Activation of STING with Synthetic Cyclic Dinucleotides and Synergy with Checkpoint Inhibition

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Disclosures

Sarah McWhirter and many contributors to the work described here are paid employees of Aduro Biotech or Novartis, hold stock in the respective companies, and in some cases are inventors on several patent applications related to STING agonists.
Presentation Summary

• Overview of Synthetic Cyclic Dinucleotides (CDNs)
  – Rationale for targeting the STING (Stimulator of Interferon Genes) pathway
  – Compound selection

• ADU-S100: Pre-clinical Efficacy Data
  – Potent tumor-initiated T cell priming and durable anti-tumor immunity
  – Mechanisms of STING-mediated antitumor immunity

• ADU-S100 and Checkpoint Inhibition in Pre-clinical Models
  – Synergistic anti-tumor efficacy with ADU-S100 in combination with anti-PD1
  – Immune correlates associated with ADU-S100 and anti-PD1 treatment
**STING** (Stimulator of Interferon Genes)

- Activates innate immunity in response to sensing nucleic acids in the cytosol.
- Downstream signaling is activated through binding of cyclic dinucleotides (CDNs).
- CDNs are synthesized by bacteria or by host enzyme cGAS in response to binding cytosolic dsDNA.
- Bacterial and host-produced CDNs have distinct phosphate bridge structures, which differentiates their capacity to activate STING.
- IFN-β is the signature cytokine of activated STING.
CDNs Activate *in situ* Tumor-Initiated T cell Priming

**Rationale for intratumoral delivery of CDNs**

- T cell inflamed tumors in humans are correlated with an IFN-β transcriptional signature in the TME
- STING plays a critical role in activating immune cells, including dendritic cells, in the tumor microenvironment
- Tumor-derived DNA induces IFN-β by tumor resident DCs through STING
- Intratumoral injection of CDNs induces IFN-β, activating tumor-resident DCs which stimulates priming of tumor specific CD8+ T cells in mice
- Approach to stimulate priming of CD8+ T cells specific for any individuals’ unique neo-antigens

Clinical compound ADU-S100: An Improved CDN Agonist of STING

(R,R) dithio diastereoisomer, non-canonical mixed-linkage [2,3]-cyclic di-AMP analog

- Phosphorothioate increases resistance to phosphodiesterases to enhance potency
- Mixed-linkage configuration allows for broad activation of human STING alleles
- Adenosine-based CDN facilitates formulation-independent trafficking to the cytosol
- Enhanced potency over natural CDN ligands
- ADU-S100 selected from series of CDN analogs based on balance of efficacy and tolerability / reduced toxicity in non-clinical studies
- Reduced injection site “reactogenicity” with adenosine analog compared with guanosine containing analogs in animal models
Impact of Phosphate Linkage Structure on STING Signaling

"Canonical Linkage"
3’3’ cGAMP

"Mixed Linkage"
2’3’ cGAMP

Bacterial Product

cGAS Pathway

ds-DNA

cGAS

2’, 3’ cGAMP

STING

NF-κB
IRF-3
STAT6

2’3’ cGAMP binds at least 10 fold more strongly to hSTING (WT allele) than the canonical cGAMP compound
STING Polymorphisms Inform Design of CDNs that Activate Human STING

Five STING alleles are found in humans

<table>
<thead>
<tr>
<th>STING variant</th>
<th>Allele frequency</th>
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<tbody>
<tr>
<td>WT</td>
<td>R GR R</td>
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<tr>
<td>Q</td>
<td>R GR Q</td>
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(Based on analysis of 1000 Human Genome Project)

Corrales and Glickman et. al, Cell Reports (2015)
STING Polymorphisms Inform Design of CDNs that Activate Human STING

Stimulation of Human STING-expressing 293T cells

Fold IFN-β LUC induction (RLU)

Corrales and Glickman et. al, Cell Reports (2015)
Clinical compound ADU-S100: An Improved CDN Agonist of STING

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ADU-S100 STING Co-Crystal Structure
IT ADU-S100 Therapy Induces a Potent Distal Effect

B16 Melanoma Flank and Lung Tumor Model

Tumor Challenge
5e4 B16 SC Flank

B16 IV Challenge

CDN IT

Harvest Lungs

Days 0 7 14 17 21 28

Primary Tumor Growth

Days Post Tumor Challenge

Tumor Volume (mm³)

B16 IV

IT Injections

Groups: n=8
- HBSS
- ADU-S100
- DMXAA

Lung Metastasis

Lung Tumor Nodules

HBSS

ADU-S100

DMXAA

IV Only

Corrales and Glickman et. al, Cell Reports (2015)
ADU-S100 Elicits Durable Anti-Tumor Immunity

4T1 Mammary Carcinoma Flank Model

**4T1 Primary Tumor Growth**

- **HBSS**
- **ADU-S100**

**Re-Challenge Tumor Growth**

- **Naive**
- **ADU-S100**

**4T1 CD8⁺ T Cell Responses**

- **Unstim**
- **AH1**

**IT vs SC Injection**

- **HBSS**
- **ADU-S100 IT**
- **ADU-S100 SC**

Endogenous tumor antigen-specific T cells

*Corrales and Glickman et. al, Cell Reports (2015)*

IT injection route required for efficacy
Durable Anti-Tumor Efficacy of ADU-S100

**B16.F10 flank model**

Anti-Tumor Response

Day 57

Tumor site

ADU-S100

Vitiligo

Survival

- **Groups (n=8)**
  - HBSS
  - R,R dithio 2',3' c-di GMP 25 μg
  - ADU-S100 25 μg

- **Groups (n=8)**
  - HBSS
  - ADU-S100 100 μg

*Corrales and Glickman et. al, Cell Reports (2015)*
ADU-S100 Induces IFN-β and TNF-α in Tumor-Resident Immune Cells

*B16.F10 melanoma model*

Inject B16-F10 flank tumor

↓

Harvest tumor at 1 hr or 5 hr

↓

4 hr culture with Golgi inhibitors

↓

Intracellular cytokine staining

1 hour

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5 hours

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<td>TNFα</td>
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% of CD45+, IFNβ+

% of CD45+, TNFα+

1 hr

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<tr>
<td>TNFα</td>
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5 hr

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**** IFNβ

**** TNFα
B16-SIY melanoma model

Impact of TNF-α blockade on regression of CDN-injected tumor

**HBSS + IgG**

**HBSS + Enbrel**

**ADU-S100 + IgG**

**ADU-S100 + Enbrel**

TNFα Mediates Primary Tumor Regression Induced by ADU-S100
ADU-S100 Treatment Induces Maturation of APCs in Tumor Draining Lymph Nodes

*B16.F10 melanoma model*
The Hematopoietic Compartment is Necessary for ADU-S100 Induced Tumor-Specific CD8\(^+\) T cell Responses

_B16.SIY model_

7d post IT Splenic IFN\(\gamma\) ELISPOT (+/- SIY peptide stimulation)
ADU-S100 Elicited CD8 T cells Contribute to Protection Against Tumor Rechallenge

4T1 flank model

I.T. ADU-S100 day 14
- cure tumors 48/60
- CD8 deplete
- rechallenge
- monitor survival

naïve mice

ADU-S100 cured mice

ADU-S100 injection
- 48/60 cleared
- cured mice

11/11 protected
2/12
CD8 T cells from ADU-S100 Treated Mice are Necessary and Sufficient to Protect Against Tumor Challenge

4T1 flank model, adoptive transfer study

ADU-S100, or saline

1 week

peak of T cell response +/- CD8 depletion, transfer whole spleens or isolated CD8s into naïve recipient

1 day

challenge with tumor cells

Tumor Volume [mm³]

no transfer

transferred population: whole spleen

CD8 T cells

cell source: saline treated

cell source: ADU-S100 treated mice

transferred population: whole spleen whole spleen CD8 depleted CD8 T cells

days after implantation
Rationale for Combining STING Agonist and anti-PD1

- Predicted synergy with ADU-S100/anti-PD1 based on unique MOA of each IO agent in tumor immuno-surveillance
- Lack of response to CPI therapy can be attributed to low level of TILs
- TIL infiltration of TME correlates with IFN-β transcriptional profile and STING activation
- Relapse to CPI therapy can be due to loss of function mutations and in some cases can be overcome by activation of STING
Correlation of Systemic Immunity with Abscopal Effect

4T1 dual flank model

Impact of CDN dose and PD-1 blockade on distal non-injected tumor growth

Schema

- Day 0: Tumor Challenge
  - 4T1 SC both flanks
- Day 8: ADU-S100 IT
- Day 31: αPD-1 or IgG2a control
  - 2X Weekly IP
- Sac at Max

ADU-S100

Innate Response
Adaptive Response?

Innate Response?
Adaptive Response

α-PD-1
Increased Efficacy with Immune Checkpoint Blockade

4T1 dual flank model
Combination Effect with Low Dose ADU-S100 and α-PD-1 Treatment is CD8-dependent

4T1 dual flank model

- HBSS + IgG
- Low Dose ADU-S100 + IgG
- Low Dose ADU-S100 + αPD-1
- HBSS + αCD8α
- Low Dose ADU-S100 + αCD8α
- Low Dose ADU-S100 + αPD-1 + αCD8α

Distal Tumor
Rationale for Combining STING Agonist and anti-PD1
ADU-S100/MIW815 Phase I Clinical Trial Design

**FIH STING Agonist Clinical Trial**

**Part 1**

(>21 patients)

- ADU-S100
- Safety & Biomarker Evaluation
- Primary Objective: Safety and Tolerability

- UV-induced tumors
- > Additional cycles to progressive disease
- q.weekly

**Part 2**

(approximately 50 patients each arm)

- ADU-S100

- UV-induced tumors
- > Additional cycles to progressive disease
- ADU-S100

- non UV-induced tumors
- > Additional cycles to progressive disease
- ADU-S100

- Two-part study to assess the safety/tolerability in patients with cutaneously accessible, treatment-refractory primary or metastatic solid tumors or lymphomas
  - Part 1: dose escalation in cohorts of 3-6 pts
  - Part 2: dose expansion arms to better characterize safety/efficacy

- Future trial is planned with ADU-S100 in combination with anti-PD1

Clinical trials.gov identifier: NCT02675439
Summary and Conclusions

• Aduro has developed a synthetic CDN STING agonist, ADU-S100/MIW815, that elicits potent and durable anti-tumor immunity when administered IT in pre-clinical syngeneic tumor models.

• Direct activation of STING in pre-clinical models by IT injection of ADU-S100 induces an acute TNF-α mediated reduction of the injected tumor and triggers CD8+ T cell mediated adaptive immunity that is necessary and sufficient to protect against tumor re-challenge.

• STING signaling within the hematopoietic compartment is required for CDN-mediated induction of anti-tumor immunity.

• Combining ADU-S100 IT with PD-1 blockade in animal models enhances treated and distal anti-tumor regression and increases the percentage and function of anti-tumor CD8+ T cells in the distal tumor.

• A Phase I trial is ongoing to evaluate the safety and efficacy of ADU-S100 in patients with advanced/metastatic solid tumors or lymphomas.