

### 1. Introduction

CD276 (B7-H3) is an immunoregulatory molecule that is reported to be widely expressed in pediatric embryonal tumors, pediatric sarcomas, and tumors infiltrating blood vessels (Majzner et al. 2017; Majzner et al. 2018). CD276 protein is expressed at low levels on several normal tissues, including cerebral cortex, liver and germinal lymph nodes. m276 is a fully-human IgG1 that binds with similar affinity to both mouse CD276 (24 nM kD) and human CD276 (29 nM kD) (Seaman et al. 2017).

To generate an antibody-drug conjugate, m276 was site-specifically conjugated to the DNA damaging agent pyrrolobenzodiazepine (PBD) via a cleavable valine-alanine linker, providing m276-PBD conjugated to the DNA damaging agent pyrrolobenzodiazepine (PBD) via a cleavable valine-alanine linker, providing m276-PBD with a Drug-to-Antibody Ratio (DAR) of 2.

Here we report the antitumor activity of m276-PBD against preclinical xenograft models of pediatric solid tumors.

### 2. Study Methods

- **m276-PBD** was administered intraperitoneally injection at a dose of 0.5 mg/kg, once weekly x 3 consecutive weeks
- **Solid tumor testing** used subcutaneous xenografts. Events were defined as 4-fold increase in tumor volume from the first day of treatment. The Kaplan-Meier method was used to compare time-to-event between treated and control groups.
- **The objective response categories** are as described by Houghton, et al. 2007.
  - **Tumor progression disease:** >50% tumor progression throughout study and >5% tumor growth
  - **PD:** when PD and the mouse’s time to event ≤ 200% the median time-to-event in control group
  - **OS:** when PD has, additionally, time to event >200% of the Kaplan-Meier (KM) median time-to-event in control group
  - **SD:** stable disease, ≤50% tumor regression throughout study and ≤25% tumor growth at end of study
  - **PR:** partial response, >50% tumor regression at any point during study but <50% tumor regression throughout study
  - **CR:** complete response, disappearance of measurable tumor mass during study
  - **MCR:** maintained complete response in tumor mass to determine for at least 3 consecutive weekly readings at any time after treatment has been completed
  - **Neuroblastoma testing** used 2 animals per model to evaluate for tumor regression, while the other histologies used standard testing procedures (n=8-10) to evaluate for tumor regression and for time to event.

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- **m276-PBD** was well tolerated, with a toxic death rate < 2% and with mean body weight loss of 9.5%.

- **The translatable our results to the clinical setting is dependent upon the extent to which the drug levels achieved in mice can be replicated in humans.** Additional experiments to define the dose-response to m276-PBD across a range of histologies will help to define the minimum exposure levels associated with tumor-regressing activity.

### 3. CD276 Expression in PPTC models

- **CD276 expression** was high in most solid tumors (median 41 FPKM) with highest expression observed in osteosarcoma (median 82 FPKM).
- **Neuroblastoma, rhabdomyosarcoma, Wilms tumor and embryonal brain tumors** also had elevated levels of expression, whereas ALL models showed low expression.

### 4. Results (continued)

- **RNAseq data for PPTC models show elevated CD276 expression levels for a wide range of pediatric solid tumors, which is consistent with protein expression data from clinical specimens (Majzner et al., 2019).**

- **m276-PBD showed very high levels of activity when tested against PPTC pediatric solid tumor preclinical models at 0.5 mg/kg administered weekly x 3**

- **Objective responses (PR/CR/MCR)** were observed in 23 of 52 (44%) models treated with m276-PBD, with 23/25 models showing regrowth by day 56 and with follow-up still ongoing.

- **There was no clear relationship between CD276 expression by RNAseq and response to m276-PBD, with CR and MCR responses observed in models with CD276 expression ranging from 20 to 168 FPKM, and with SD/PR responses observed at expression levels from 14 to 131 FPKM.

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- **Conclusion:** Our results confirm CD276 as a high priority target for pediatric solid tumors and support further evaluation of CD276-targeted ADCs with direct DNA-damaging payloads for these cancers.

### 5. References

- Seaman S, Zhu Z, Saha S, Zhang YM, Yang MY, Hilton MB, et al. CD276 expression levels for a wide range of pediatric solid tumors, which is consistent with protein expression data from clinical specimens (Majzner et al., 2019).