A payload screening and ADC evaluation platform for STING agonist antibody drug conjugate

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Abstract

Antibody drug conjugates (ADCs) are a class of rapidly developing targeted biotherapeutic drugs, which consist of three elements: antibody, linker, and payload. ADCs combine the high specificity of antibodies with the potency of payloads, delivering the highly efficient small molecule within the target tumor cells.

As one of the new payload types, stimulator of interferon genes (STING) agonist can initiate the innate immune response, enhancing the attack on tumor cells. STING agonist ADCs, allowing tumor-localized activation of STING, may achieve greater anti-tumor activity and better tolerability.

By combining the STING binding assay, different THP-1 reporter assay, and cytokine release detection, ICE Bioscience can support for STING agonist activities screening. Western blotting assay can be applied for demonstrating the molecular mechanisms of candidates. For specific screening and evaluation of HER2 STING agonist ADCs, cell binding assay, cell internalization assay, HER2 overexpression reporter assays, co-culture assays of tumor cell lines or STING knockout tumor cell lines with THP-1, hPBMC, as well as whole blood can be utilized at different project stages.

Background

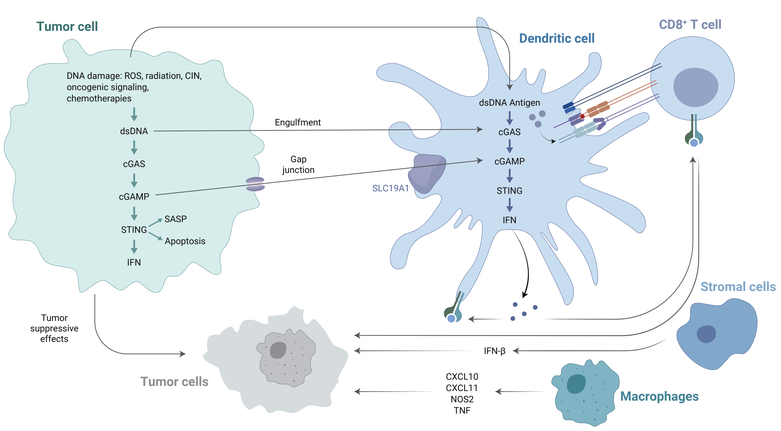

Figure 1. Overview of cGAS-STING signaling pathway and its antitumor mechanism.
The cGAS-STING pathway plays a critical role in the innate immune system and antitumor immunity. As a natural immunosensor, cGAS can detect and bind to cytoplasmic dsDNA to form a complex, generating a second messenger cGAMP, which can activate the STING protein. STING activation in tumor cells may promote apoptosis in tumor cells. Meanwhile, STING also trigger the transcriptional activation of NF-κB, which regulate the expression and secretion of pro-inflammatory cytokines. STING activation also facilitate communication between tumor cells and neighboring immune cells to modulate antitumor immunity. Moreover, DCs can capture tumor-associated antigens and subsequently activate CD8+ T cells to kill tumor cells. Macrophages can produce TNF-α and express high levels of chemokines such as CxCL10, CxCL11, and NOS2. Stromal cells can express IFN-β to inhibit tumor angiogenesis.

Specific assay list in ICE supporting for STING agonist screening

Biochemical and potency screening

- STING binding assay
- STING-ISRE-Luc reporter assay
- pSTING/pIRF3/pTBK1 WB assay
- qPCR detection of chemokines
- THP1-Dual reporter assay
- THP1-Dual KI-hSTING-R232 reporter assay
- PBMC activation
- Cytokine release detection: ELISA, MSD
- ...

ADMET screening

- Hydrophilic (log P)
- Permeability
- Lysosome stability
- Efflux ratio
- ...

Cardiac safety assessment

- hERG

STING agonist activities screening

1. Binding activity and potency screening with reporter assays

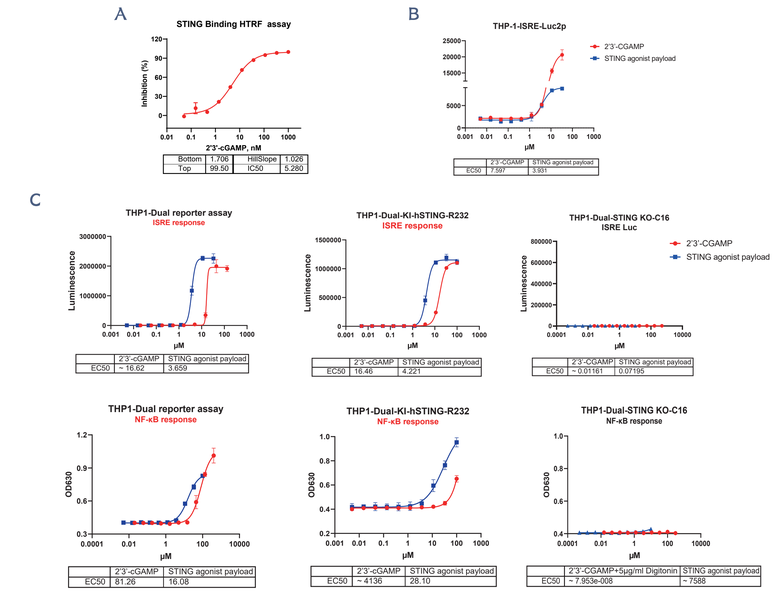

Figure 2. Binding activity and potency evaluation of STING agonist. A. Biochemical binding assay for STING agonist screening. B. THP1-ISRE reporter assay for STING agonist screening. C. THP1-Dual reporter assay for STING agonist evaluation. 2'3'-cGAMP was used as systematic control.

2. Cytokine release analysis

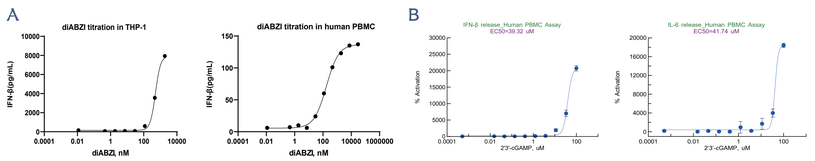

Figure 3. Cytokine release analysis. A. IFN-β in different cell culture supernatants (THP-1 and human PBMC) induced by STING agonist diABZI were detected by ELISA. B. 2' ,3' -cGAMP activate the release of IFN-β and IL-6 in human PBMC, which were detected by ELISA.

3. Molecular mechanism study: Western blotting assay

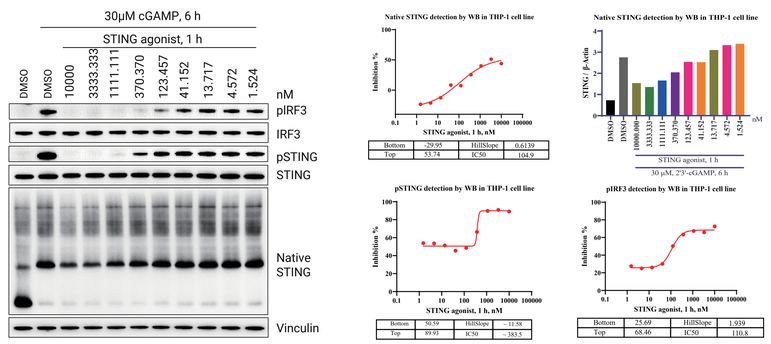

Figure 4. Western blotting analysis of native STING, phosphorylated STING (pSTING) and phosphorylated IRF3 (pIRF3) in STING agonist-treated THP-1 cells.

Specific assay list in ICE supporting for STING-ISAC project

- Cell binding assay and internalization assay
- Co-culture assays: tumor cells + THP-1, hPBMC, whole blood; STING-ISRE-Luc THP-1, THP1-Dual, THP1-Dual KI-hSTING-R232
- Cytokine release assay: ELISA, MSD

HER2 STING agonist ADC screening and evaluation

1. Cell list in ICE can be used supporting for STING-ISAC project

- Normal cell line: THP-1
- Reporter cell lines: THP1-ISRE-Luc2p, THP1-Dual, THP1-Dual KI-hSTING-R232
- HER2 overexpression cell lines: THP1-ISRE-HER2-OE-17#, THP1-Dual™-HER2-OE-1#, THP1-Dual™ KI-hSTING-R232-HER2-OE-20#
- STING-KO cell lines: THP1-STING-KO-25#, THP1-ISRE-Luc STING KO, THP1-Dual STING-KO-16#, SKOV3-GFP STING-KO-3#

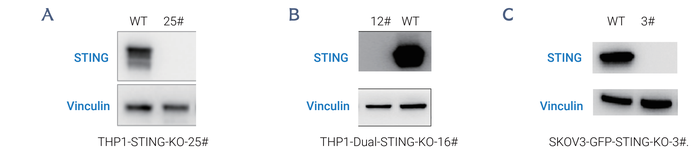

Figure 5. Verification of STING-KO cell lines, detected by western blotting. A. THP1-STING-KO-25#. B. THP1-Dual STING-KO-16#. C. SKOV3-GFP STING-KO-3#.

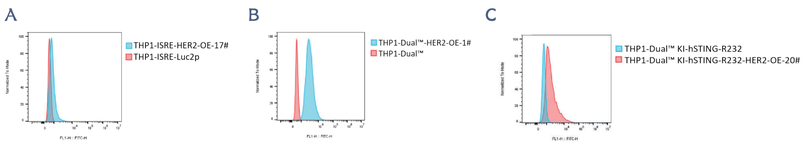

Figure 6. Verification of HER2 over-expression cell lines, detected by flow cytometry. A. THP1-ISRE-HER2-OE-17#. B. THP1-Dual™-HER2-OE-1#. C. THP1-Dual™ KI-hSTING-R232-HER2-OE-20#.

2. Cell binding assay and cell internalization assay

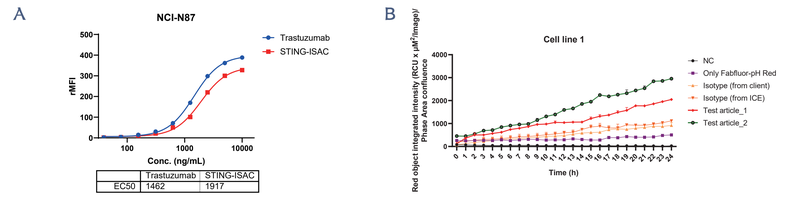

Figure 7. Cell binding assay and cell internalization assay. A. Cellular affinity comparison of HER2 STING-ISAC and the corresponding antibody (Trastuzumab) on NCI-N87 cell lines. B. Kinetic monitoring of antibody internalization with Incucyte S3. Note: The test articles are not Trastuzumab or HER2 antibody.

3. Co-culture assays

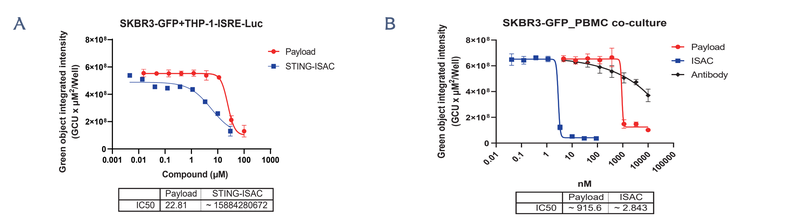

Figure 8. Co-culture cell killing activity of STING-agonist payload and HER2 STING-ISAC. A. Co-culture of SKBR3-GFP and THP1. B. Co-culture of SKBR3-GFP and human PBMC.

References

Gan Y, Li X, Han S, Liang Q, Ma X, Rong P, Wang W, Li W. The cGAS/STING Pathway: A Novel Target for Cancer Therapy. Front Immunol. 2022 Jan 3;12:795401.