Immuno-Oncology: Targeting STING

Johannes Diesel

MacMillan Group Meeting

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## “STING Fever”

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<tr>
<th>Company</th>
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<th>Delivery</th>
<th>Program</th>
<th>Stage</th>
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<td>Aduro/ Novartis</td>
<td>ADU-S100</td>
<td>IT</td>
<td>Small-molecule STING agonist</td>
<td>Ph1/2</td>
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<td>Merck</td>
<td>MK-1454</td>
<td>IT</td>
<td>Small-molecule STING agonist</td>
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<td>IT/ IV</td>
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<td>IV</td>
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<td>Bristol-Myers Squibb (IFM)</td>
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<td>Abbvie (Mavupharma)</td>
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<td>E. coli engineered to produce high levels of the STING agonist</td>
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<td>Adenovirus that produces the bacterial STING agonist c-di-GMP</td>
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<td>Small-molecule STING agonists/ nucleic acid-based STING activators</td>
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Outline

- cGas-STING Pathway
- Structure of the STING Protein and the cGAMP-STING Complex
- Cyclic Dinucleotides (CDNs) as unique class of secondary messengers
- STING Agonists
  - CDN STING agonists
  - non-CDN STING agonists
cyclic GMP-AMP synthase (cGAS) is a DNA sensor activated by cytosolic DNA

cGAS generates the second messenger 2’,3’-cGAMP, a cyclic dinucleotide (CDN)

cGAMP is recognized by ER-bound adapter protein stimulaor of interferon genes (STING)
activated STING translocates to perinuclear Golgi compartments

cGas-STING pathway

dying cancer cell

tumor derived DNA

cGAS

cGAMP

Type I IFNs

translocation

TBK1

IRF3

ER

nucleus

aggregation

palmitoylation &

activated STING aggregation and recruiting of kinases TBK1 and IKK

phosphorylated STING binds IRF3, which dimerizes and translocates into the nucleus

transcription of genes encoding interferons (IFNs) is initiated.

IFNs exert cytotoxic effects on cancer cells and link innate and adaptive immune response.

STING pathway is downregulated in various cancer cell lines (cancer immune evasion).

STING agonists have large potential as effective anti/tumor agent

most promising are combination therapies with checkpoint inhibitors
Structure of the STING Protein

STING protein forms a V-shaped homodimer consisting of cytoplasmic C-terminal ligand binding domain (LBD) and N-terminal transmembrane domain.

Downstream signalling depends on C-terminal tail region. Ser366 is phosphorylated by TBK1 to form STING-IRF3 complex.

cGAMP-STING Complex

Two distinct binding positions of the asymmetric ligand to the symmetric binding pocket

Main binding interactions are with Arg238 via charge reinforced hydrogen bonds to the phosphate groups and nucleobase π-stacking with Tyr167

Cyclic Dinucleotides (CDNs)

2',3'-cGAMP binds STING 100-fold stronger than 3',3'-cGAMP

Cyclic Dinucleotides (CDNs)

Rational for difference in total binding energies $\Delta G_{\text{total}}$ of 2',3'-cGAMP and 3',3'-cGAMP

$$\Delta G_{\text{total}} = \Delta H_{\text{total}} - T \Delta S_{\text{total}}$$

$$\Delta S_{\text{total}} = \Delta S_{\text{protein}} + \Delta S_{\text{ligand}} + \Delta S_{\text{water}}$$

$$\Delta S_{\text{ligand}} = S_{\text{ligand(bound)}} - S_{\text{ligand(free)}}$$

- $\Delta H_{\text{STING/2',3'-cGAMP}} \sim \Delta H_{\text{STING/3',3'-cGAMP}}$ (based on ITC data)
- $\Delta S_{\text{protein}}$ is comparable as both 2',3'-cGAMP and 3',3'-cGAMP induce the same conformational change (based on X-ray structures)
- the ligands have the same volumes ($\sim 520 \, \text{Å}^3$) hence $\Delta S_{\text{water}}$ is similar

**different binding affinities originate from difference in $\Delta S_{\text{ligand}}$**
Rational for difference in total binding energies $\Delta G_{\text{total}}$ of 2',3'-cGAMP and 3',3'-cGAMP
Cyclic Dinucleotides (CDNs)

Rational for difference in total binding energies $\Delta G_{\text{total}}$ of 2',3'-cGAMP and 3',3'-cGAMP

$2',3'\text{-cGAMP binding to STING requires significantly less entropy cost}$

$S_{2',3'\text{-cGAMP}}(\text{free}) \ll S_{3',3'\text{-cGAMP}}(\text{free})$

C. Chen et al. PNAS, 2015, 112, 8947.
**CDN STING Agonists**

2',3'-cGAMP

**Assay: Differential Scanning Fluorimetry (DSF)**
- measures stabilization of protein by ligand binding against thermal unfolding
- unfolding temperature is measured by increase of fluorescence of a dye binding to hydrophobic protein parts, which are exposed upon protein unfolding

- Aduro 2',3'-cGAMP analog

STING WT DSF $\Delta T_M = 16.2 \, ^\circ C$

- increased binding affinity
- increased cellular uptake
- increased metabolic stability

STING WT DSF $\Delta T_M = 27.3 \, ^\circ C$


The Thio Effect

- decreased rate of hydrolysis caused by lower solvent stabilization of the pentavalent charged intermediate
- thio effect is widely applied in RNA-based drug discovery
- stereogenic phosphorus atom results in diastereomer formation

CDN STING Agonists

**Aduro**

STING WT DSF $\Delta T_M = 19.2 \ ^\circ\text{C}$

**Boehringer Ingelheim** (locked nucleic acid)

STING WT DSF $\Delta T_M = 30.3 \ ^\circ\text{C}$

“late eluting” diastereomer

**GSK**

**BMS**

**CDN Synthesis - Jones Protocol**

1. tBuNH₂
2. DCA/H₂O
3. pyr, G

CDN Synthesis - Jones Protocol

1. DMOCP
2. MeNH₂
3. Et₃N · HF

overall yield: \((R_p,R_p)\) 19\%, \((R_p,S_p)\) 17\%

efficient access to gram scale quantities of c-diGMP

8 steps are in one flask, <10h

bis-phosphorothioate analogs of c-diGMP accessible

mixture of up to 4 diastereomers, requires separation

protecting group manipulations

functional group interconversions
CDN Synthesis - Baran and BMS Protocol

(-)-limonene oxide

1 step

(-)-Ψ reagent

**CDN Synthesis - Baran and BMS Protocol**

![Chemical Structures]

24%, single diastereomer

previously 4%, stereorandom

- avoids sensitive P(III)-reagents
- high step efficiency
- stereoselective


diol chemoselectivity controls diastereoselectivity

CDN Synthesis - Merck Biocatalytic Approach

variety of CDN STING agonists have advanced to clinical trials

exclusively for solid tumors allowing intratumoral administration

innovation needed to allow systematic administration to patients with multiple heterogenous tumors

synthetic small molecules may be advantageous by providing improved permeability and easier synthetic access
Non-Nucleotide STING Agonists

Amidobenzimidazol (ABZI)

- HTS of small molecules that compete with binding of radiolabeled cGAMP
- ABZI identified with IC\textsubscript{50} = 14 μmol
- Two molecules ABZI bind to the STING cGAMP binding site

Non-Nucleotide STING Agonists

ABZI, IC$_{50}$ = 14 μmol

Non-Nucleotide STING Agonists

Non-Nucleotide STING Agonists

Jencks’ Principle

linked fragments reflect the sum of binding energies of two unconnected fragments if unfavorable interactions of the linker with the protein are avoided and the binding orientation is maintained.


Conformational state of STING protein determined by hydrogen deuterium exchange (HDX) MS

intravenous administration of diABZI leads to adaptive CD8\(^+\) T cell response in vivo
Non-Nucleotide STING Agonists

- Orally available non-nucleotide based STING Agonist

\[
\text{MeO} \quad \text{MeO} \\
\text{MeO} \quad \text{MeO} \\
\text{O} \quad \text{O} \\
\text{OH} \\
\]  

benzothiophene oxobutanoic acid (MSA-2)

- HTS of 2.4 million small molecules in phenotypic cell based assay
- MSA-2 induced IFN production only in STING containing THP-1 cells
- MSA-2 induced phosphorylation of STING pathway mediator proteins TBK-1 and IRF-3

Non-Nucleotide STING Agonists

MSA-2 is bound as a non-covalent dimer to STING

X-ray structure of MSA-2 bound to human STING

Benzothiophene proton shifts change concentration dependend

Non-Nucleotide STING Agonists

Different administration routes of MSA-2 and effect on MC38 (colon carcinoma) tumor growth

Non-Nucleotide STING Agonists

- MSA-2 enhances antitumor activity of anti-PD-1 immune checkpoint inhibitor in tumor models that are poorly responsive to PD-1 blockade

- MSA-2 and anti-PD-1 are synergistic in inhibiting tumor growth

→ both innate and adaptive immune function contribute to STING agonist-driven tumor regression