Tumor Targeting of a STING Agonist with an Antibody-Drug Conjugate Elicits Potent Anti-Tumor Immune Responses

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INTRODUCTION

STimulator of **I**nterferon **G**enes (STING) has emerged as an innate immune pathway capable of inducing anti-tumor immune activity by stimulating antigen presenting cells and type I interferon production, leading to T-cell priming and activation.[1] In murine models, both intratumoral (IT) and intravenous (IV) administration of STING agonists have been shown to induce tumor regressions and generate immunological memory;[2,3] clinical studies are underway. However, application of IT or IV administered STING agonists have several limitations, such as accessibility of tumors for IT injections, tumor exposure, and nonspecificity. Here, we employed our proprietary Synthemer technology to generate novel STING antibody-drug conjugates (ADC), which consists of a STING agonist incorporated into an optimized chemical scaffold for bioconjugation, designed to provide optimal drug-like properties.

We hypothesize that a STING antibody-drug conjugate (ADC) – in which a STING agonist is conjugated to an antibody – will overcome these limitations and exhibit several advantages, including:

- ***Active / Targeted-Delivery:*** By actively delivering STING agonist into the desired cell types in the TME, ADCs overcome any solubility/permeability issues of the free agonist and achieve tumor specificity.
- ***Systemic Delivery:*** ADCs are administered systemically, and therefore are capable of carrying STING agonist to all tumor lesions.
- ***Improved Therapeutic Index:*** Targeted delivery increases tumor-specific immune activation and minimizes systemic inflammation.
- ***Enhanced Pharmacokinetic Properties:*** Significantly better pharmacokinetic properties of ADCs ensure increased exposure of tumors to the STING agonist.

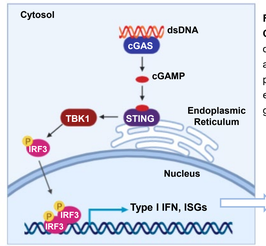


Figure 1. Depiction of the STING pathway[4] (adapted from Corrales et al)[1]. cGAS recognizes and binds to cytosolic dsDNA and synthesizes cGAMP, a natural STING agonist that activates STING, which in return activates TBK1-mediated phosphorylation of IRF3 transcription factor, leading to expression of type I interferon and other interferon-stimulated genes and eventually to an anti-tumor immune response.

Anti-Tumor Immune Responses

Synthemer Technology for Optimal ADC Synthesis

Building ADCs with the modular, fully synthetic Synthemer technology

- 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.

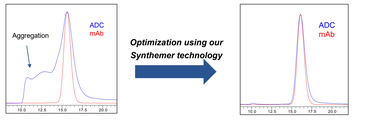


Figure 2. Mersana's novel Synthemer technology enables optimal ADC synthesis

RESULTS

Targeted STING ADC Exhibits More Than 100x Increased Potency Compared to the Free Payload *In Vitro*

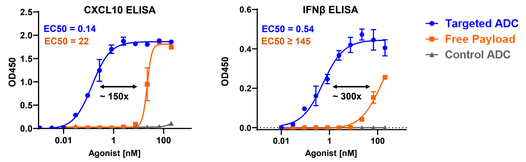
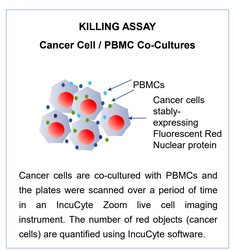
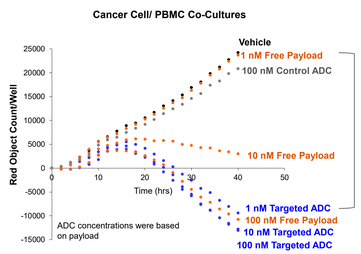
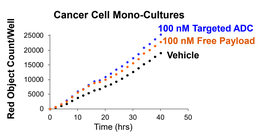


Figure 3. STING pathway activation by targeted ADC *in vitro*. Cells were treated with the indicated test articles for 16 hours and supernatants were analyzed by a CXCL10 or IFNβ ELISA kit. Targeted ADC exhibits ~150 – 300x higher potency compared to the free payload. Control ADC has no significant activity. ADC concentrations were based on payload.

Targeted STING ADC Treatment Induces Robust Killing of Cancer Cells by PBMCs







Neither ADC nor free payload induced cancer cell death in mono-cultures.

Targeted ADC induced robust killing of cancer cells by PBMCs at 100x lower payload concentration. Similar results were obtained using PBMCs from several different donors.

Representative IncuCyte images of cancer/PBMC co-cultures (10 nM test articles, 40 hours)

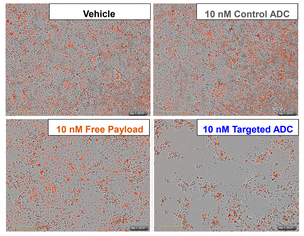


Figure 4. PBMC killing assay. Fluorescent Red Nuclear Protein-expressing cancer cells were co-cultured with PBMCs and after addition of the indicated test articles the plates were placed in an IncuCyte Zoom live cell imaging instrument in an incubator (37 °C, 5% O_2) and scanned every 4 hours over 2 days. The number of red objects (cancer cells) were quantified using the IncuCyte software. Red object numbers were normalized to T=0 hr for each well. ADC concentrations shown were based on payload.

Targeted STING ADC Outperforms Systemically Administered STING Agonist and Elicits Potent Anti-tumor Immune Responses *In Vivo*

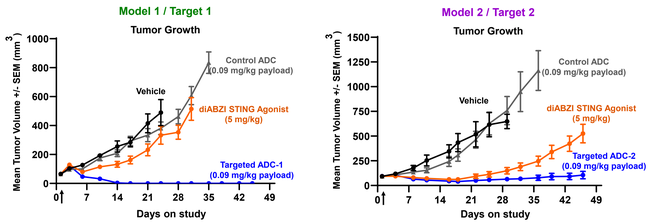
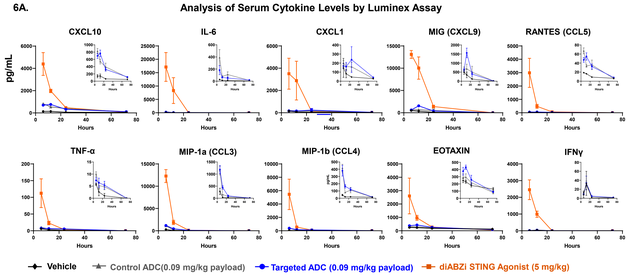


Figure 5. Comparison of the efficacy of two different ADCs (targeting different antigens) to a systemically administered diABZI STING agonist[3] in two different tumor models. Single dose of test articles were administered intravenously at the indicated doses and the tumor volumes were measured two times a week. As shown above, the targeted ADCs at a ~50x lower payload dose outperformed the systemically administered diABZI STING agonist. Control ADCs did not show any efficacy. ADCs were well tolerated at the efficacious doses (data not shown).

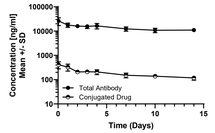
Targeted STING ADC Induces Significantly Lower Levels of Systemic Cytokines at Doses Resulting in Sustained Tumor Regressions Unlike the Systemically Delivered STING Agonist



Systemically delivered diABZI STING agonist induced markedly high levels of systemic cytokines despite the lack of sustained anti-tumor activity (Fig. 5). In contrast, targeted STING ADC did not cause significant systemic cytokine elevation while maintaining extended plasma exposure (Fig. 6B) and potent tumor specific STING activation/sustained tumor regression (Fig. 7). Control ADC induced very low levels of systemic cytokines similar to the targeted STING ADC.

Figure 6A. Luminex analysis of systemic cytokine concentrations in serum samples of tumor-bearing mice treated with a single dose of 0.09 mg/kg (IV) ADCs and 5 mg/kg (IV) of diABZI STING agonist. Cytokines were analyzed using Milliplex mouse cytokine/chemokine magnetic bead panel on a FlexMap3D Luminex instrument. **6B.** Total antibody and antibody-conjugated drug concentrations of STING ADC in non-tumor bearing mouse plasma were measured using an MSD-ECL sandwich immunoassay and an immunocapture-mass spectrometry technique respectively.

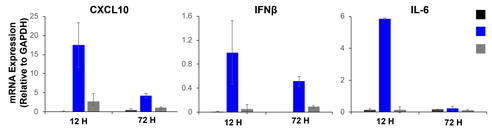
6B. Plasma Concentration of STING ADC



Extended plasma exposure to STING ADC did not result in extended exposure to systemic cytokines

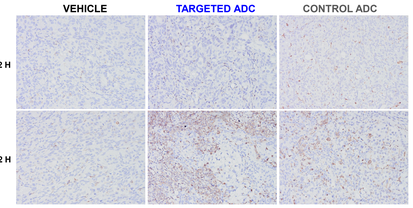
Targeted STING ADC Activates STING Pathway in Tumors and Induces Marked Immune Cell Infiltration

7A. qPCR Analysis of FFPE Tumor Tissue



Targeted STING ADC activated STING pathway in tumors significantly better than control ADC

7B. CD45 Immunohistochemistry Staining of FFPE Tumor Tissue



Targeted STING ADC led to significantly more CD45+ immune cell infiltration into tumors compared to the control ADC.

Figure 7. Comparison of tumor specific activity of targeted ADC to control ADC. Tumor-bearing mice were intravenously injected with a single dose of the targeted or control ADCs (0.09 mg/kg payload). Tumors were harvested 12 hours (group1, N=5) and 72 hours (group 2, N=5) after dosing and processed into FFPE samples. **7A.** qPCR analysis of the FFPE samples for CXCL10, IFNβ, and IL-6 mRNA expression. GAPDH was used as a reference gene. **7B.** CD45 immunohistochemistry staining of the FFPE sections.

CONCLUSIONS

We have generated STING ADCs with desirable physicochemical properties and demonstrated:

- Over 100-fold increased potency relative to free agonist
- Significantly lower induction of serum cytokines relative to a systemically administered STING agonist
- Sustained tumor regressions after a single intravenous administration of STING ADCs

- These data suggest that STING ADC may confer an improved therapeutic index vs free agonist (IT or IV).

- ADC-mediated systemic delivery of STING agonist can activate an immune response locally

REFERENCES

1. Corrales et al. ***JCI*** 2016, 126: 2404-2411
2. Sivick et al. ***Cell Reports*** 2019, 25: 3074-3085
3. Ramanjulu et al. ***Nature*** 2018, 564: 438-443
4. Image was created with BioRender.com