Abstract ID: 1738

XMT-2056, a well-tolerated, Immunosynthen-based STING-agonist antibody-drug conjugate which induces anti-tumor immune activity

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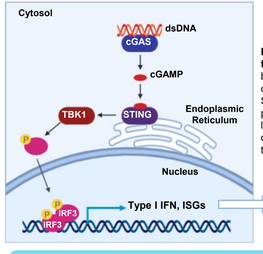
Abstract - STING pathway agonism has emerged as a potential therapeutic mechanism to stimulate an innate anti-tumor immune response. However, the systemic administration of a free STING agonist may be limited by toxicity, and broad biodistribution may not be ideal. Antibody-drug conjugates (ADCs) constitute a proven therapeutic modality that enables tumor-targeted delivery and thus is ideally suited to systemic administration with reduced toxicity. To develop an optimized STING agonist ADC platform, we designed a novel STING-agonist specifically tailored for use in an ADC. Determination of the co-crystal structure confirmed that the agonist binds to the closed, or 'active', conformation of the STING homodimer.

The resulting Immunosynthen platform, which was developed specifically for the selected STING agonist payload, was used to generate XMT-2056, a tumor antigen-targeted STING-agonist ADC with excellent drug-like properties and >100-fold increased potency as compared to the free STING-agonist payload. In mice, XMT-2056 induced robust anti-tumor immune activity, with only minimal increases in systemic cytokine levels, and exhibited significant benefit over the benchmark free STING-agonist payload in both regards. Additionally, *in vitro* and *in vivo* studies demonstrate that XMT-2056 is able to activate the STING pathway in both tumor-resident immune cells and tumor cells, offering a potential advantage over other innate immune activating pathways. XMT-2056 was well-tolerated in non-human primates at significantly higher exposure levels than those required for anti-tumor activity, and the ADC exhibited favorable pharmacokinetics after repeat doses. Together these data support the clinical development of XMT-2056.

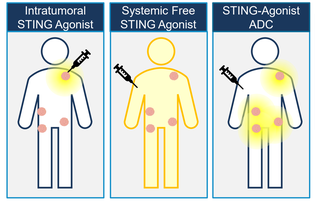


STING Pathway

Depiction of the STING pathway (adapted from Corrales et al[1]). cGAS recognizes and binds to cytosolic dsDNA and synthesizes cGAMP, a natural STING agonist that activates STING, which in return activates TBK1-mediated phosphorylation of IRF3 transcription factor, leading to expression of type I interferon and other interferon stimulated genes and eventually to an anti-tumor immune response.

Anti-Tumor Immune Responses

STING Agonist ADC Approach Could Address Administration Issues, Systemic Tolerability, and Activity



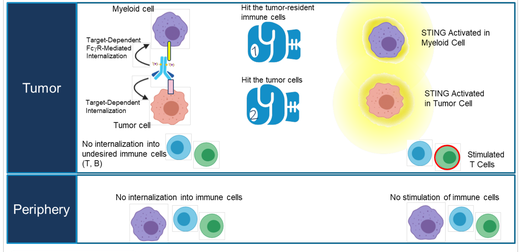
- Systemic administration with targeted delivery to the tumor
- Improved anti-tumor activity compared to free agonist
- Improved tolerability compared to free agonist

Systemic immune activation

Tumor, no immune activation

Tumor with STING-Mediated Innate Immune Activation

STING Agonist ADCs Deliver a One-Two Punch, Activating STING in Myeloid *and* Tumor Cells[2]



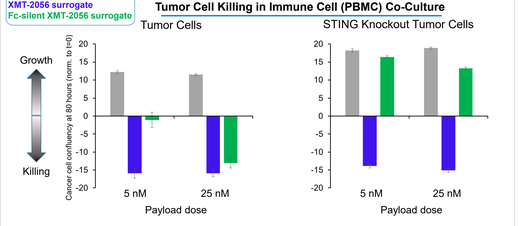
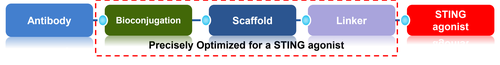


Figure 1. Tumor-targeted Fc silent ADC activity is diminished in STING knock out cancer:PBMC co-cultures. STING wt and ko cancer cells were co-cultured with PBMCs and cancer cell killing activity of the XMT-2056 surrogate Fc wt or Fc silent ADCs, and control ADC were assessed by IncuCyte analysis. XMT-2056 surrogate with wt Fc induced significant killing of both STING wt and ko cancer cells in PBMC co-cultures, whereas Fc silent XMT-2056 surrogate induced significant killing of only STING wt cells but had no activity in STING ko cancer cell:PBMC co-cultures. These data demonstrate the contribution of tumor-intrinsic STING pathway activation to the anti-tumor activity of the tumor cell-targeted STING agonist ADCs in *in vitro* co-cultures of human cancer cell : primary immune cell co-cultures. For a more in-depth discussion on tumor intrinsic activity with a STING agonist ADC, please refer to abstract 1773.

Highly Modular Approach Allowed for Optimal ADC Synthesis

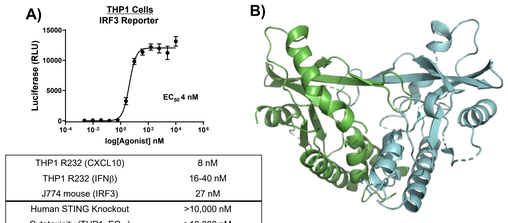
Critical Attributes Matched to Antibody and Target



Building ADCs with a modular approach:

- Flexibility in design enables optimization of ADC for optimal pharmacological and pharmacokinetic properties.
- Modular components enable fine-tuning of drug-to-antibody ratio (DAR).
- Amenable to many bioconjugation methods.
- Ultimately the ADC is optimized for the target, antibody, and payload.

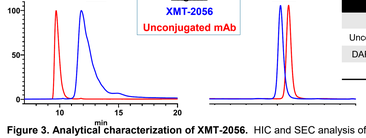
Identification of an Optimal Payload for ADC Delivery



THP1 R232 (CXCL10)	8 nM
THP1 R232 (IFNβ)	16-40 nM
J774 mouse (IRF3)	27 nM
Human STING Knockout	>10,000 nM
Cytotoxicity (THP1, EC_{50})	>10,000 nM
Safety Screen 44 - Panlabs	No activity at 1 μM

Figure 2. Characterization of a STING agonist. A) THP1 cells engineered with a secreted luciferase reporter for IRF3 activity were incubated with compound for 2h before analysis showed dose-dependent activity of the STING agonist. Additional characterization of the STING agonist shows similar activity in activating mouse STING relative to hSTING activity and the high specificity of the compound. **B)** High resolution co-crystal structure obtained demonstrating the agonist binds to the closed, or active, conformation of STING.

XMT-2056 Demonstrates Good Physicochemical Properties



Attribute	Value
mAb (mg/mL)	>10.0
Unconjugated mAb (%)	<1
DAR (UV-Vis method)	~8
HMW (%)	<1

Figure 3. Analytical characterization of XMT-2056. HIC and SEC analysis of XMT-2056 compared to unconjugated mAb. Drug-to-antibody ratio (DAR) was determined by UV-Vis spectrometry. Amount of unconjugated mAb was determined by HIC analysis. Amount of high molecular weight species (HMW) was determined by SEC analysis.

XMT-2056 is a Potent STING Agonist *In Vitro* Demonstrating Significant Improvement Over the Free Payload

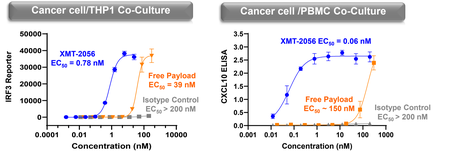


Figure 4. *In vitro* analysis of XMT-2056. Cancer cells were co-cultured with either engineered THP1 cells or PBMCs, treated with XMT-2056 and/or the free payload, and evaluated for STING activation by IRF3 or CXCL10 levels, respectively. XMT-2056 shows a significant approvement in activity compared to the free payload. In mono-culture experiments, XMT-2056 showed no activity at the highest dose in both assays (data not shown), demonstrating the necessity of both the cancer and immune cell for STING activation.

XMT-2056 Demonstrates Excellent *In Vivo* Efficacy and PK

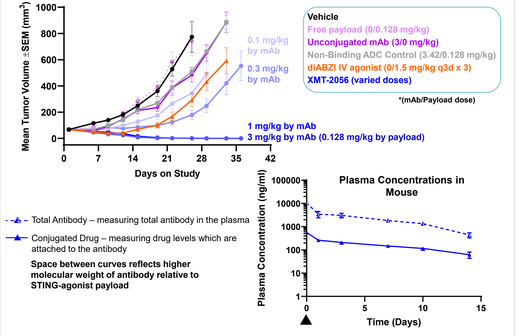


Figure 5. XMT-2056 induced durable and complete tumor regressions in xenograft mouse models at significantly lower doses by payload than the IV administered diABZI STING agonist[3] and demonstrated excellent *in vivo* PK properties. Single doses of XMT-2056 administered intravenously resulted in tumor regression in a dose dependent manner. Control ADC, unconjugated mAb, and free payload at equivalent doses show little to no effect demonstrating the advantage of the ADC. The diABZI IV agonist (administered as described in reference 3) showed only modest effect on tumor growth inhibition. PK assessment in non-tumor bearing mice showed excellent exposure over the course of the study.

Doses of STING ADC that Result in Complete Tumor Regressions Induce Significantly Lower Levels of Serum Cytokines than the IV STING Agonist

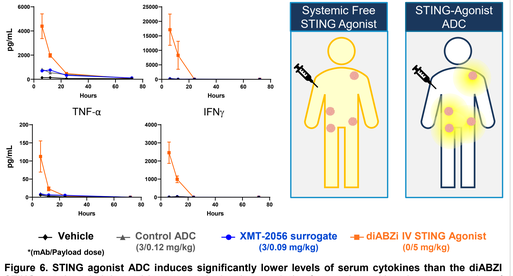
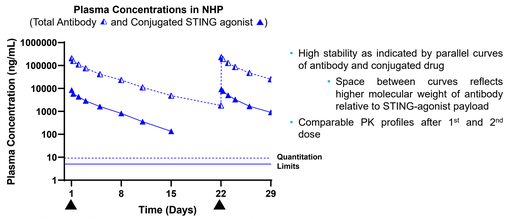


Figure 6. STING agonist ADC induces significantly lower levels of serum cytokines than the diABZI STING agonist administered intravenously. Luminex analysis of systemic cytokines in serum samples of tumor bearing mice treated with a single dose of XMT-2056 surrogate or control ADC and the diABZI IV STING agonist. Systemically delivered diABZI STING agonist induced 6-100x higher systemic cytokines than targeted STING agonist ADC despite the lack of sustained anti-tumor activity.

XMT-2056 Shows Good Exposure After Multiple Doses and is Well Tolerated in Non-Human Primates



- High stability as indicated by parallel curves of antibody and conjugated drug
  - Space between curves reflects higher molecular weight of antibody relative to STING-agonist payload
- Comparable PK profiles after 1st and 2nd dose

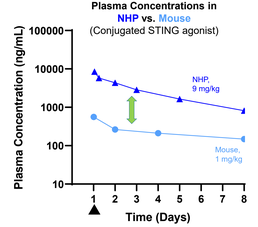
Figure 7. XMT-2056 shows good pharmacokinetics within NHP. Animals were treated with XMT-2056 at 9 mg/kg on days 1 and 22 (q3w x 2) with a scheduled necropsy on day 29. Assessments included clinical pathology, serum cytokines, and histopathology.

NHP Results

Single-dose and repeat-dose studies at 9 mg/kg antibody

Intravenous administration

- No clinical signs, no mortality
- High exposure, high ADC stability in circulation
- Transient elevation of 5 cytokines out of 24 tested
- No adverse changes in clinical pathology
- No adverse findings in histopathology



Exposure of XMT-2056 at a well-tolerated dose in NHPs is ~10-fold higher than the exposure required for sustained tumor regression in mouse

Conclusions

- XMT-2056 delivers a STING agonist payload which binds to the closed conformation of the protein and designed with properties suitable for ADC development.
- XMT-2056 has potent *in vitro* STING activity with >100-fold improvement in activity over the free payload, exhibiting a 'one-two punch' unique to STING agonist ADCs.
- XMT-2056 shows excellent *in vivo* efficacy after a single IV dose, while having minimal effect on systemic cytokines.
- XMT-2056 has good exposure in NHPs and is well tolerated at a dose level ~10-fold higher than required for sustained tumor regression in mice models.
- These data support further development of XMT-2056 with expected FIH dose in early 2022.

References:

1. Corrales et al. ***JCI*** 2016, 126: 2404-2411.
2. Cetinbas et al. SITC 2020 poster; Cetinbas et al. AACR 2021 poster. Both posters available at www.Mersana.com.
3. Ramanjulu et al. ***Nature*** 2018, 564: 438-443.