XMT-1660, a B7-H4-Targeted Dolasynthen Antibody-Drug Conjugate for the Treatment of Breast Cancer

Abstract
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Abstract

XMT-1660 is a novel DAR-6 Dolasynthen-based antibody drug conjugate carrying a DolaLock payload with controlled bystander effect and targeting B7-H4, a tumor antigen that is broadly expressed on the cell surface in breast, ovarian and endometrial cancers. B7-H4 (VTCN1) exerts immunosuppressive effects by suppression of T cell proliferation and is expressed on tumor-associated macrophages (TAMs) as well as epithelial tumor cells. XMT-1660 is comprised of an anti-B7-H4 antibody site-specifically conjugated to Dolasynthen, with a total of 6 DolaLock Auristatin F-HPA (AF-HPA) anti-tubulin payloads per antibody (DAR-6).

To select the optimal ADC, three ADCs using the same antibody and DolaLock payload were compared: site-specific Dolasynthen-based DAR-2 and DAR-6 ADCs, and a stochastically conjugated Dolaflexin-based DAR-12 ADC. *In vitro*, no significant differences were observed among the 3 ADCs: all exhibited specific recognition of B7-H4 and elicited potent cytotoxicity against B7-H4-expressing cancer cells. *In vivo*, XMT-1660 consistently exhibited more anti-tumor activity than the other ADCs in TNBC models and ER+/HER2- models after single, equivalent doses based on payload. XMT-1660 demonstrated dose-dependent anti-tumor activity and induced sustained tumor regressions after a single administration. XMT-1660 and the Dolasynthen DAR-2 ADC both exhibited improved pharmacokinetics in mouse relative to the Dolaflexin DAR-12 ADC.

These data indicate that XMT-1660 exhibited a superior preclinical profile to the other ADCs and more generally demonstrate the importance of DAR-ranging studies to identify the optimal ADC for a given target. These results, as well as results from exploratory toxicology studies in non-human primates, strongly support the clinical development of XMT-1660.

B7-H4 Is a Promising Target for a DolaLock ADC

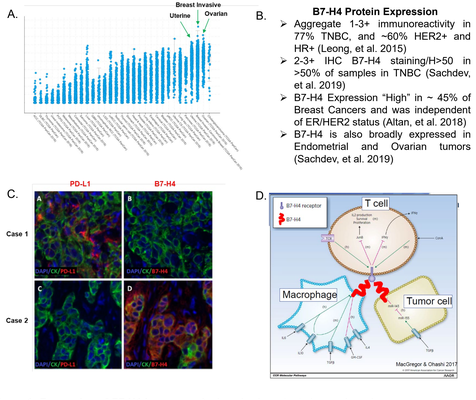


Figure 1: Expression of B7-H4 in cancer. A. Invasive breast, uterine, and ovarian cancers are among the highest expressors of B7-H4 mRNA in the TGCA database. B. Summary of published B7-H4 protein expression data indicates expression of B7-H4 across different breast cancer subtypes, as well as endometrial and ovarian tumors C. Representative data from a published study from Altan, et al. (2018) showing low correlation between expression of B7-H4 and PD-L1 (coexpression in 1% and 4% of two different cohorts) D. Expression of B7-H4 is on both tumors and tumor-associated macrophages in several indications, including breast (as reviewed in MacGregor and Ohashi, 2017), suggesting the potential for multiple mechanisms of antitumor activity by a DolaLock ADC.

Structure of XMT-1660

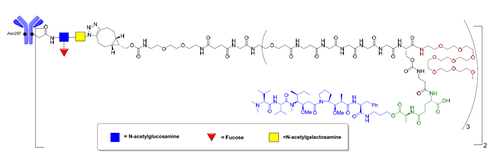


Figure 2: Structure of XMT-1660. Two molecules of Dolasynthen, each bearing three DolaLock payloads (AF-HPA; shown in blue), are conjugated to anti-B7-H4 monoclonal hIgG1 antibody. XMT-1604, via click chemistry at Asn297 (EU numbering) after glycan remodeling with Synaffix GlycoConnect™ technology. The result is a site-specific ADC with drug-to-antibody ratio (DAR) of 6.

The Anti-B7-H4 Antibody Does Not Inhibit B7-H4 Function

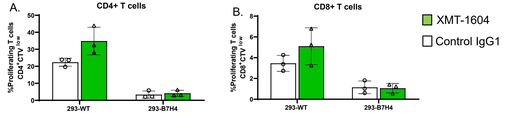


Figure 3: XMT-1604, the B7-H4 Antibody component of XMT-1660 does not affect T cell proliferation. CD4+ (A) and CD8+ (B) T cells cultured in the presence of HEK-293 cells engineered to overexpress B7-H4 showed reduced proliferation, as measured by Cell Trace Violet assay in comparison to control HEK-293 cells. Binding of cells with XMT-1604 did not reverse T cell suppression.

B7-H4 ADCs Evaluated in These Studies

- Dolasynthen is a fully synthetic ADC platform that can be used to precisely modulate DAR, and is compatible with site-specific bioconjugation
- Dolaflexin is a polymeric ADC platform that can enable the generation of higher DAR ADCs through stochastic conjugation methods
- Both platforms use the DolaLock payload (AF-HPA), with controlled bystander effect (Clardy, et al. 2018)

In these studies, we compared three B7-H4-targeted DolaLock ADCs with the same targeting antibody:
- XMT-1660 = site-specific bioconjugation at glycan-remodeled Asn297; DAR = 6.
- Dolasynthen DAR-2 = site-specific bioconjugation at glycan-remodeled Asn297; DAR = 2.
- Dolaflexin DAR-12 = stochastic bioconjugation at cysteines; Average DAR of 12

B7-H4 ADCs Exhibit Potent, Target-Dependent Cytotoxicity

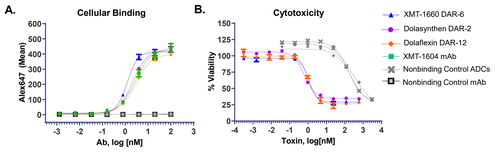


Figure 4: Cellular Binding and Cytotoxicity. **A.** All B7-H4 ADCs in these studies retain their ability to recognize B7-H4 on the cell surface, and induce potent and specific cytotoxicity (B) as assessed with HEK-293 cells engineered to overexpress B7-H4.

XMT-1660 DAR-6 Outperformed Other DolaLock ADCs in Triple Negative Breast Cancer Models MX-1 and HBCx-24

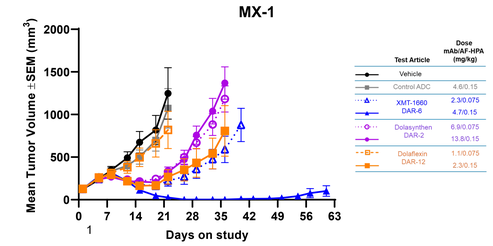


Figure 5: Anti-tumor activity in MX-1 xenografts. NCr nu/nu mice bearing MX-1 tumors were randomized into groups and administered a single IV dose (black arrowhead) of either XMT-1660, the Dolasynthen DAR-2 ADC, the Dolaflexin DAR-12 ADC or the control ADC. XMT-1660 outperformed the other ADCs at matched payload doses.

Figure 6: Anti-tumor activity in HBCx-24 xenografts. Athymic nude mice bearing HBCx-24 tumors were randomized into groups and administered a single IV dose (black arrowhead) of either XMT-1660, the Dolasynthen DAR-2 ADC, the Dolaflexin DAR-12 ADC, the control ADC, or the unconjugated antibody. XMT-1660 outperformed the other ADCs at matched payload doses. Unconjugated mAb XMT-1604 did not have any effect on tumor growth.

XMT-1660 Outperformed Other B7-H4 DolaLock ADCs in an ER+/HER2- PDX Model

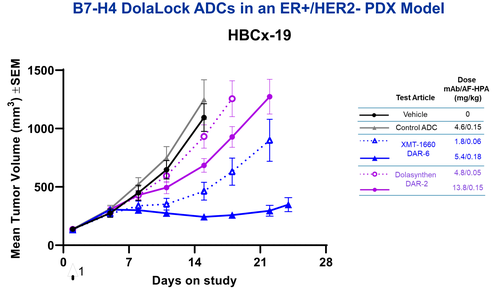
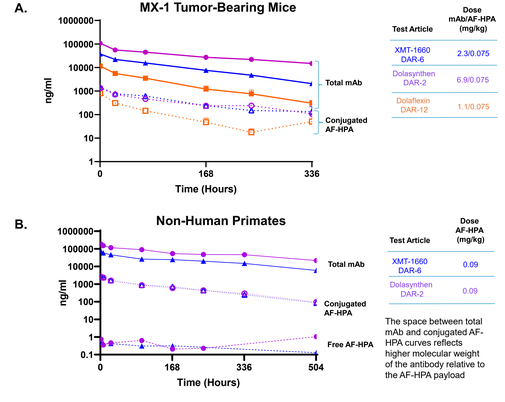


Figure 7: Anti-tumor activity in HBCx-19 xenografts. Athymic nude bearing HBCx-19 tumors were randomized into groups and administered a single IV dose (black arrowhead) of XMT-1660 DAR-6, the Dolasynthen DAR-2 ADC, or the control ADC. XMT-1660 DAR-6 outperformed the Dolasynthen DAR-2 ADC at both dose levels.

B7-H4 DolaLock ADCs Are Highly Stable *In Vivo*



The space between total mAb and conjugated AF-HPA curves reflects higher molecular weight of the antibody relative to the AF-HPA payload

Figure 8: Pharmacokinetic studies of XMT-1660 DAR-6 and other conjugates. Pharmacokinetics after a single IV dose of ADCs at 0.075 mg/kg by payload in MX-1 tumor-bearing mice (A), and 0.09 mg/kg by payload in non-human primates (B). Conjugates showed suitable PK in both models. Low amounts of free AF-HPA in non-human primates dosed with XMT-1660 and Dolasynthen DAR-2 ADCs indicate stability of Dolasynthen ADCs *in vivo*. Free AF-HPA was not measured in the mouse study. Dolaflexin DAR-12 was not tested in non-human primates..

The "Perfect Storm" Hypothesis

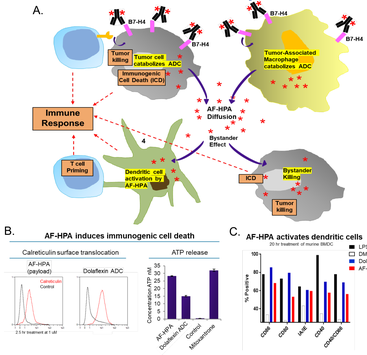


Figure 9: Targeting the function of B7-H4-expressing tumor-associated macrophages (TAMs) is hypothesized to contribute to antitumor activity. Targeting B7-H4 on TAMs may contribute to ADC uptake and processing in the tumor microenvironment (Li, et al. 2017), resulting in additional antitumor activity, by bystander killing and immune cell activation (A). In support of this, prior work (Traore, et al. 2018) has demonstrated the ability of AF-HPA to activate immunogenic cell death (B) and dendritic cell activation (C).

Conclusions

1. B7-H4 is a suitable target for DolaLock ADCs due to expression across different solid tumors, including PD-L1-negative tumors.
2. XMT-1660 is a B7-H4-targeted site-specific ADC, DAR-6, that uses the AF-HPA-based Dolasynthen platform and the Synaffix GlycoConnect™ platform.
3. We evaluated B7-H4 ADCs with three DARs and XMT-1660 DAR-6 consistently outperformed the other ADCs *in vivo*.
4. XMT-1660 demonstrated a desirable PK profile in tumor-bearing mice and non-human primates.
5. Targeting of B7-H4 on tumor-associated macrophages as well as tumor cells could add to the antitumor activity of XMT-1660.
6. Overall data, including efficacy, PK, and NHP tolerability supports further development of XMT-1660 and a clinical trial for the treatment of B7-H4-expressing tumors, such as breast, endometrial, and ovarian.

References

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. Clardy, S., et al. (2018) Unique Pharmacological Properties of Dolaflexin-Based ADCs- A Controlled Bystander Effect. Poster presented at the Annual AACR Meeting
3. Leong, S., et al. (2015). An Anti-B7-H4 Antibody–Drug Conjugate for the Treatment of Breast Cancer. Mol. Pharmaceutics, 12, 1717−1729
4. Li, F., et al. (2017). Tumor-Associated Macrophages Can Contribute to Antitumor Activity through FcgR-Mediated Processing of Antibody–Drug Conjugates. Molecular Cancer Therapeutics, 16, 1347-1354
5. MacGregor, H., and Ohashi, P. (2017). Molecular Pathways: Evaluating the Potential for B7-H4 as an Immunoregulatory Target. Clin Cancer Res; 23(12); 2934–41
6. Sachdev, et al. (2019). Phase 1a/1b Study of First-in-Class B7-H4 Antibody, FPA150 as Monotherapy in Patients with Advanced Solid Tumors. Poster presented at the Annual ASCO Meeting
7. Traore, et al. (2018). Synergy of an anti-HER2 ADC TAK-522 (XMT-1522) in combination with anti-PD1 mAb in a syngeneic breast cancer model expressing human HER2. Poster presented at the annual AACR meeting

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