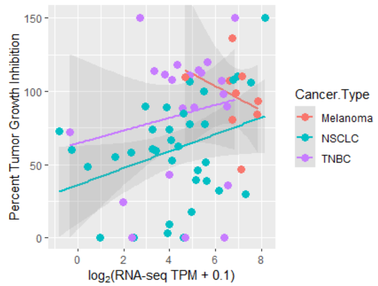
Expression-Response Relationship is Indication Specific

NSCLC response has a weak but significant correlation with CD228 RNA expression

- TNBC shows a trend between CD228 and response, but this is not statistically significant
- There is no correlation between CD228 expression and response for melanoma
- Similar results were obtained if CD228 IHC or proteomics were used instead of RNA

CD228 expression was compared across melanoma, NSCLC, and TNBC models versus average tumor volume as a measurement for SGN-CD228A activity. Similar results were obtained with IHC and proteomics.

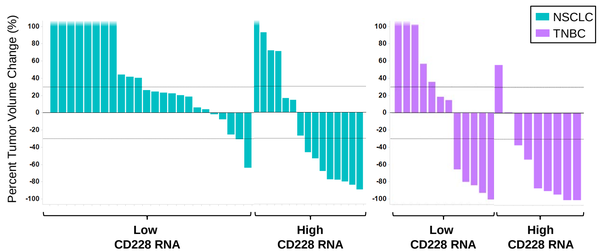
NSCLC: 0.42 (p=0.0071)
TNBC: 0.23 (p=0.31)
Melanoma: -0.31 (p=0.41)



Models with High CD228 RNA Have Improved Activity

Separating either NSCLC or TNBC into high and low CD228 RNA improves ORR

- RNA threshold was applied to NSCLC and TNBC models
- In high CD228 expressing models the ORR improved in NSCLC from 26% to 53% and in TNBC from 59% to 78%



PDX models were divided into "low" and "high" CD228 RNA expression and the percent tumor volume change is plotted for both NSCLC and TNBC. Melanoma was not included because all the models fell above the chosen CD228 RNA threshold. The RNA threshold was arbitrarily selected, but it represents the highest 1/3 of CD228 expressing NSCLC models and approximately the top 1/2 of CD228 expressing TNBC models.

Table 4: SGN-CD228A ORR improves in high expressing CD228 models

RECIST criteria	%ORR (based on CD228 RNA)
All TNBC models	59% (n=22)
Low TNBC models (RNA<5.20 $\log_2$ TPM + 0.1)	46% (n=13)
High TNBC models (RNA>5.20 $\log_2$ TPM + 0.1)	**78%** (n=9)
All NSCLC models	26% (n=39)
Low NSCLC models (RNA<5.20 $\log_2$ TPM + 0.1)	8% (n=24)
High NSCLC models (RNA>5.20 $\log_2$ TPM + 0.1)	**53%** (n=15)

B) Percent tumor growth inhibition (TGI) is determined by normalizing the growth of treated **(T)** and control **(C)** animals, **f** denotes the final/end of study tumor volume. **(100 x [1-(T_f-T_i)/(C_f-C_i)])**

Conclusions

- CD228 is expressed in a variety of tumor types, including melanoma, NSCLC, mesothelioma, breast, pancreatic and colorectal cancers
- A single dose of SGN-CD228A showed anti-tumor activity in most NSCLC, TNBC, mesothelioma, and melanoma PDX models tested
- TNBC and melanoma models were the most responsive to SGN-CD228A
- CD228 expression was measured by RNA, IHC, and proteomics, which all showed good concordance with one another
- CD228 RNA levels had a weak but statistically significant correlation to SGN-CD228A anti-tumor activity in NSCLC models, but not in TNBC or melanoma.
  - For melanoma, the lack of correlation is likely due to high overall expression and activity and/or the small sample size
  - For TNBC, there was a trend between expression and activity, but it was not statistically significant, which could also be due to sample size or other factors
- Despite the lack of a strong correlation between expression and activity, improved ORR was observed in TNBC and NSCLC models with the highest CD228 RNA expression (26% to 53% in NSCLC and 59% to 78% in TNBC)
- SGN-CD228A is in Phase I single agent clinical trial (NCT04042480)

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