WuXi AppTec STING Pathway Related Service

WuXi AppTec Research Service Division, Oncology & Immunology Unit

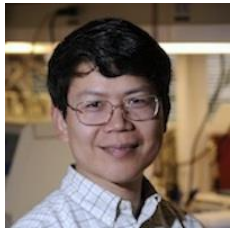
2021.01

OncoWuXi Newsletter

Outline

- STING Background
- *In vitro* STING pathway related functional assays
- *In vivo* anti-tumor efficacy study of STING agonist in CT26/4T1 syngeneic models
- *In vivo* anti-tumor efficacy study of STING agonist in B16F10 syngeneic model
- Cytokine analysis of B16F10 syngeneic model post STING-1 treatment
- Immunoprofiling of B16F10 syngeneic model post STING-1 treatment

Background: The STING-cGAMP-cGAS chronicle



2008
Three labs first discovered STING as a 42 kDa dimeric adaptor protein

2009
Intracellular DNA-mediated, type-I-IFN-dependent STING pathway

2009-2011
STING activation by C-di-GMP

2012-2013
The discovery of the upstream sensing of cytosolic dsDNA (cGAS, cGAMP)

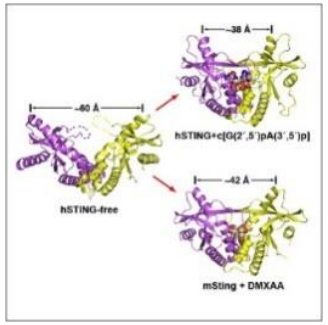
2014
mSTING but not hSTING in response to DMXAA+FAA

2018
STING agonist (ADU-S100/MIW815) was reported capable of mediating cancer progress in humans

2020
Non-nucleotide small-molecule systemic STING agonists (SR-717 & MSA-2)

2008 2009 2009-2011 2012-2013 2014 2015 2017 2018 2020

2012-2013
IRF3 phosphorylation by TBK1 in STING pathway



2014
Role of STING in tumor cell recognition and anti-tumor system

2015
Pre-clinical experiment of STING agonist in metastasis melanoma

2017
Anti-PD(L)1 was proofed depends on cGAS-STING, cGAMP showed to have anti-tumor function, and boosts anti-PD(L)1 therapy

2018
Preliminary results of clinical combination trial of STING agonist (MK-1454) with anti-PD-1 therapy (Pembrolizumab)

Background: The STING signaling pathway

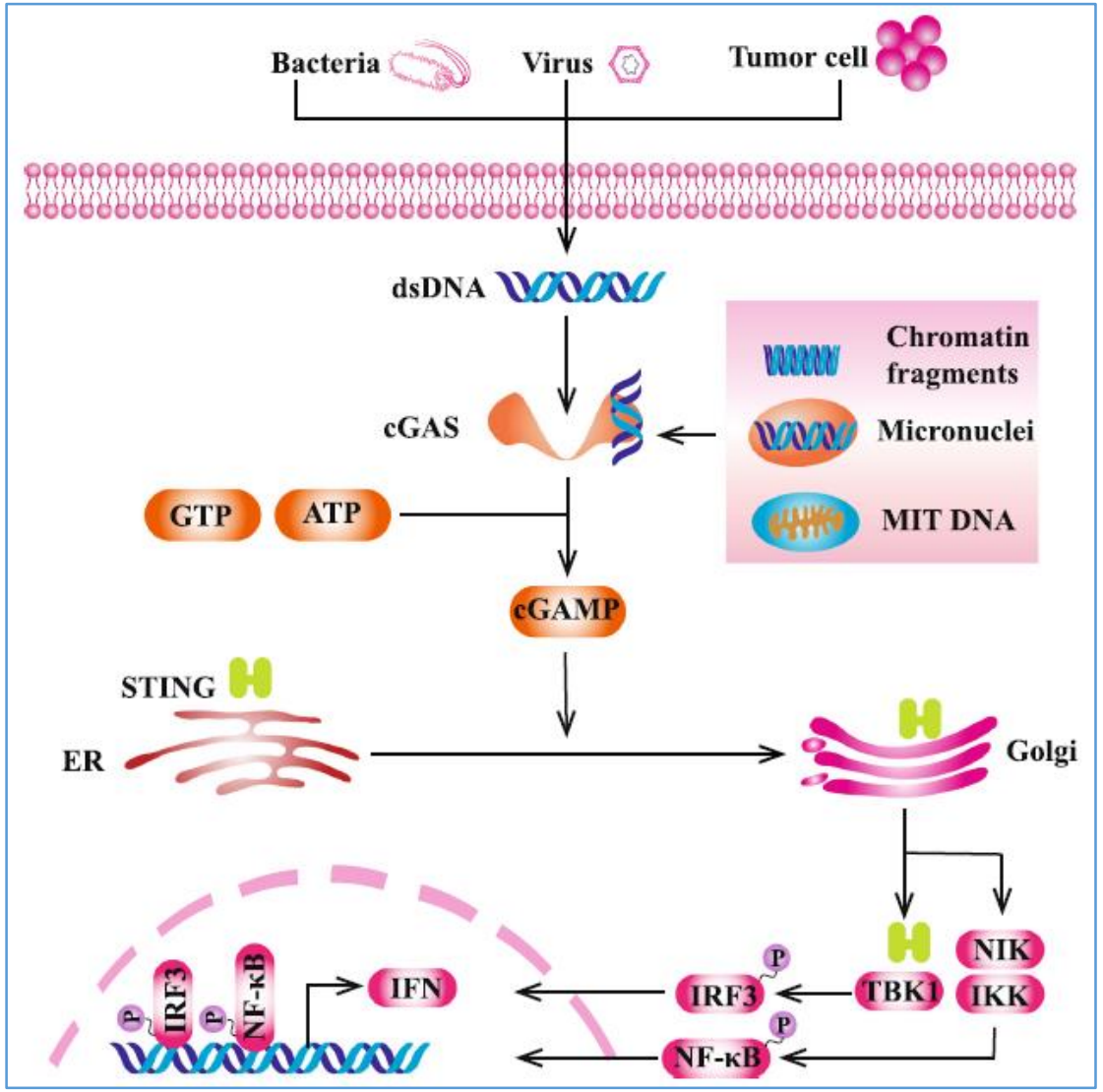
- Stimulator of interferon genes (STING), cytosolic DNA sensor anchored in endoplasmic reticulum (ER), is highly expressed in several APCs, such as macrophages and DCs, as well as plasmacytoid DCs, MDSCs, T-cells, and various endothelial or epithelial subtypes

- The STING pathway is predominantly activated by cyclic dinucleotides (CDNs), a product derived from the intracellular enzyme, cyclic GMP-AMP synthase (cGAS), upon invasion by pathogens and exposure to self-DNA, which leads to the production of type I interferons and pro-inflammatory cytokines

  - ✓ STING could recruit and activate TANK-binding kinase 1 (TBK1) which further phosphorylates interferon regulatory transcription factor 3 (IRF3) and upregulates the expression of type I IFN

  - ✓ STING could also activate NF-κB pathway by binding to IκB kinase (IKK) and NF-κB-inducing kinase (NIK), which further collaborates with TBK1-IRF3 pathway to induce the expression of type I IFN

- Type I IFN has multiple immune-stimulatory functions promoting the maturation, migration, and activation of multiple immune cells such as DCs, T cells, and NK cells



J Hematol Oncol, 2019, 12(1), 35

Background: STING pathway and cancer therapy

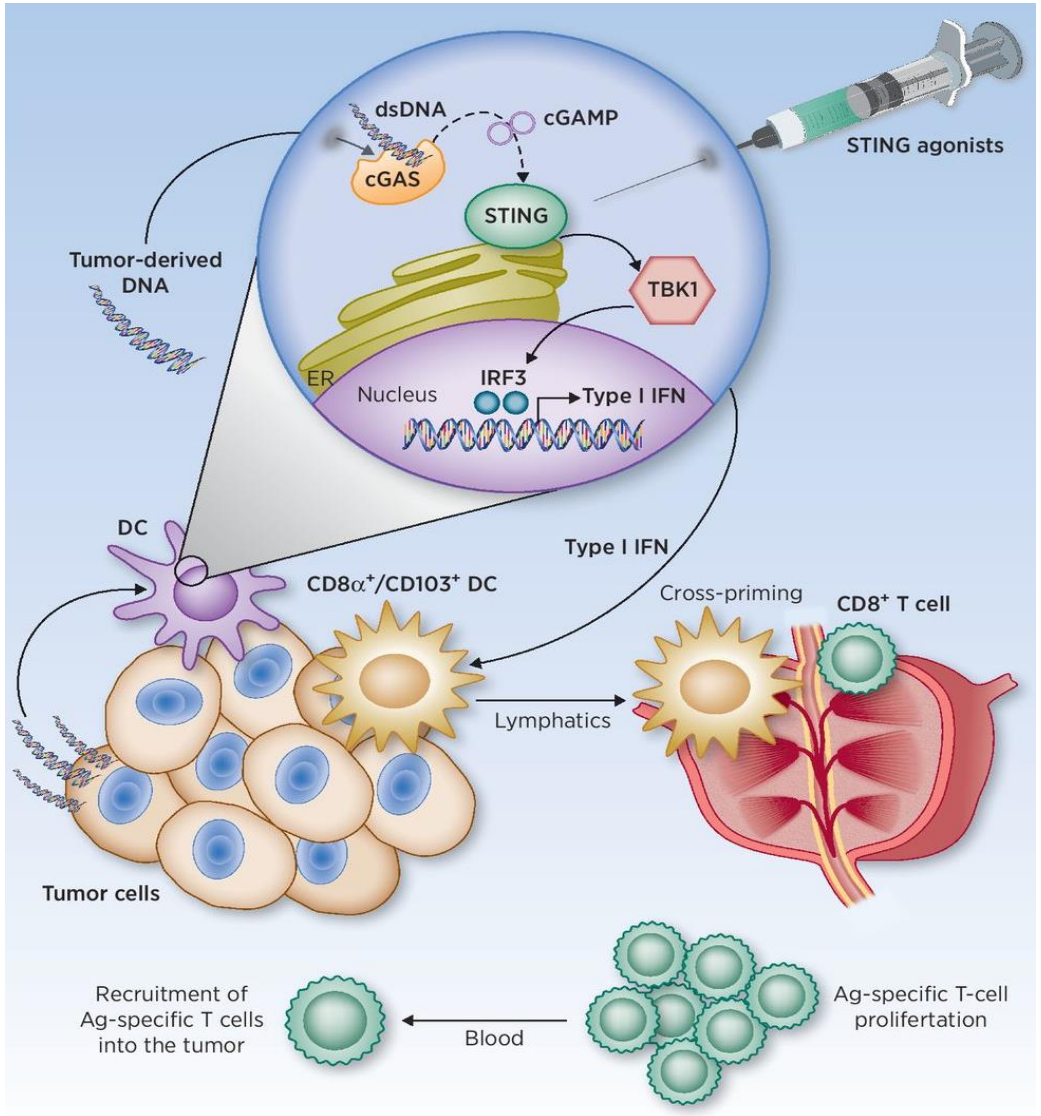
- DNA leakage not only activates STING pathway in tumor cell, but also promotes STING activation in DCs by DNA uptake or cGAMP transfer

- Activation of cGAS-STING signaling pathway can be deliberately stimulated by the use of direct STING agonists, when compounds are therapeutically administrated into the tumor microenvironment

- *In-vivo* studies using gene-targeted mice demonstrated a crucial role of STING-dependent type I IFNs production, and its signaling on basic leucine zipper transcription factor ATF-3 (BATF3) lineage of DCs for spontaneous antitumor T-cell responses *in vivo* and recruitment of effector T cells into the tumor microenvironment

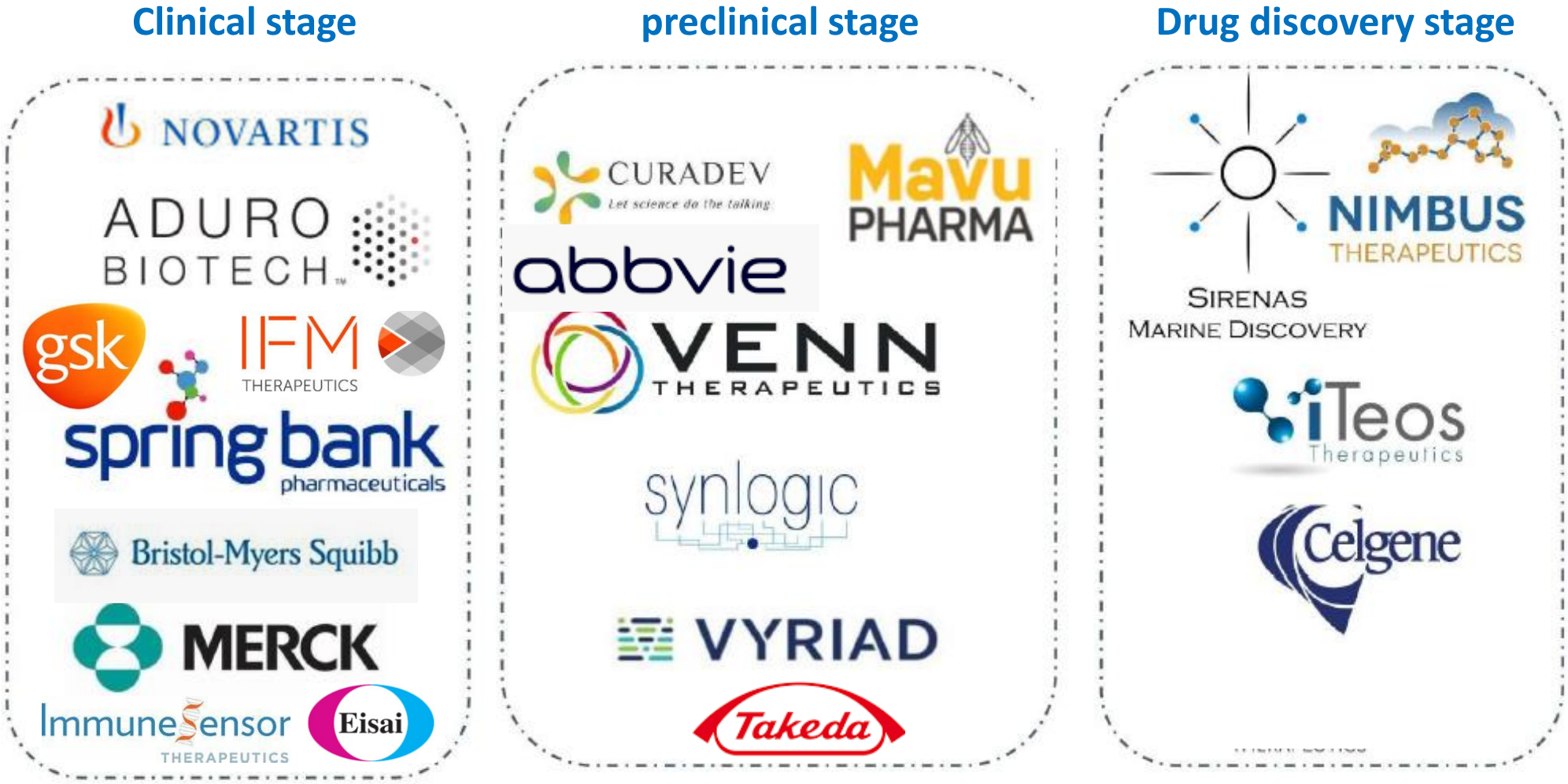
- Two major hypotheses have been prompted for the DC activation by cancer cells: tumor-derived DNA activates the DCs, or tumor derived cGAMP directly activates the STING pathway via protein STING, thereby leading to the production of type I IFNs

- The type I IFN signaling pathway contributes to:
  - ✓ CD8α+ DC survival and antigen retention
  - ✓ Up-regulation of CCR7, MIP-3β, and Th-1 chemokines to reinforce the lymph node-homing
  - ✓ Significant enhancement of tumor antigen specific T cell responses through the activation of STAT1



J Hematol Oncol, 2020, 13, 81
AACR, 2015

Background: The current industrial pipelines on STING agonists



Cited from Evaluated Pharma database

Background: The STING agonists in clinical trials (10 trials)

Drug	Company	Cancer Type	Phase	Trial Start Date	Status (Estimated Completion)	Pertinent Findings of Trial	NCT Code
ADU-S100 (i.t.) +/− ipilimumab (i.v.)	Aduro Biotech; Novartis	Advanced/metastatic solid tumours; lymphomas	I	04/16	Terminated 12/19	Undisclosed	NCT02675439
ADU-S100 (i.t.) + PDR001(i.v.) (spartalizumab)	Novartis	Solid tumours; lymphomas	Ib	09/17	Terminated 12/19	Data cut-off: 5th April 2019 - 12/53 SD, 4/53 PR, 1/53 CR - Responders: median reduction of 73% in 1° lesion diameter - 78% TRAEs, 12.2% of TRAEs = grade3/4 - No DLTs - MTD not determined - T1/2 = 10–23 min	NCT03172936
ADU-CL-20 (i.t.) + anti-PD-1 (i.v.)	Aduro Biotech	Metastatic/recurrent HNSCC	II	08/19	Ongoing (2022)	Undisclosed	NCT03937141
MK-1454 (i.t.) +/− pembrolizumab (i.v.)	Merck & Co	Advanced/metastatic solid tumours; lymphomas	I	02/17	Ongoing (2021)	Data cut-off: 31st July 2018 - TRAEs 83% monotherapy, 82% combination - 7% in combination discontinued due to TRAEs - MTD not yet determined - Combination 6/25 (24%) → PR (3 HNSCC, 1 TNBC, 2 anaplastic thyroid carcinoma) - Combination: median reduction of 83% in 1° lesion diameter - T1/2 = 1.5 h	NCT03010176

J. Clin. Med. 2020, 9, 3323

Background: The STING agonists in clinical trials (10 trials)

Drug	Company	Cancer Type	Phase	Trial Start Date	Status (Estimated Completion)	Pertinent Findings of Trial	NCT Code
MK-2118 (i.t.; s.c.) +/− pembrolizumab (i.v.)	Merck & Co	Advanced/metastatic solid tumours; lymphomas	I	09/17	Ongoing (2022)	Undisclosed	NCT03249792
BMS-986301 (i.t.) +/− nivolumab (i.v.), ipilimumab (i.v.)	Bristol-Myers Squibb	Advanced solid tumours	I	03/19	Ongoing (2023)	Undisclosed	NCT03956680
GSK3745417 (i.v.; s.c.) +/− pembrolizumab (i.v.)	GSK	Advanced solid tumours	I	03/19	Ongoing (2024)	Undisclosed	NCT03843359
SB-11285 (i.v.) + nivolumab (i.v.)	Spring Bank Pharmaceuticals	Advanced solid tumours	Ia/Ib	09/19	Ongoing (2022)	Undisclosed	NCT04096638
IMSA-101 (i.t.) +/− ICI (i.v.)	ImmuneSensor Therapeutics	Advanced solid tumours	I/IIa	09/19	Ongoing (2023)	Undisclosed	NCT04020185
E7766 (i.t.)	Eisai Inc.	Advanced solid tumours; lymphomas	Ia/Ib	03/20	Ongoing (2022)	Undisclosed	NCT04144140

J. Clin. Med. 2020, 9, 3323

Background: The limitations and challenges of the STING agonists based therapies

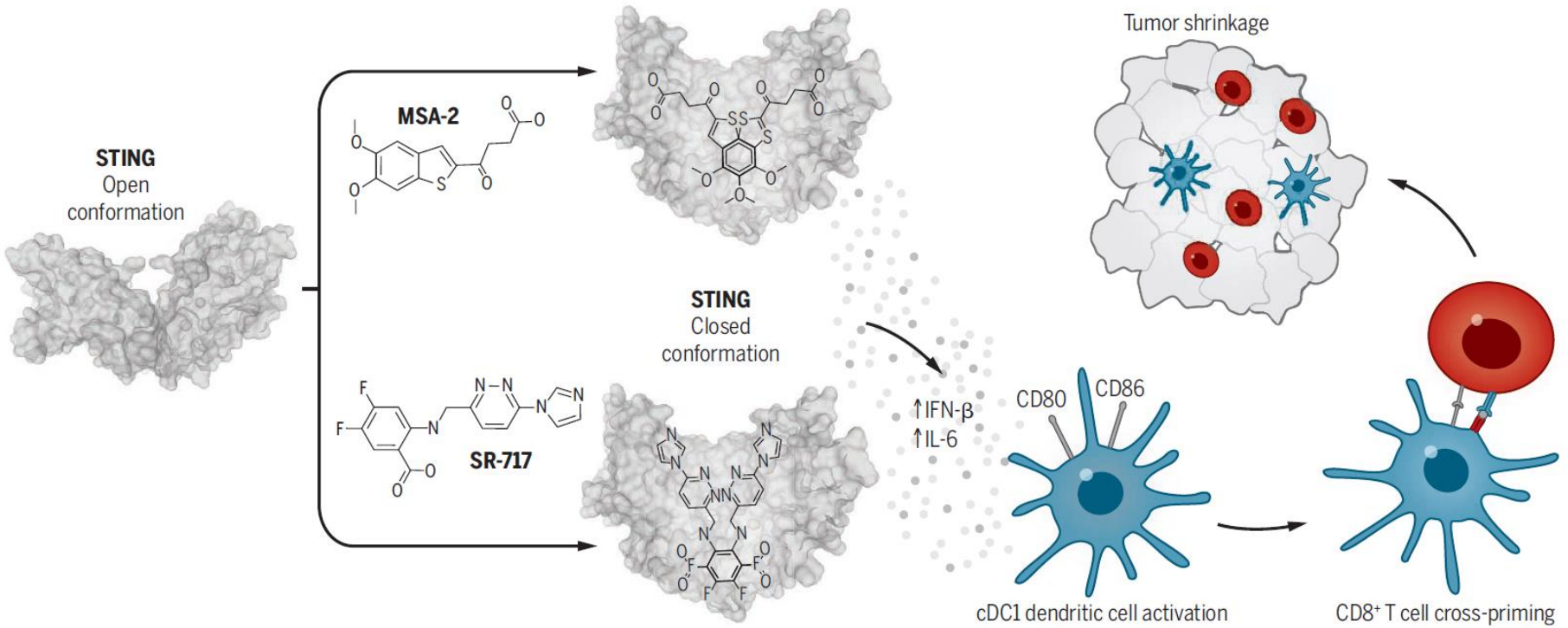
1. The metabolic instability and poor permeability of CDNs
2. I.T. delivery concern: limits the use & not cover the host's entire tumor antigen spectrum
3. Safety: inflammation, cytokine storm, normal B cell and tissue toxicity, autoimmunity
4. STING activation induce T cell stress and death & tolerogenic immune response
5. SNPs in STING with implications for the selection of appropriate STING agonists

J Hematol Oncol, 2020, 13, 81
J. Clin. Med. 2020, 9, 3323

Background: The current strategies to overcome the limitations of the first generation of STING agonists (CDNs)

New delivery modalities	• **Cancer vaccines**: STINGVAX, CT26, SCCFVⅡ, Panc02 *(Aduro Biotech)* • **Nanostructures**: SB11285 *(Spring Bank, iTeos therapeutics)* • **ADC**: CRD5500 conjugated with Trastuzumab *(Curadev);* SB11285 *(Spring Bank)*
STING agonists formulated for systemic administration	• **Comp3**: i.v. *(GSK)* • **CRD5500**: i.v. or s.c. *(Curadev licensed to Takeda)* • **SR-717:** i.p. *(The Scripps Research Institute)* • **MSA-2:** p.o. *(LifeMine Therapeutics)*
The STING pathway enhancer	• **Mavupharma** (MAVU-104): a first-in-class, orally active, small molecule inhibitor of ENPP1, a phosphodiesterase that negatively regulates the STING pathway *(AbbVie)*
Injection of viruses or bacteria to produce endogenous c-di-A/GMP	• IT Injection of engineered E. coli specifically engulfed by APCs, SYNB 1891 *(Synlogic)* • IT Injection of adenovirus *(Venn Therapeutics)*

Recent promising non-nucleotide small-molecule systemic STING agonists



- Direct mimetic of the natural STING ligand cGAMP that stabilize STING in its closed conformation
- cGAS–STING pathway-targeted cell-based screening led to the identification of SR-717
- A phenotypic cell- based screen that detects stimulation
- of IFN-β secretion led to the identification of MSA-2

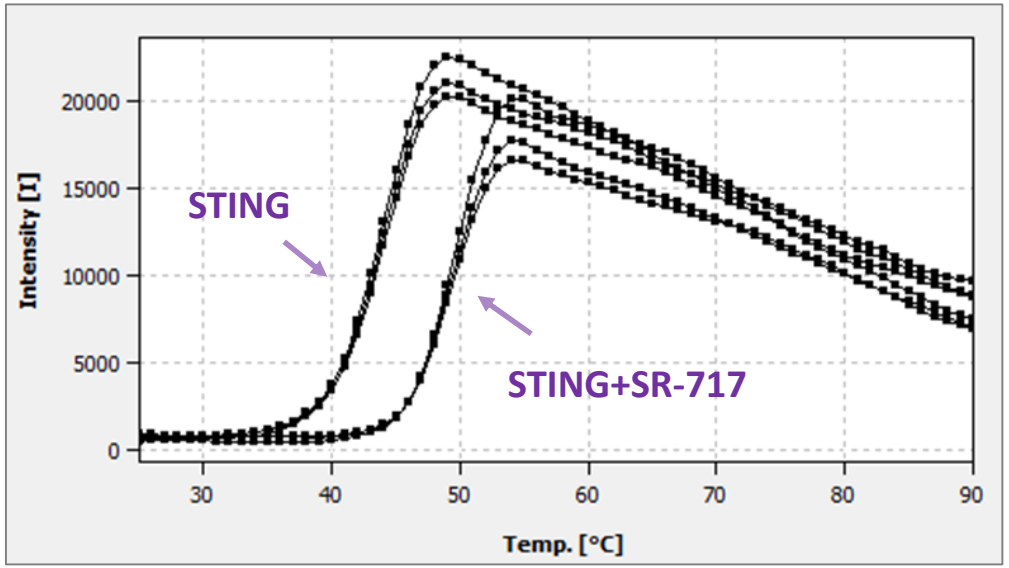
Science, 2020, 369, 993–9
Science, 2020, 369, eaba6098

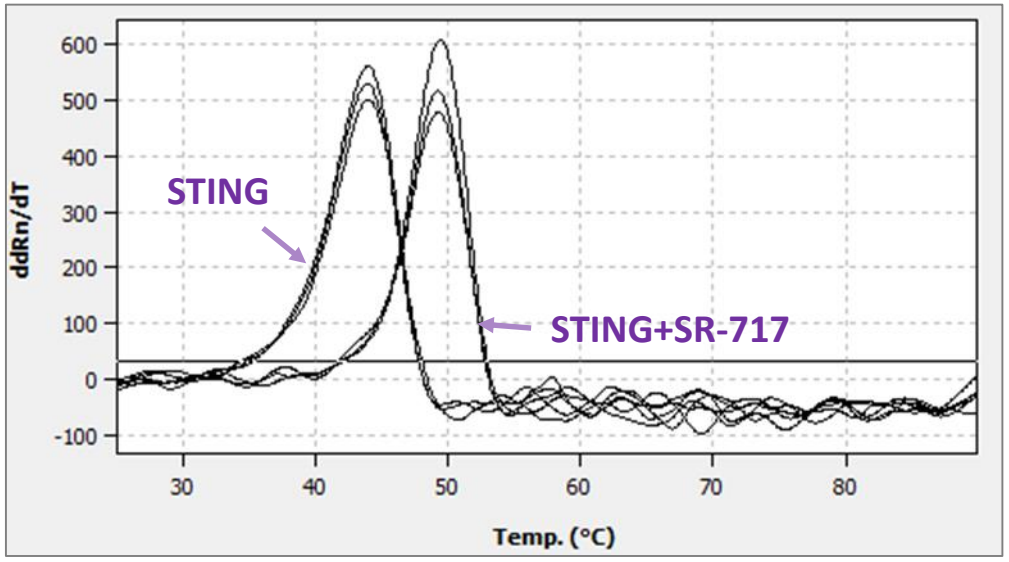
Several important considerations

- Greater toxicity: engaging APCs outside the TME may release high amounts of IFN-β and other inflammatory cytokines
- The effect of systemic STING agonists on specific immune cell sub-populations
- The dose and schedule of systemic administration
- Tumor types and patients that have the potential to respond to these agents

In vitro STING pathway related functional assay

Protein based binding of SR-717 to the recombinant STING-DCL8 protein by Thermal Shift assay





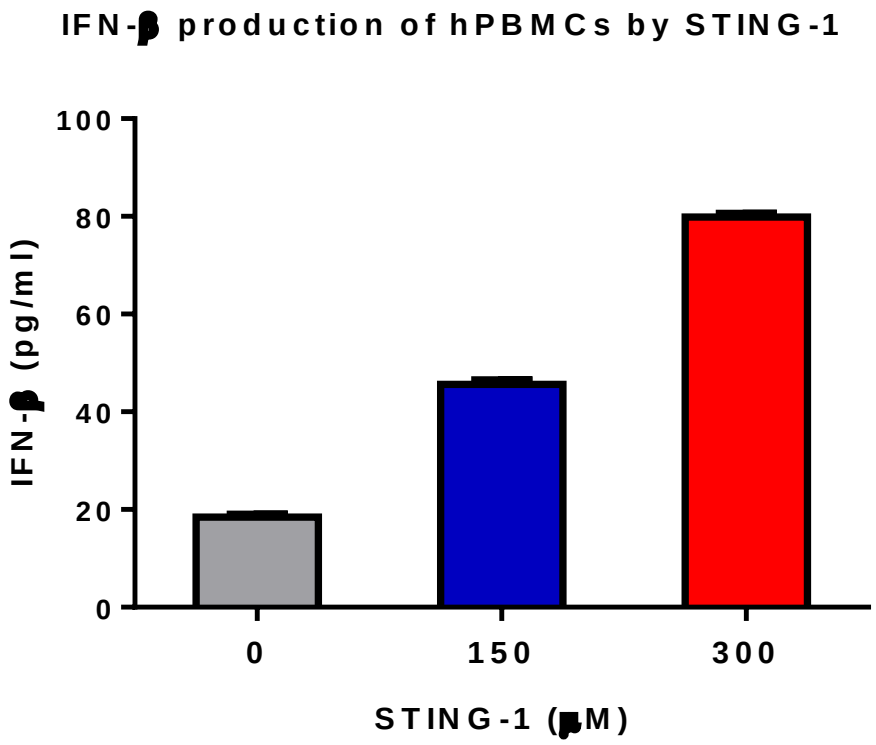
Test repeats	Tm (℃)	
	STING alone	STING + SR-717 (1 mM)
1	43.9	49.3
2	43.9	49.4
3	43.9	49.2
Mean	43.9	49.3

- SR-717 showed significant binding to STING-DCL8 protein

In vitro STING pathway related functional assay

STING activation by STING agonist (STING-1) in human PBMCs

A rapid screening method for potential STING agonists in drug discovery



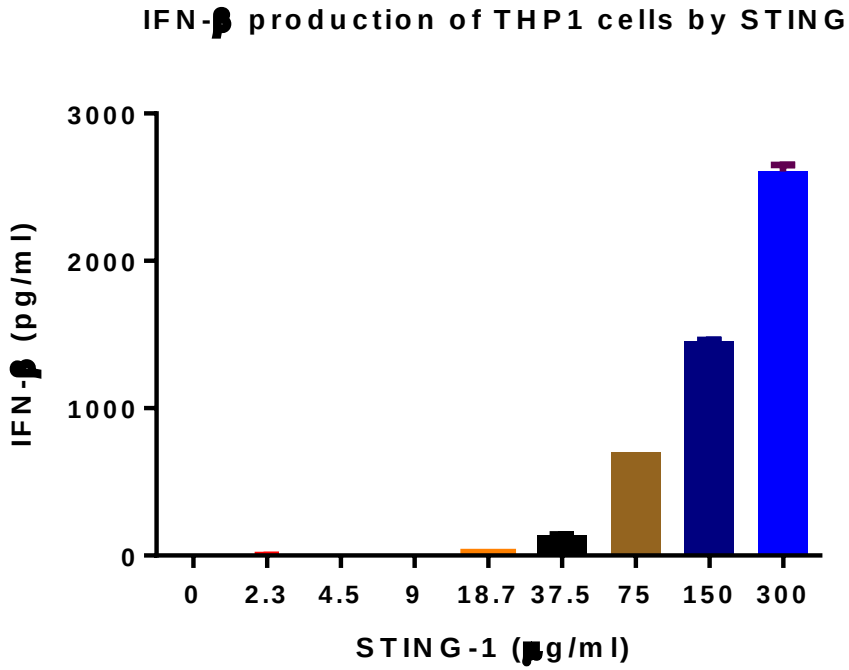
- STING-1 dose-dependently induced IFN-β production in hPBMCs

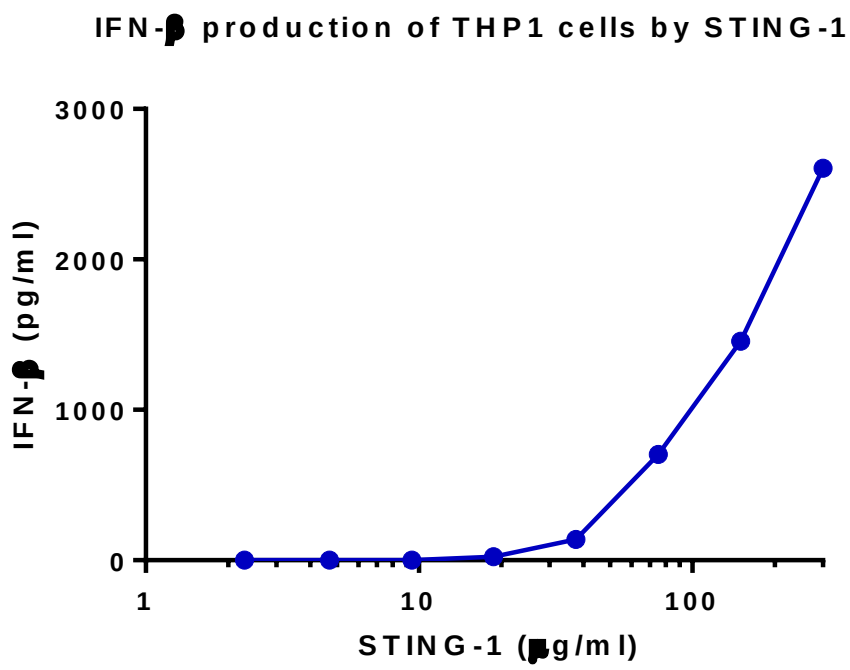
Science, 2020, 369, 993–9

In vitro STING pathway related functional assay

STING activation by STING agonist (STING-1) in THP1 cells

A rapid screening method for potential STING agonists in drug discovery



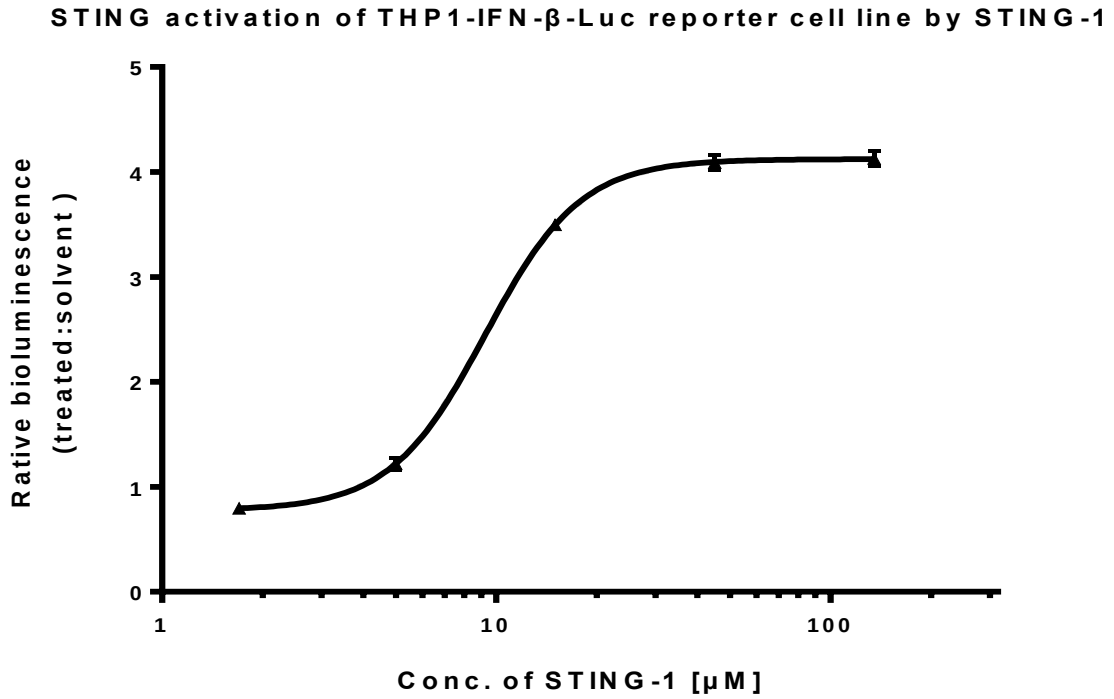


- STING-1 dose-dependently induced IFN-β production in THP1 cells

In vitro STING pathway related functional assay

STING activation by STING agonist (STING-1) in THP1-IFN-β-Luc reporter cells

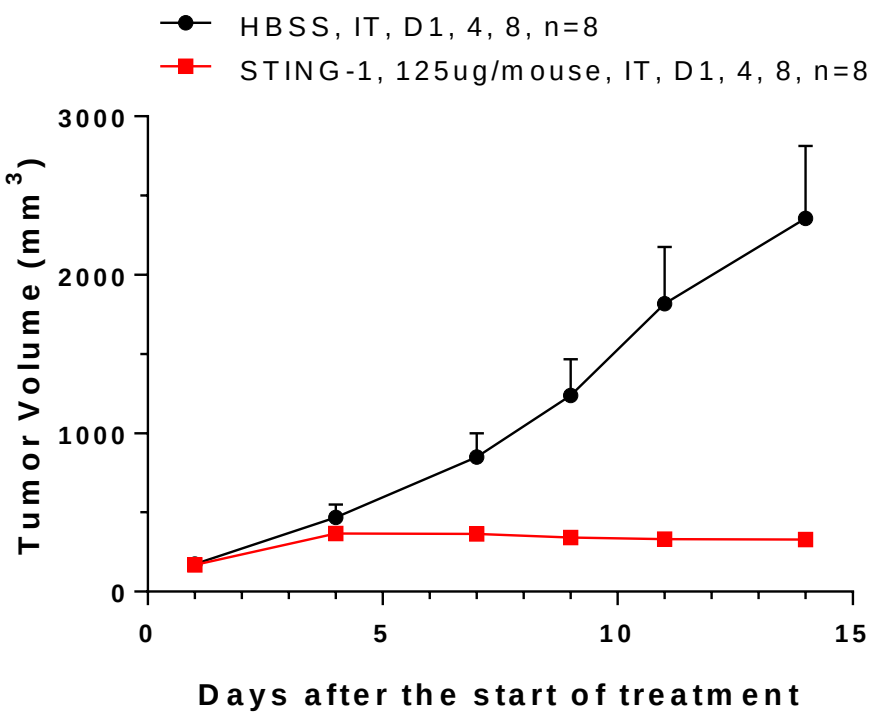
A rapid screening method for potential STING agonists in drug discovery



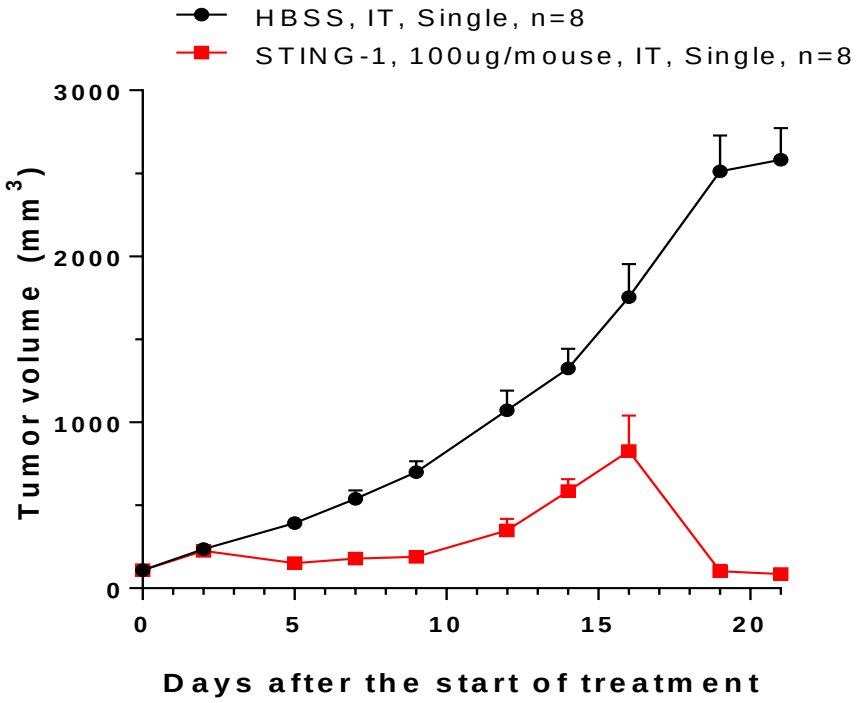
- STING-1 dose-dependently activated THP1-IFN-β-Luc reporter cells

In vivo anti-tumor efficacy study of STING-1 in CT-26 and 4T1 syngeneic models

CT-26

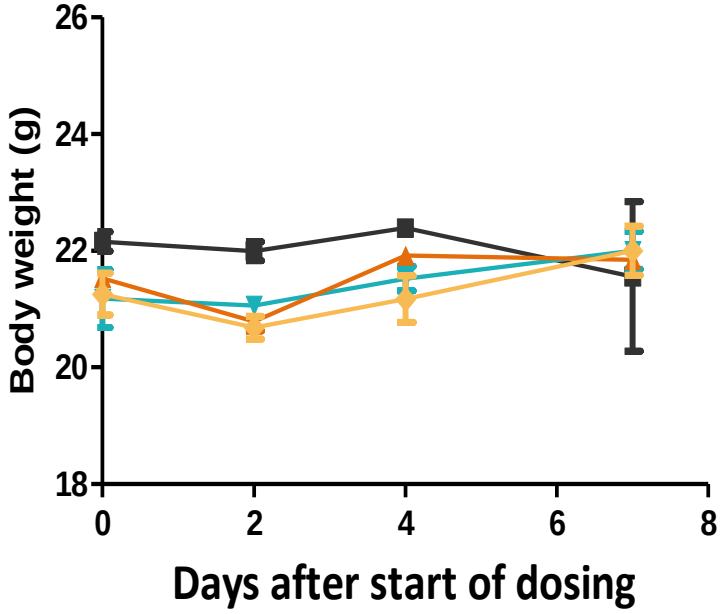


4T1



- STING-1 showed significant anti-tumor efficacy in CT-26 and 4T1 syngeneic models

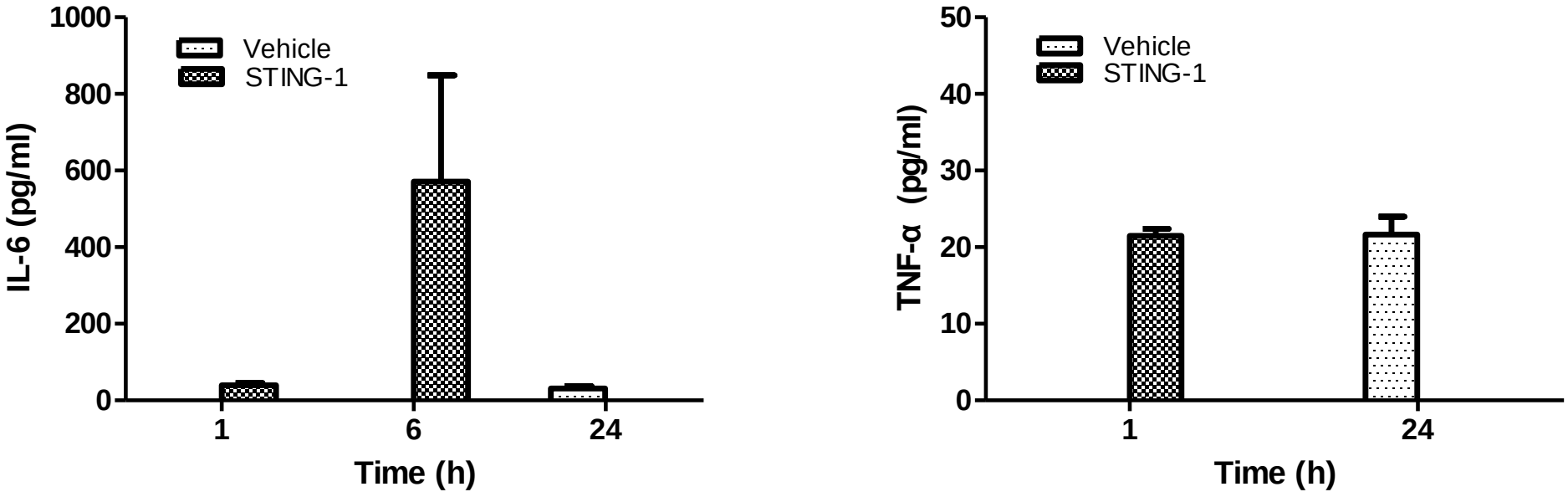
In vivo anti-tumor efficacy study of STING-1 in B16F10 syngeneic model



- STING-1 showed dose-dependent anti-tumor efficacy in B16F10 syngeneic model

Plasma cytokine analysis of B16F10 model post STING-1 treatment

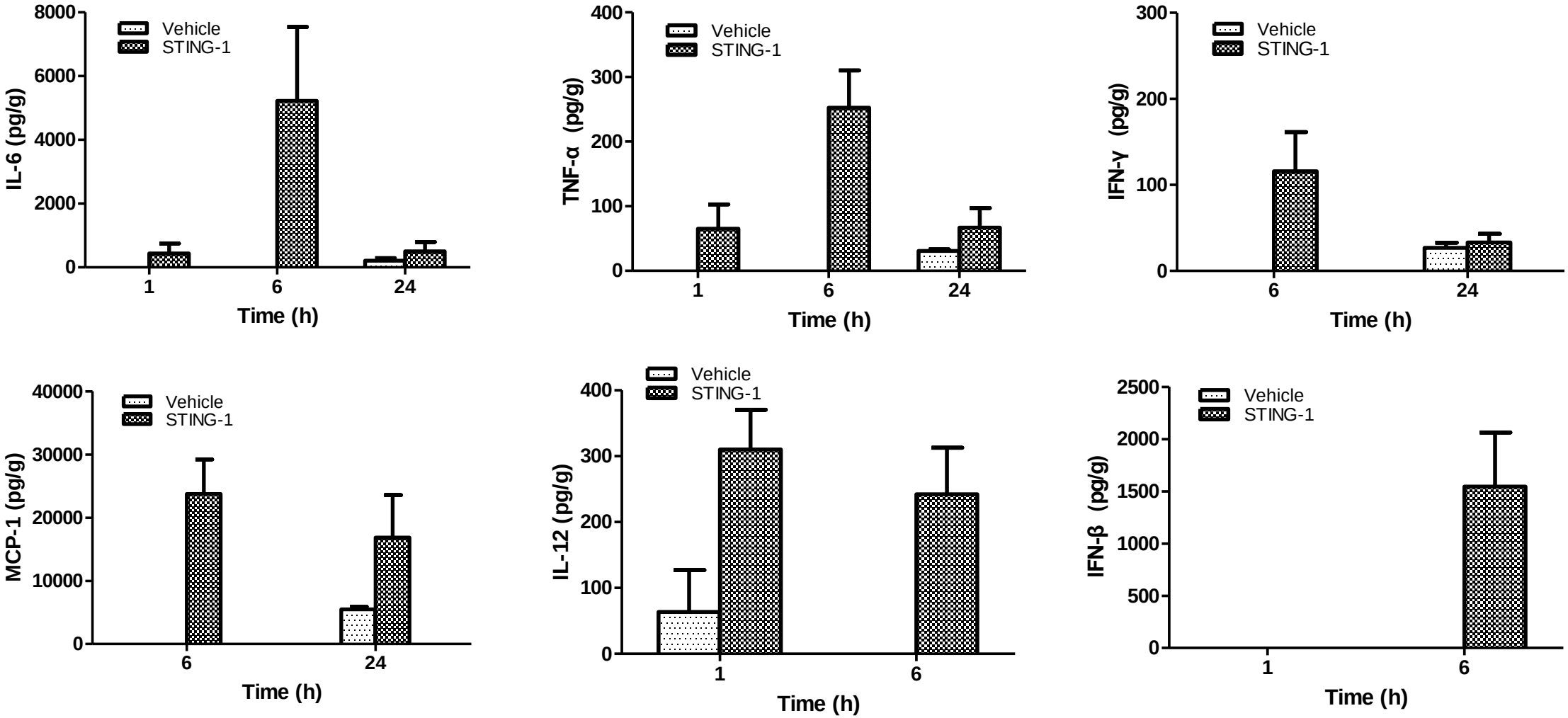
IL-6 and TNF-α release in Plasma



Detection by BD™ CBA Human Th1/Th2 Cytokine Kit II (Catalog No. 551809)

Tumor cytokine analysis of B16F10 model post STING-1 treatment

IL-6, TNF-α, IFN-γ, MCP-1, IL-12 and IFN-β levels in Tumor



Detection by BD™ CBA Human Th1/Th2 Cytokine Kit II (Catalog No. 551809)

Immunoprofiling in B16F10 model post STING-1 treatment

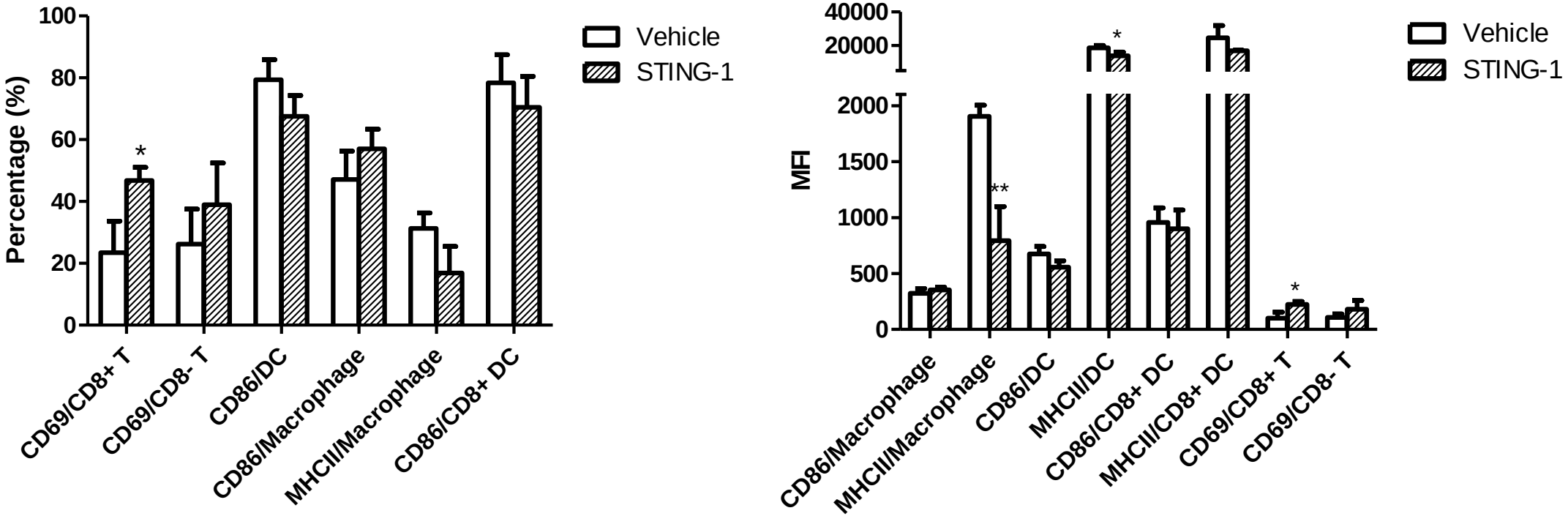
Panel design

Model	Cancer Type	Mouse
B16F10	Melanoma	C57BL/6

Channel	Fluorescein	Panel 1 (Tumor & Spleen)	Panel 2 (Blood)
FITC	FITC	F4/80	-
PE	PE	CD69	-
PerCP	PerCP-Cy5.5	CD11c	CD19
APC	APC	CD8	-
APC-R700	AF700	CD45	CD45
APC-Cy7	APC-Cy7	CD3	CD3
V450	BV421	Live/dead	Live/Dead
V500	BV510	CD86	-
BV605	BV605	MHCII	-

Immunoprofiling in B16F10 model post STING-1 treatment

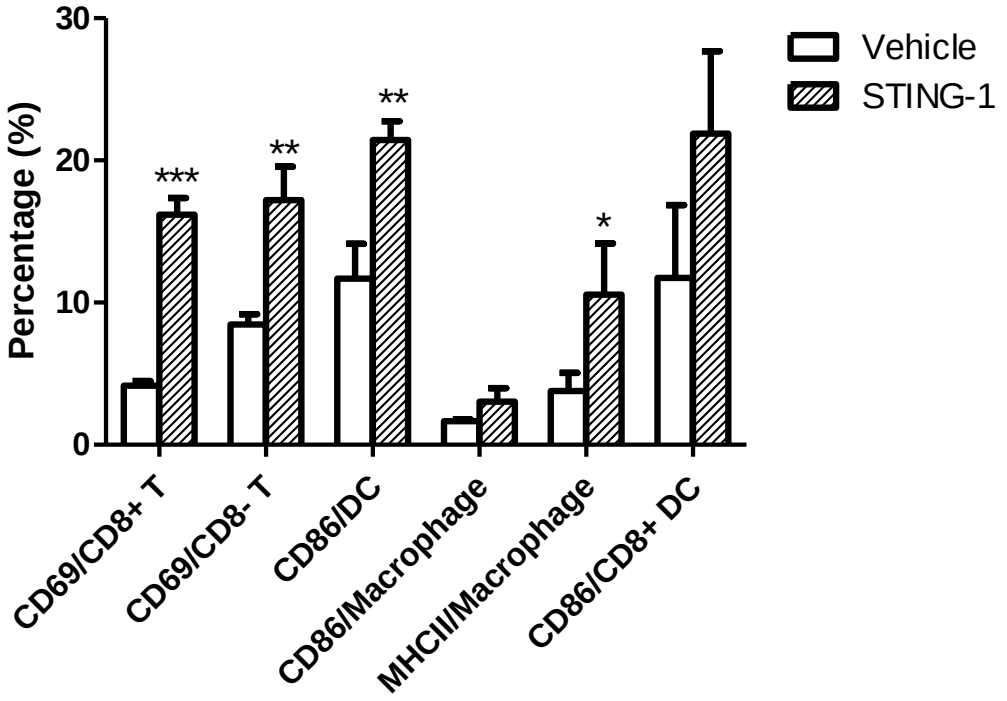
Activated marker expression in tumor infiltrating immune cells

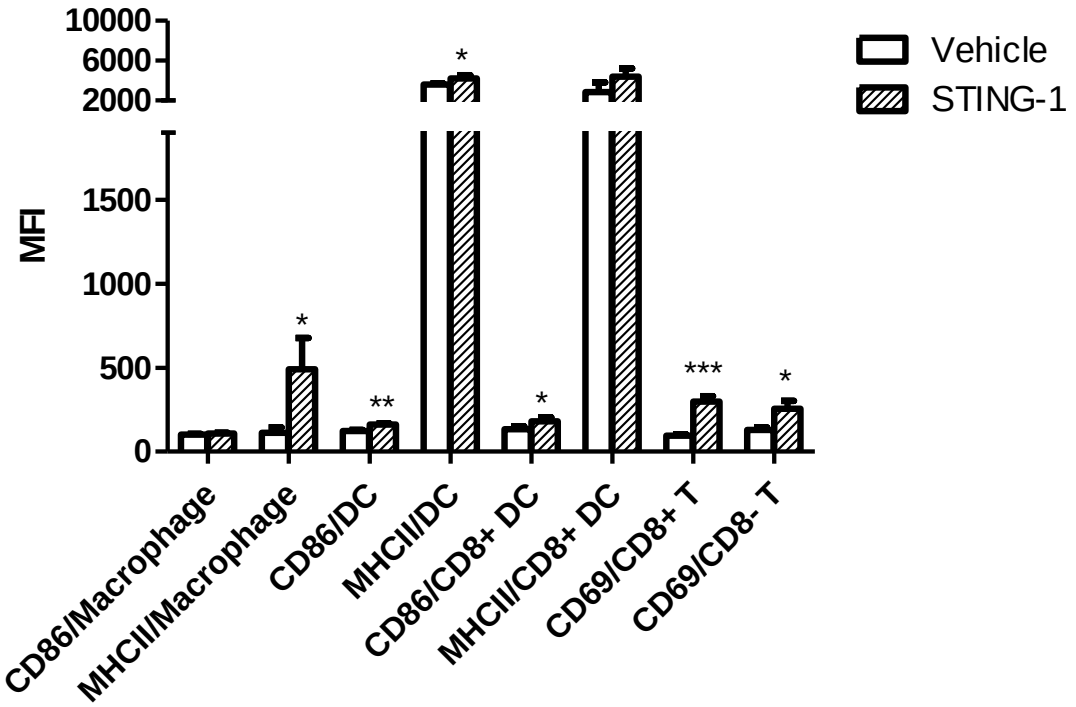


- STING-1 increased the percentage of activated CD8+ T cells (CD69+) in tumor

Immunoprofiling in B16F10 model post STING-1 treatment

Activated marker expression of immune cells in spleen





- STING-1 increased the percentage of activated $CD8^+$ T cells ($CD69^+$), DCs ($CD86^+$), and macrophage ($MHCII^+$) in spleen

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