1756

Anti-tumor effect of XMT-1660, a B7-H4 targeting antibody drug conjugate, in an unselected panel of patient derived xenograft models of breast cancer

Scott D. Collins, *Pamela S. Shaw*, *Shawn P. Fessler*, *Jason Wang*, *Rebecca Mosher* Mersana Therapeutics, Inc., Cambridge, MA

Introduction

XMT-1660 is a Dolasynthen Antibody-Drug Conjugate (ADC) targeting B7-H4 and carrying a DolaLock payload with controlled bystander effect. B7-H4 is an immunoregulatory protein that is expressed in breast, ovarian and endometrial tumors[1]. Expression of B7-H4 has been described in both tumor cells and in tumor associated macrophages, and B7-H4 shows infrequent co-expression with PD-L1, suggesting distinct immunoregulatory functions[2]. XMT-1660 is built on the Dolasynthen platform which incorporates several attributes, including site-specific bioconjugation and precise control over drug-antibody ratio (DAR). The XMT-1660 ADC contains 6 DolaLock Auristatin F-hydroxypropyl amide (AF-HPA) anti-tubulin payloads per antibody (DAR-6). Previously, we demonstrated the cytotoxic effect of XMT-1660 in cell line xenograft and patient derived xenograft models of breast cancer known to have B7-H4 target expression. The purpose of this study was to evaluate the anti-tumor activity of XMT-1660 across an unselected panel of breast cancer patient derived xenograft (PDX) models, evaluate the level of B7-H4/VTCN1 expression across this panel of breast PDX models, and assess the correlation between B7-H4 expression and anti-tumor activity following a single dose of XMT-1660.

Figure 1 Structure of XMT-1660

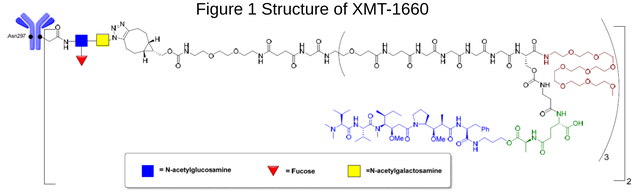


Figure 1. Two molecules of Dolasynthen, each bearing three DolaLock payloads (AF-HPA; shown in blue), are conjugated to anti-B7-H4 monoclonal hIgG1 antibody, XMT-1604, via click chemistry at Asn297 (EU numbering) after glycan remodeling with Synaffix GlycoConnect™ site specific bioconjugation technology. The result is a site-specific ADC with drug-to-antibody ratio (DAR) of 6.[3]

Methods

A panel of 30 planned breast cancer PDX models, (Champions Oncology) annotated by prior treatment history, and divided between TNBC and ER-positive subtypes, was implanted into athymic Nude-Foxn1nu mice. When tumors reached an average volume of 150-300 mm3, animals (n=3) were treated with a single administration of either XMT-1660 4.71 mg/kg (antibody dose)/0.15mg/kg (payload dose)/IV/QD X1 or saline vehicle. Tumor volumes were measured until the planned endpoint of mean tumor volume of control group of 1500 mm3 or day 28. At the endpoint, xenografts, or tumor beds (in the case of no palpable mass) were collected as formalin fixed paraffin embedded material. Model outcome was expressed as median best response (MBR), where every treated xenograft was compared back to it's day 0 size over the course of the study and MBR was defined as the median of the best response of the 3 animals evaluated for each model.

RNA was extracted from FFPE samples using the Qiagen RNeasy FFPE kit according to the manufacturer's instructions. Samples were equalized based on nanodrop reading and cDNA produced using the Thermofisher SuperScript IV VILO Master Mix with exDNase Enzyme. Gene expression assays were set up with the TaqMan Fast Advanced Master Mix. ABI assay Hs01552471_g1 was used for VTCN1. Hs99999903_m1 ACTB and Hs03929097_g1 GAPDH were used as endogenous controls. Expression data was analyzed as ΔΔ Ct of the average of animals in each vehicle treated group (generally n=3) referencing a universal RNA control.

IHC to detect B7-H4 expression was performed on a single vehicle treated animal from each model. Briefly, tissues were sectioned at 4 µ onto positively charged slides and dried overnight. Using the Leica BOND III platform, sections were baked, dewaxed and subjected to antigen retrieval (LEICA BOND III ER1+ Proteinase K). The primary B7-H4 antibody (Abcam ab209242) was used at a concentration of 0.2 µg/ml (prepared in DAKO/Agilent diluent S3022). Signal was detected using the Leica BOND Polymer Refine system/DAB chromogen. Slides were evaluated by light microscopy and H-scores and TPS scores (tumor proportion score/percent positive at any intensity) of membrane reactivity were calculated .

Results

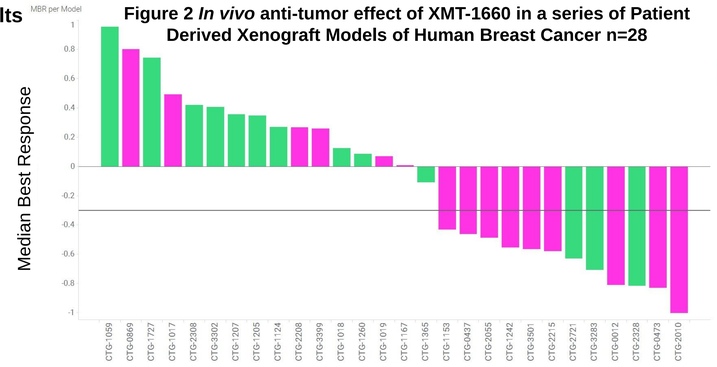


Figure 2. In this model set, 12/28 (43%) of primary breast carcinoma models achieved a median best response of 30% (shown as -0.3 on the Y Axis) or better following a single dose of XMT-1660. Tumor volume reductions were seen in 13/28 (46%) models. Of the 12 PDX in which a median best response of 30% was achieved, 10 were derived from known previously treated tumors. The anti-tumor effect of MBR of 30% or more was more frequent in TNBC models 9/15 (60%) compared to ER-positive models 3/13 (23%), (bars colored by receptor status). Two additional ER positive models, originally proposed for this study, were excluded from summary analysis due to unexpectedly rapid growth/mouse tumor phenotype or very slow growth in vehicle animals.

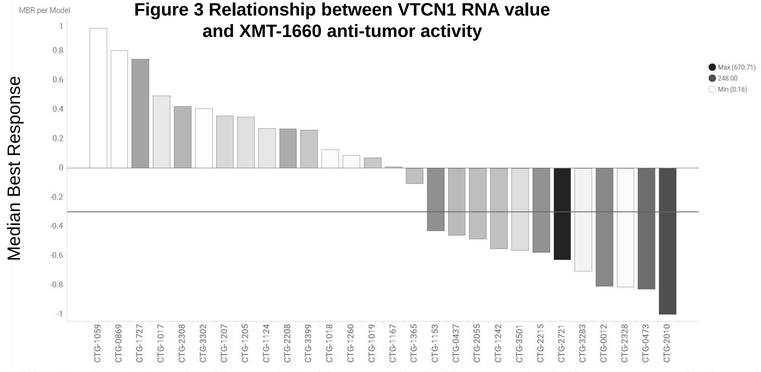
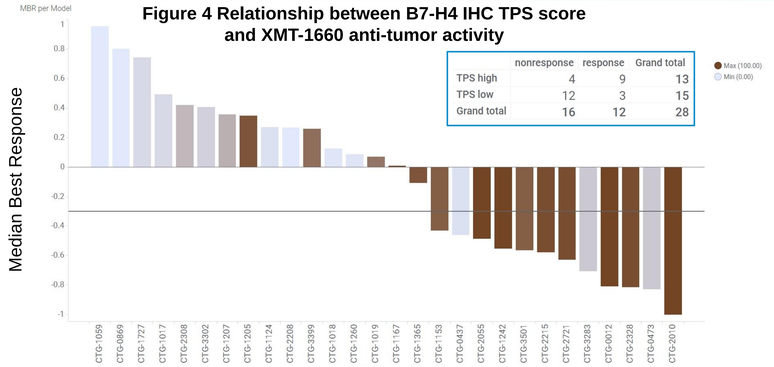


Figure 3. Higher B7-H4/VTCN1 RNA values (shown as ΔΔCt of the average of animals) were associated with greater response in this sample set. The relationship was more apparent in the TNBC models. One ER positive model had a very high RNA value compared to all other models, note shading scale is broken at this point in figure key.

4A



4B

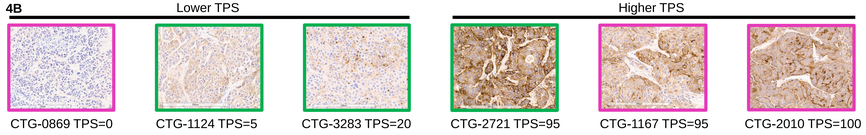


Figure 4. TPS ranged from 0-100 and was calculated based on membrane immunoreactivity; cytoplasmic reactivity, if noted, was not included in the score. Higher B7-H4 IHC TPS values were associated with response in this sample set **(4A)** A similar result was obtained when IHC was quantified using an H-Score method.
Employing a cut-off, for example TPS75 (TPS high ≥75, TPS low <75) identified 9/12 (75%) of responding models; below a cutpoint of TPS75 12/15 (80%) of models were non-responsive, when response was considered to be MBR -0.3 following a single dose of XMT-1660. Representative images from screen captures of Aperio scans are shown with associated scores **(4B)**; frame color corresponds to receptor status (green: ER+; pink: TNBC).

Summary

- A single dose of XMT-1660 elicits a range of anti-tumor activity in models of human breast cancer and shows anti-tumor activity in both TNBC and ER positive models, including in models derived from previously treated tumors.
- A relationship is seen between XMT-1660 efficacy and B7-H4 expression; stronger anti-tumor activity of XMT-1660 tends to be more frequent in models with higher B7-H4 expression.
- Work is ongoing to evaluate B7-H4 expression in human primary tumors.
- XMT-1660 is currently in IND-enabling studies and is expected to enter a Phase I dose escalation clinical study in 2022.
- The efficacy/expression relationship will be evaluated in the upcoming clinical study with a goal to identify patients most likely to respond to XMT-1660.

The authors gratefully acknowledge the contributions of: Discovery Life Sciences/QualTek for IHC work, Champions Oncology for *in vivo* work, and Stacy Higgins for QC of the *in vivo* data.

1.Altan M., et al. (2018). Association of B7-H4, PD-L1, and tumor infiltrating lymphocytes with outcomes in breast cancer. NPJ Breast Cancer, 4(40)
2 Leong S., et al. (2015). An Anti-B7-H4 Antibody–Drug Conjugate for the Treatment of Breast Cancer. Mol. Pharmaceutics, 12, 1717−1729
3 Fessler S., et al. (2021) XMT-1660, a B7-H4-Targeted Dolasynthen Antibody-Drug Conjugate for the Treatment of Breast Cancer, AACR Abstract #907