Modified STING-Activating Cyclic Dinucleotide Derivatives Significantly Enhance the Anti-Tumor Activity of Therapeutic Vaccines

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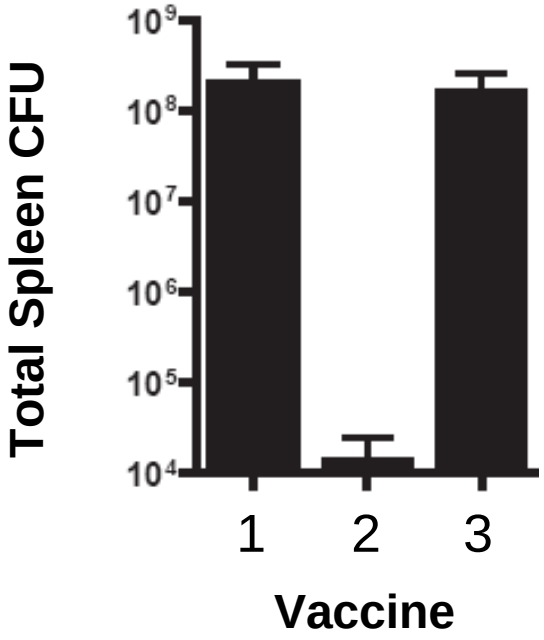
Presenter Disclosure:

Thomas W. Dubensky, Jr., PhD

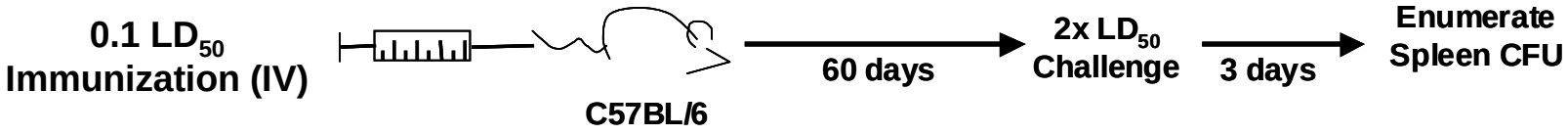
Employee and shareholder of Aduro BioTech

Cytosolic Access is Required for *L. monocytogenes* to Establish Protective Immunity



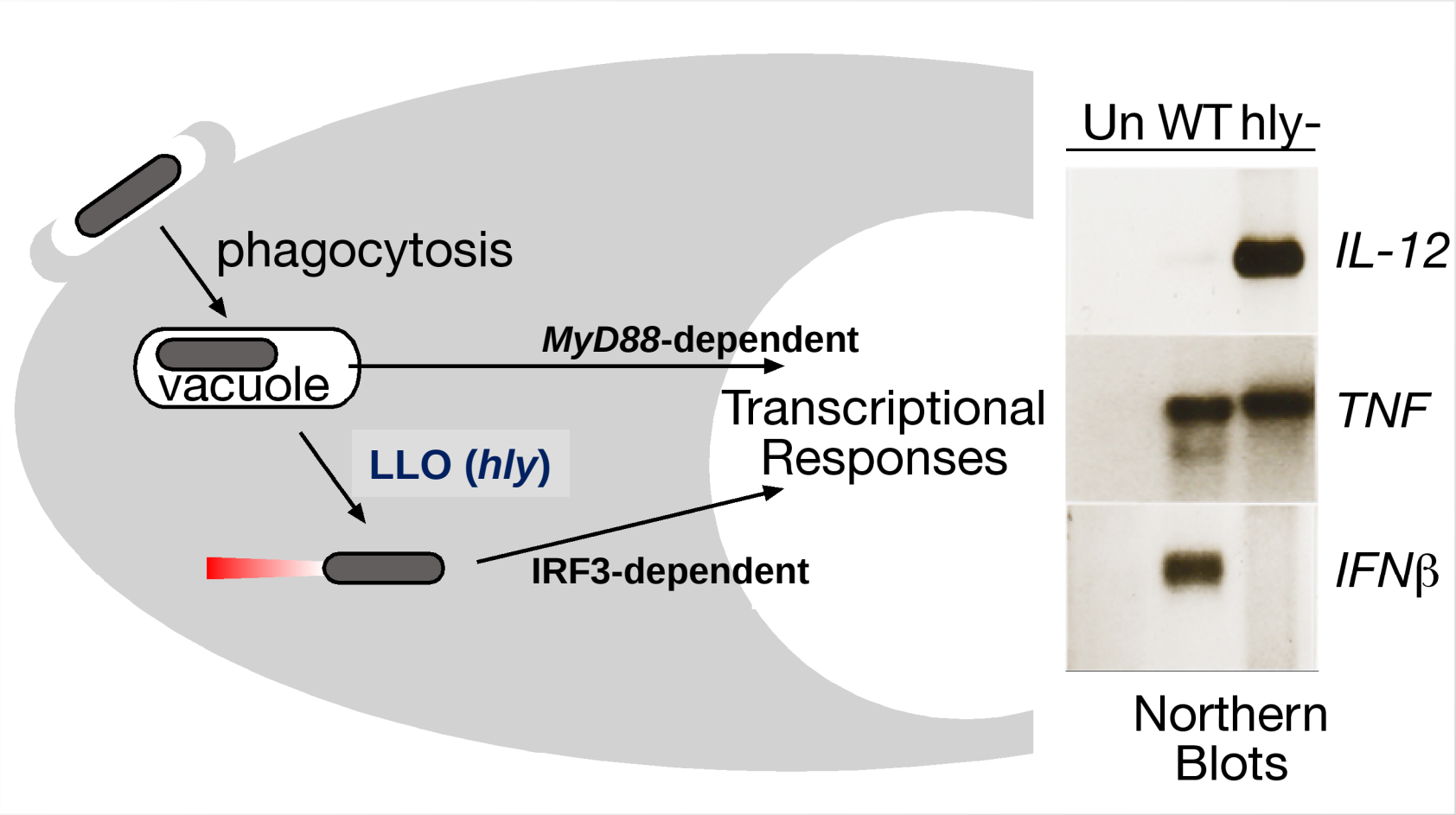
Group
1.HBSS
2.Wild-type LM
3.LM ΔLLO (Δ*hly*)

Experimental Protocol:



Bahjat, 2006, *Infect Imm*

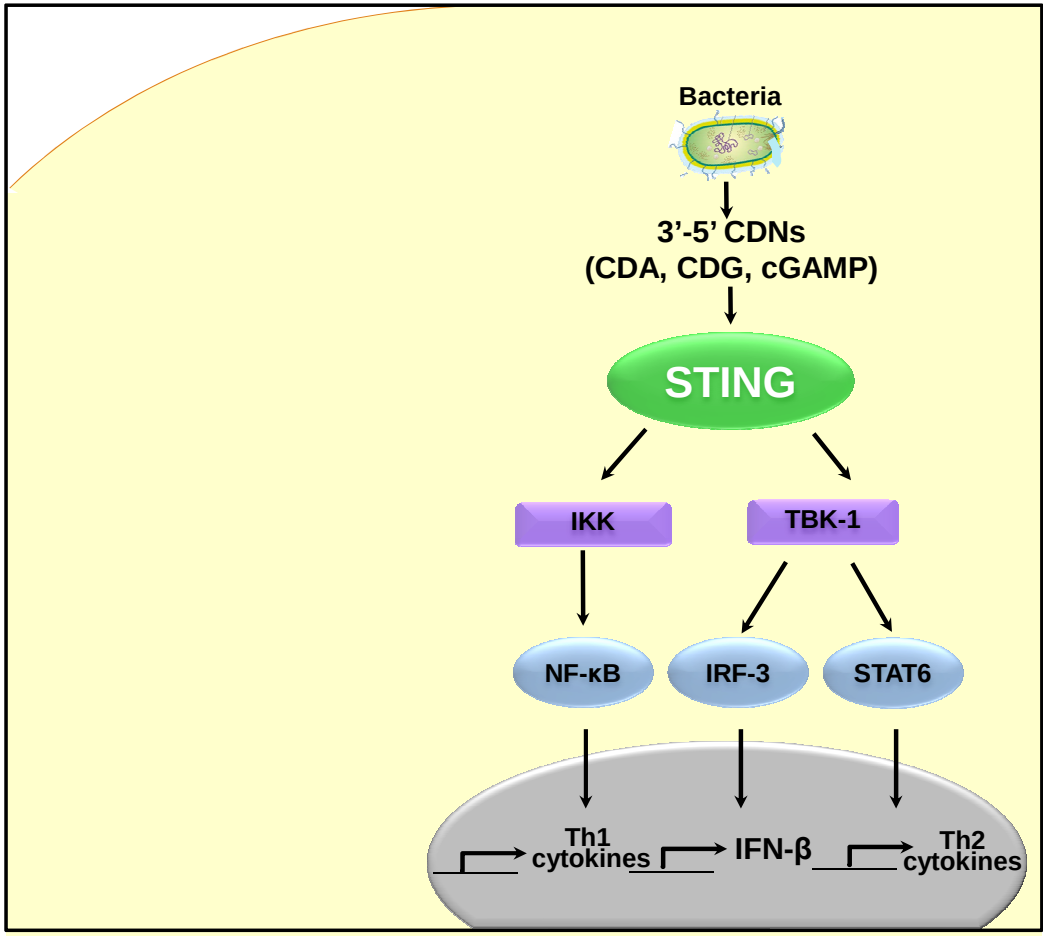
Macrophages discriminate between vacuolar and cytosolic *L. monocytogenes*



Luster, Moors and Portnoy (1994) unpublished

Cyclic Dinucleotides (CDNs) Activate Innate Immunity through the STING Pathway

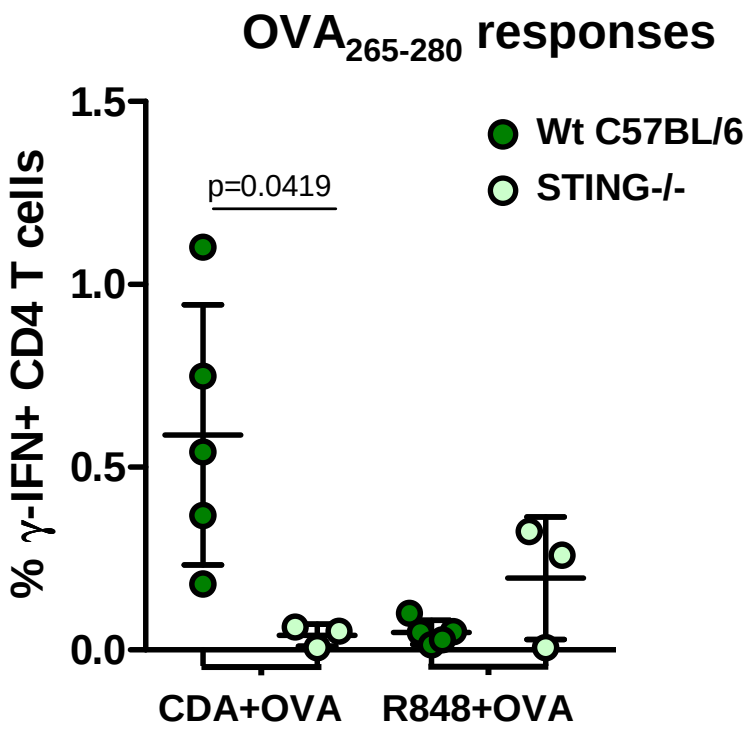
- Cyclic dinucleotides (CDNs) are bacterial second messengers
- Intracellular bacteria secrete CDNs through MDRs into the cytosol of infected cells
- cGAS synthesizes c-GMP-AMP in response to binding cytosolic DNA
- CDNs are sensed by STING (Stimulator of Interferon Genes)
- STING activates innate immunity by signaling through the TBK-1/IRF-3 axis, NF-κB and STAT6 pathways
- STING is a central mediator in the cytosol for activating innate immunity in response to sensing of nucleic acids

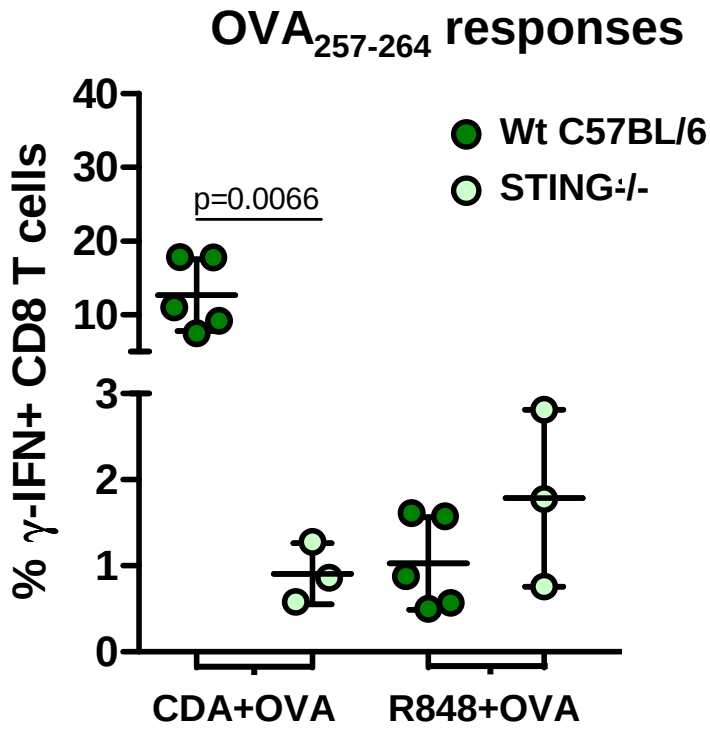


Leong, 2013, Ther Adv Vacc

The Immunogenicity of CDN-Adjuvanted Vaccines is STING-Dependent

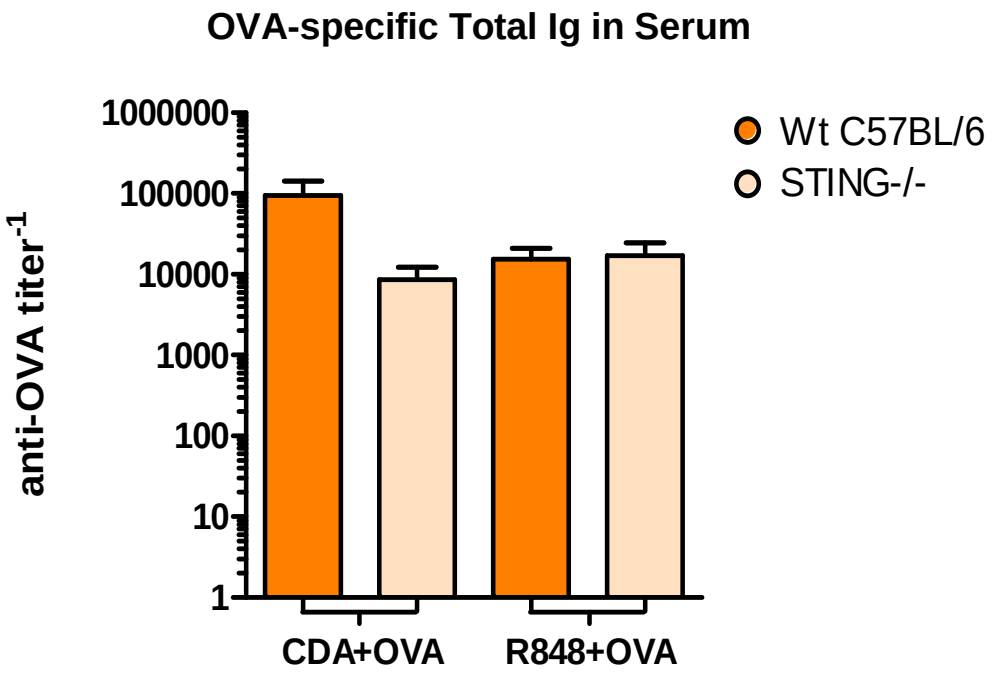
OVA-specific CD4 and CD8 T cell response at 6 days post boost vaccination with c-di-AMP (CDA) adjuvanted OVA in 2% oil-in-water emulsion

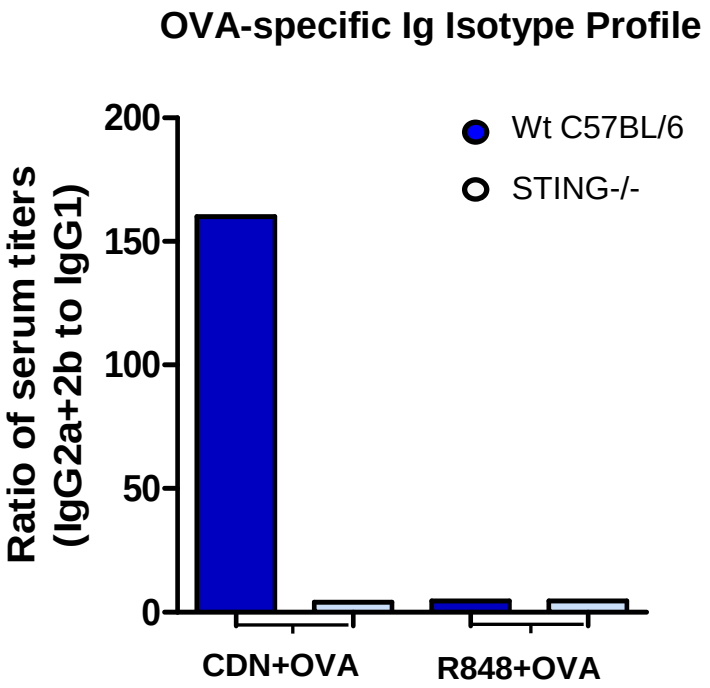




CDN-Formulated Vaccines Induce Th1-Biased STING-Dependent Humoral Immunity

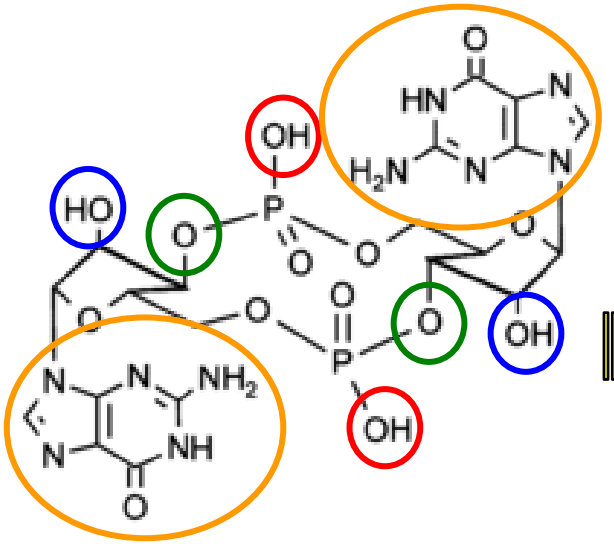
OVA-specific α-Ig and Th1 Ig Isotype profile at 6 days post boost





STING-CDN Crystal Structure Enables Rational Design of Molecules with Increased Potency

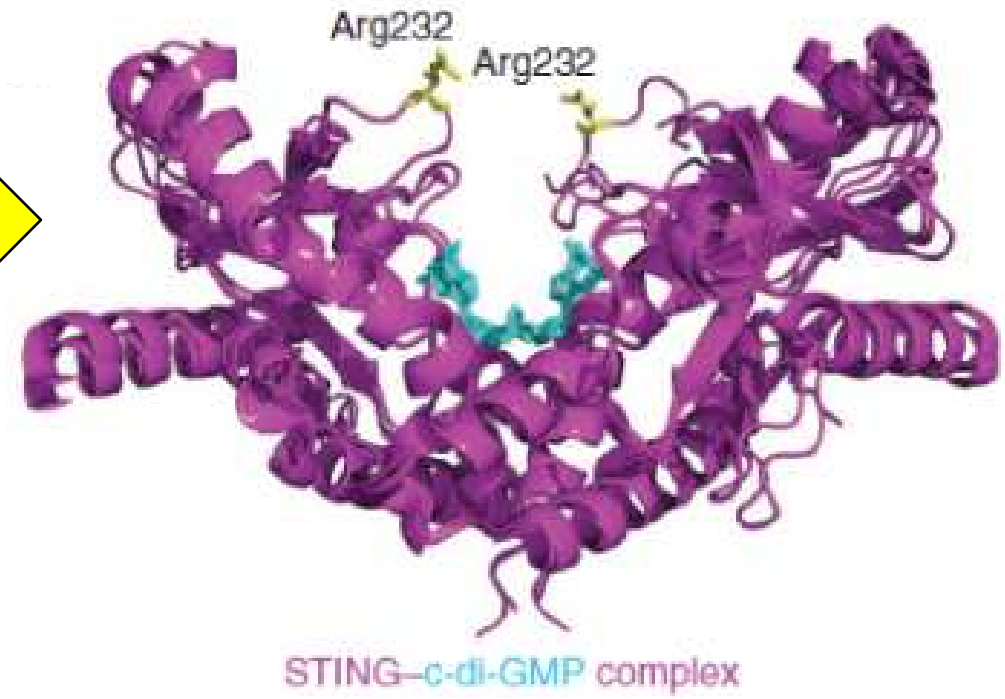
CDN structure



CDN composition & modifications

- **: Purine nucleotide base**
- **: Phosphate bridge linkage**
- **: Phosphodiesterase resistance**
- **: Formulation enhancement**

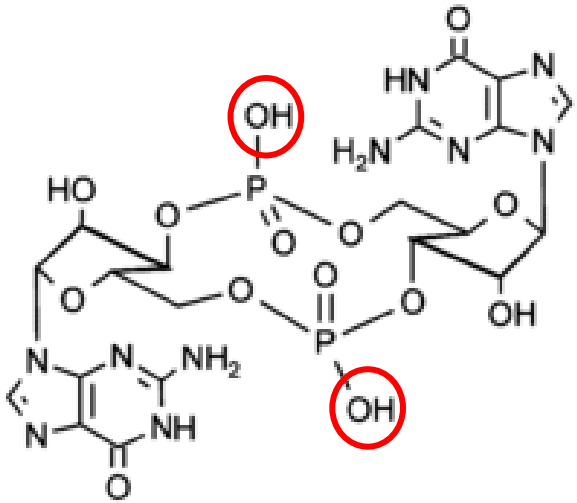
c-di-GMP STING co-crystal



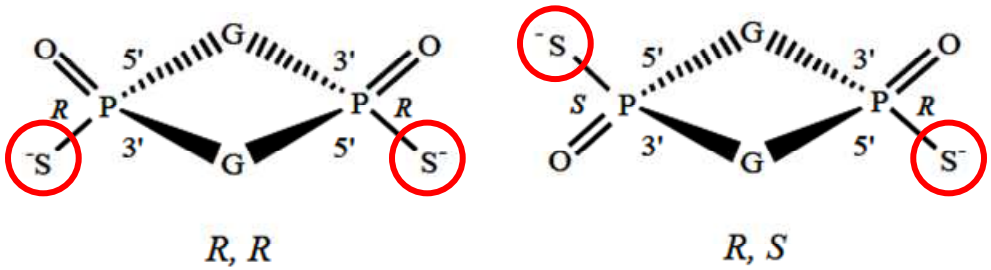
Burdette, 2012, Nat Immunol
Ouyang, 2012, Immunity

Synthetic Derivatives to Increase CDN Potency

c-di-GMP



Dithio c-di-GMP Diastereomers



R,R S2 c-di-GMP **R,S S2 c-di-GMP**

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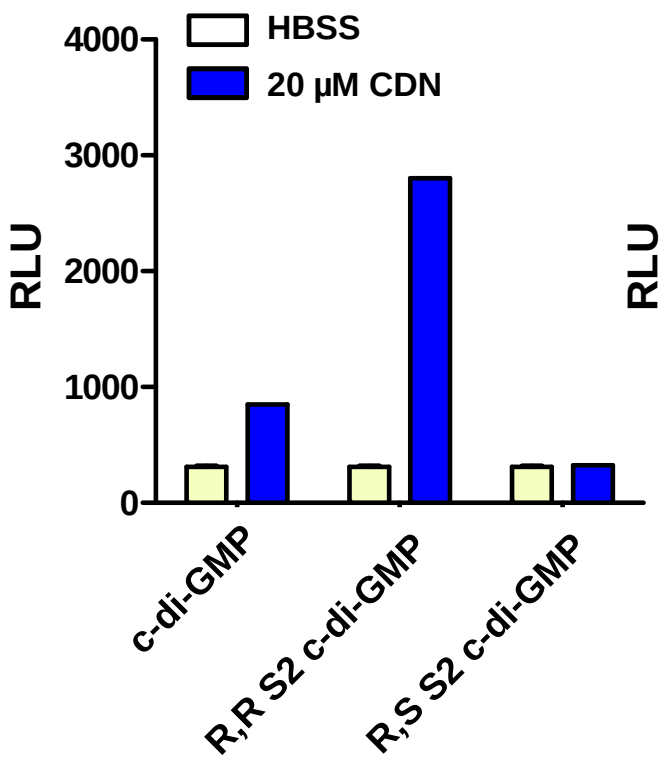
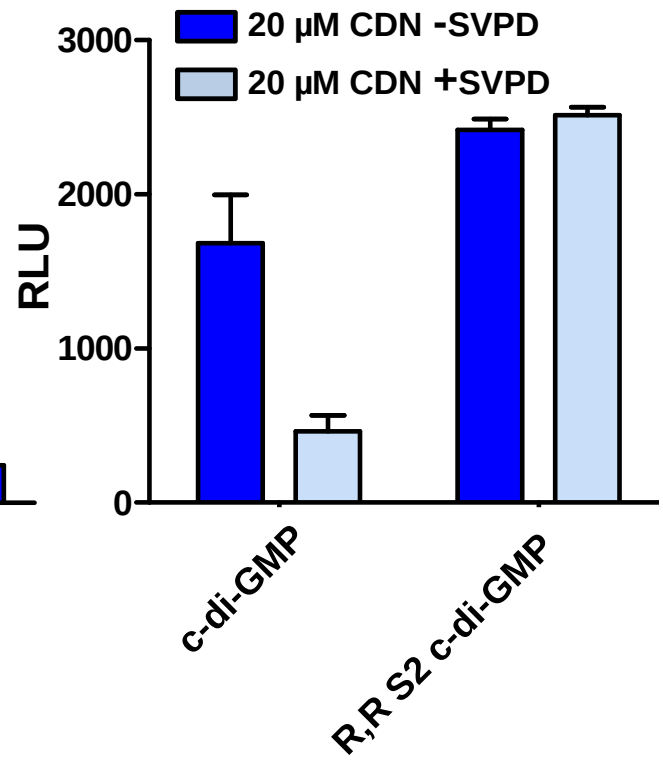
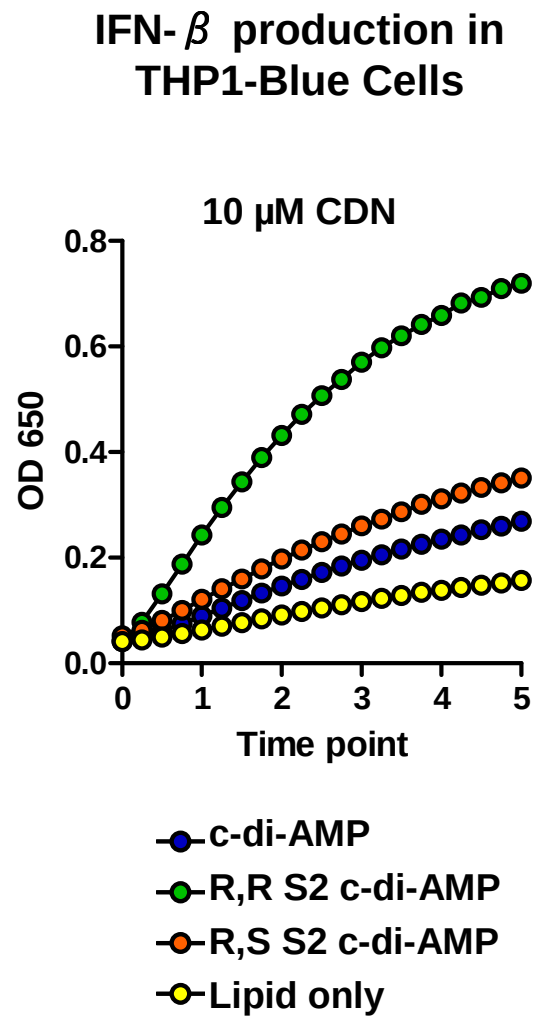
R,R Dithiophosphate CDN Diastereomers have Increased Activity

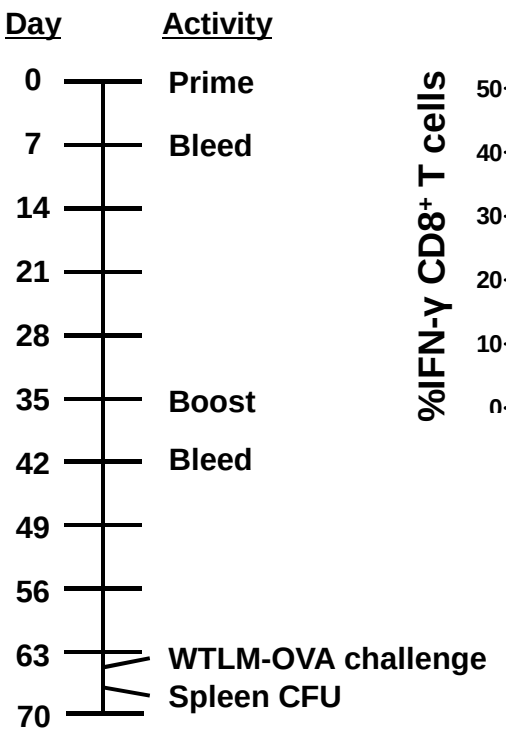




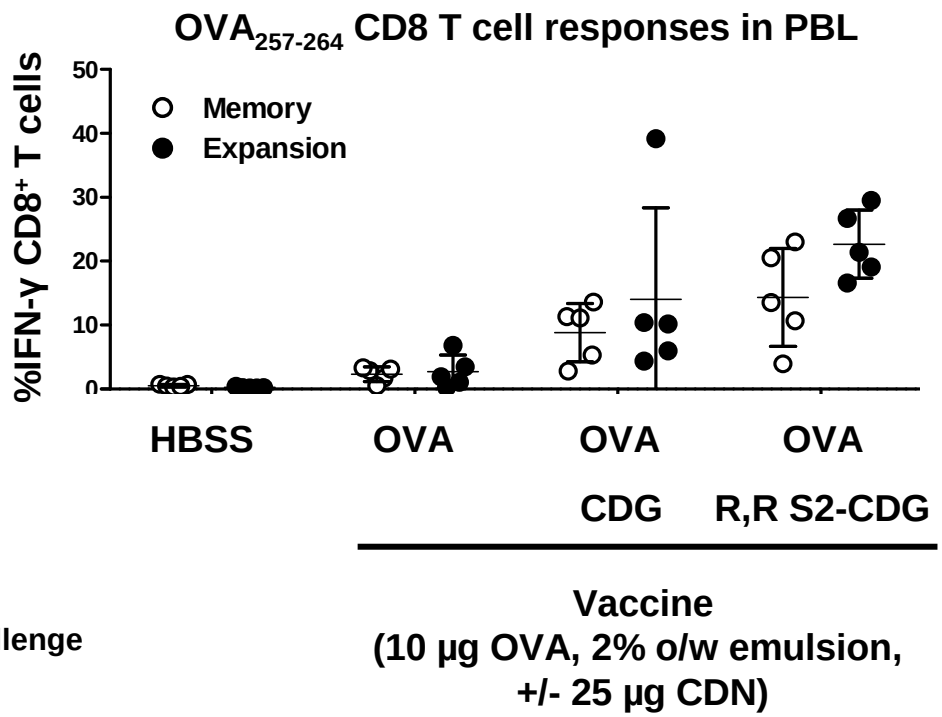


R,R Di-Thio c-di-GMP Diastereomer Increases CDN Adjuvanticity

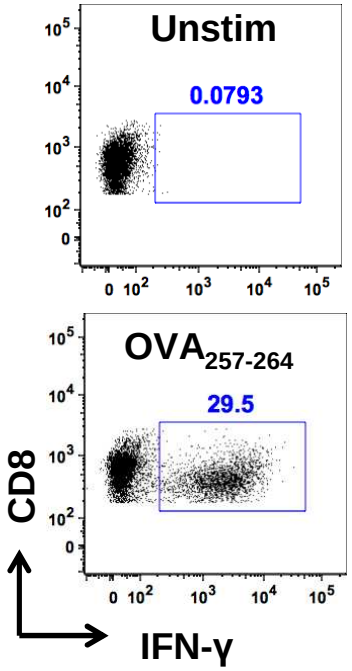
Schema



Immunogenicity & Protection



Memory CD8+ T cell response in PBL, 27 days post boost



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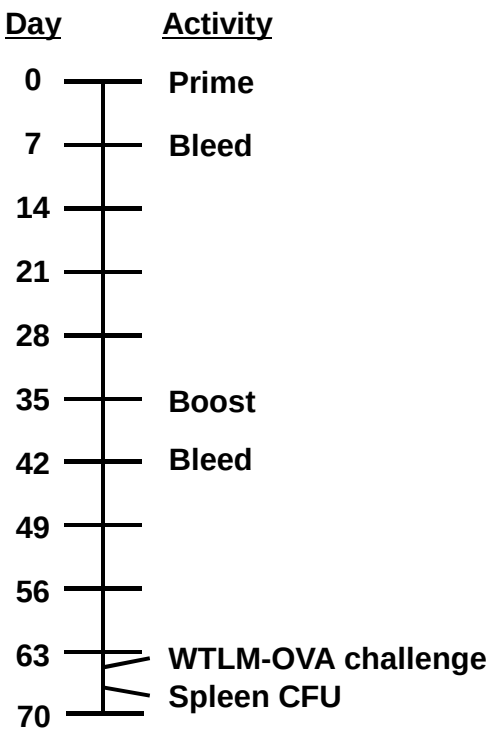

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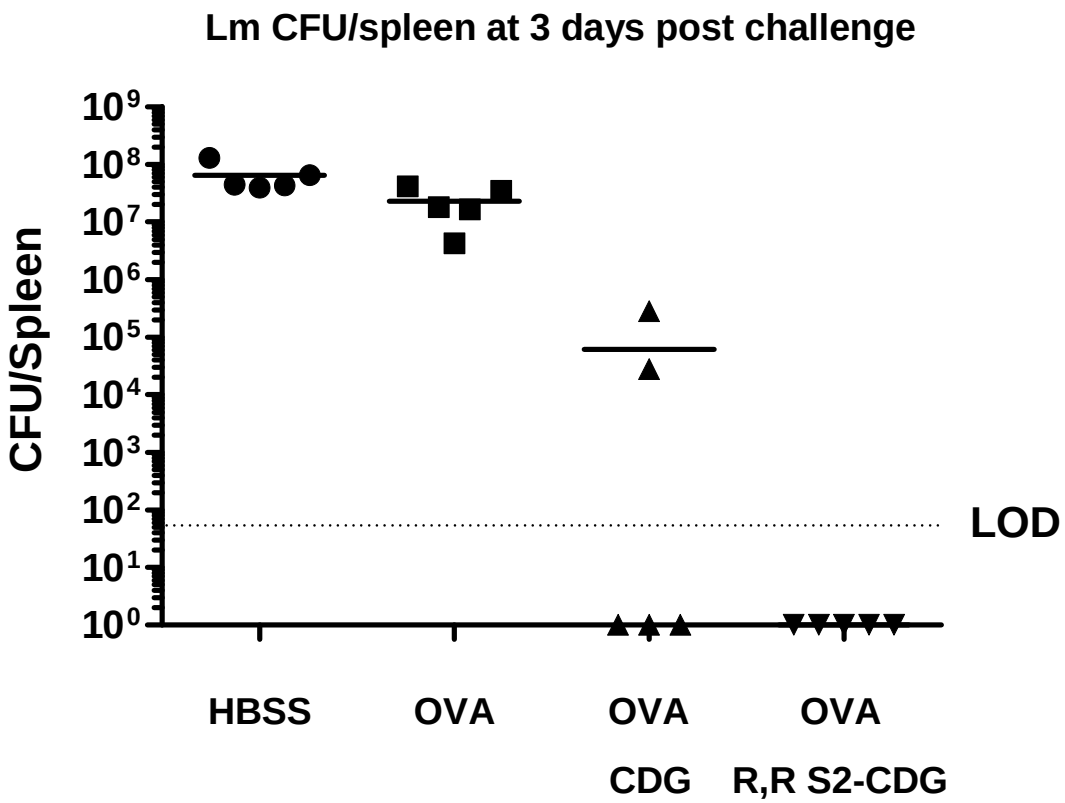

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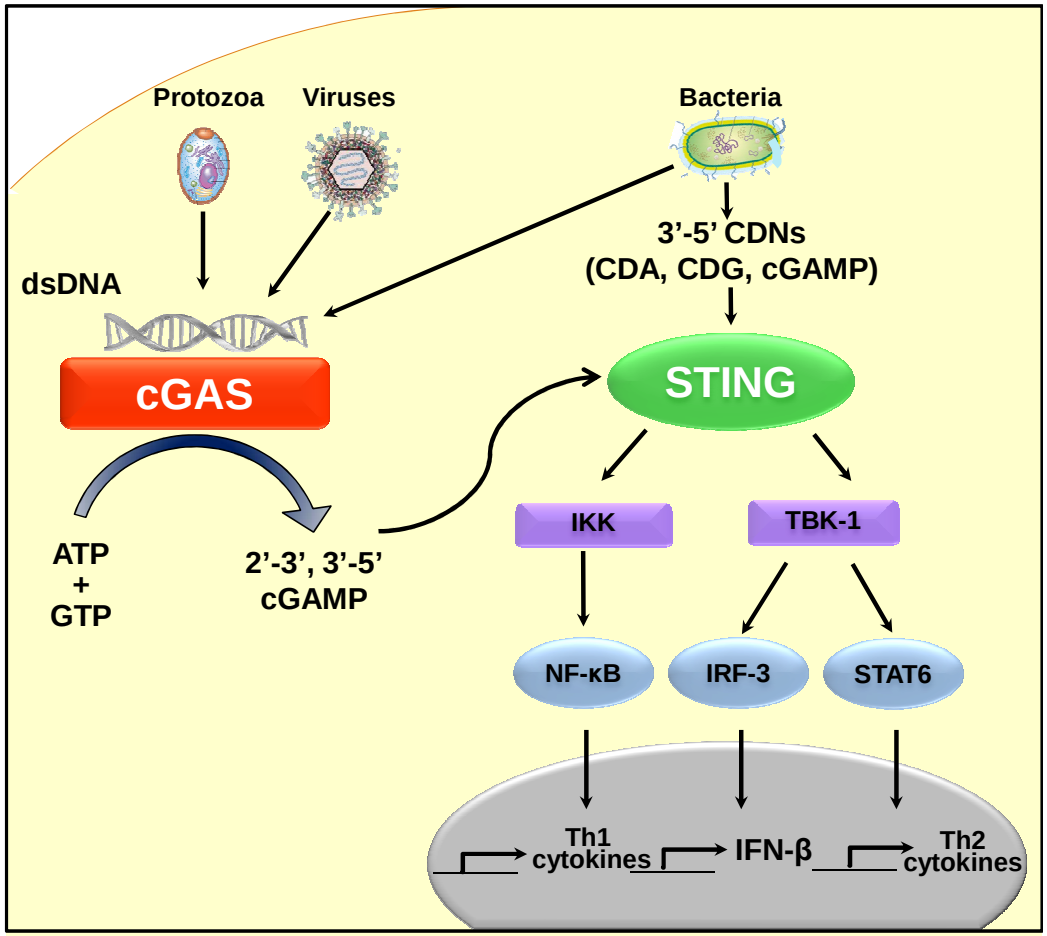
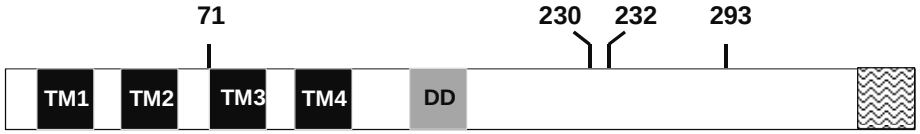


Leong, 2013, Ther Adv Vacc

Human STING Polymorphisms Affect Responsiveness to CDNs

Particular STING alleles have increased responsiveness to non-canonical (mixed linkage) CDNs

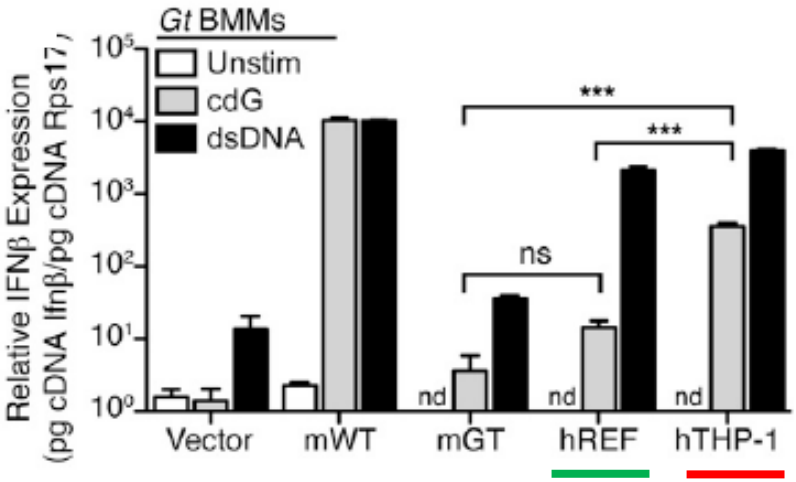
Four STING alleles are found in humans



STING variant				% homozygous
WT	R	GR	R	45%
hREF	R	GH	R	2%
Q	R	GR	Q	4%
THP-1	H	AR	Q	3%

Jin, 2011, Genes Immunity

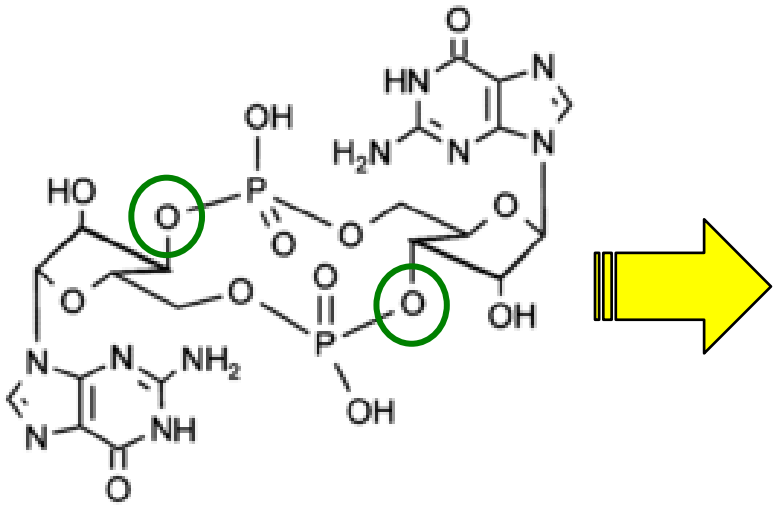
Vance laboratory data



Diner, 2013, Cell Reports

Impact of Phosphate Linkage on STING Signaling

c-di-GMP (canonical)



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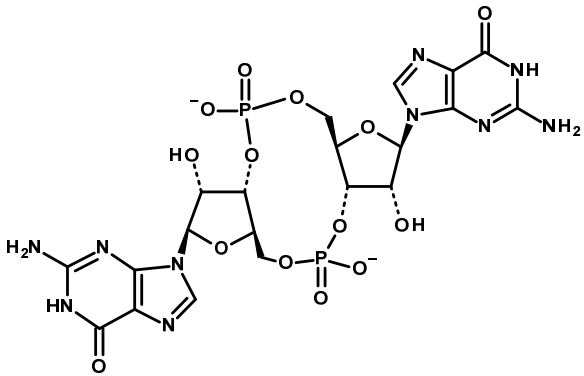
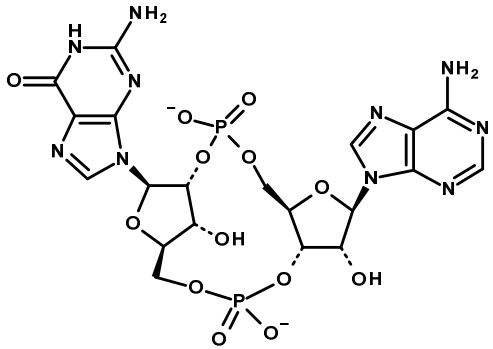
"Canonical"
cyclic[G(3',5')pG(3',5')p])

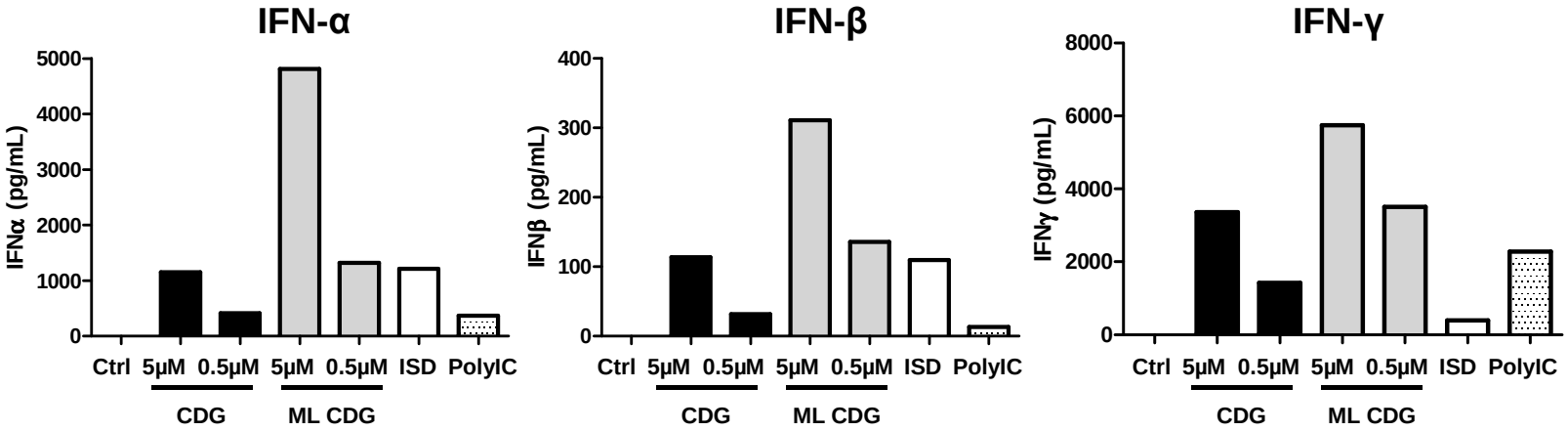


"Non-canonical"
cyclic[G(2',5')pA(3',5')p]

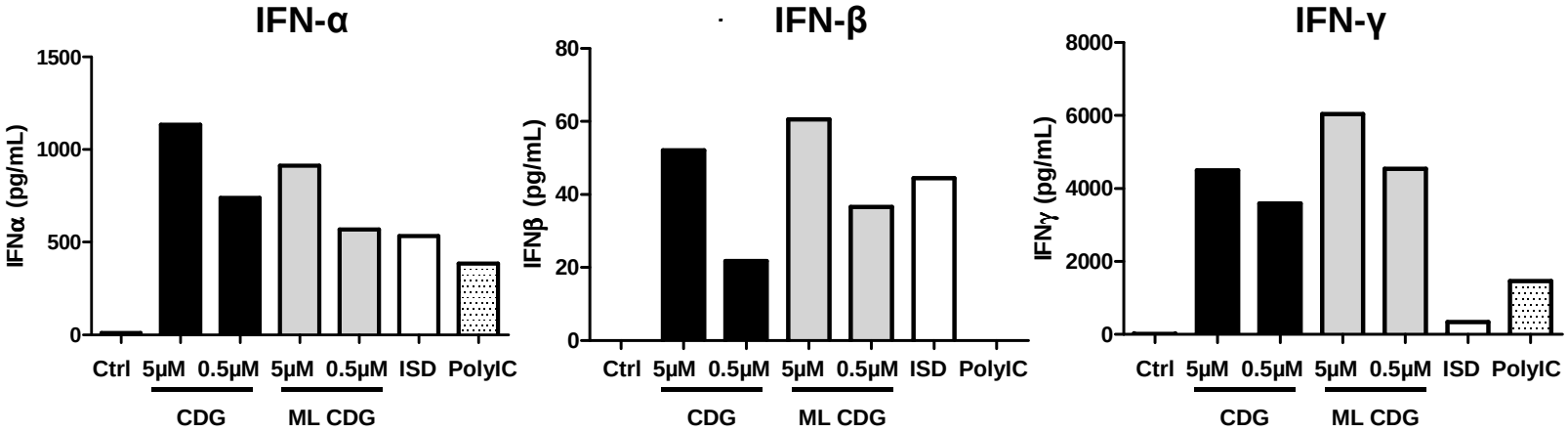


Enhanced Stimulation of Human PBMCs with Noncanonical CDNs

Donor 001: G230, R232 Homozygous



Donor 4050: G230, R/H232 Heterozygous

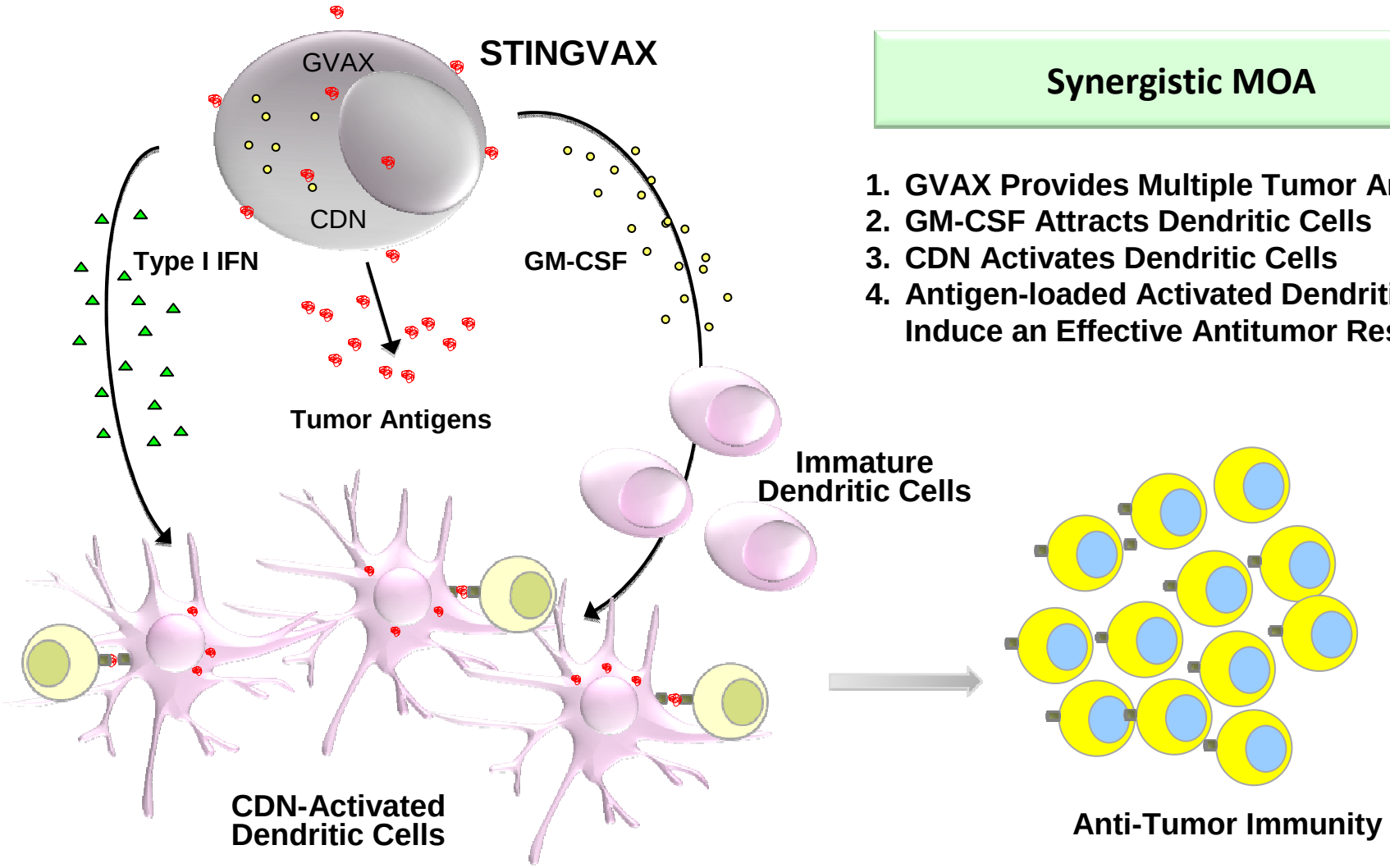


Translation of CDNs

- "**STINGVAX**"
  - **STING**-activating CDNs co-formulated with **GVAX** (irradiated GM-CSF secreting allogeneic tumor cell lines)
  - CDNs provide a potent activation stimulus for GM-CSF recruited dendritic cells
  - Straightforward approach to enhance the potency of a technology platform with multiple cell lines for diverse malignancies

- **CDN Single Agent**
  - Intratumoral injection—"*in situ*" vaccination
  - Subcutaneous (tumor distal) injection—induction of a systemic pro-inflammatory response
  - Combination with SOC chemotherapy or radiotherapy
  - Potentiate therapeutic efficacy of mAbs (e.g., Cetuximab) through ADCC (antibody-dependent cellular cytotoxicity)

STINGVAX: **STING** Activating CDNs **+ GVAX** GM-CSF Secreting Tumor Cell Vaccine

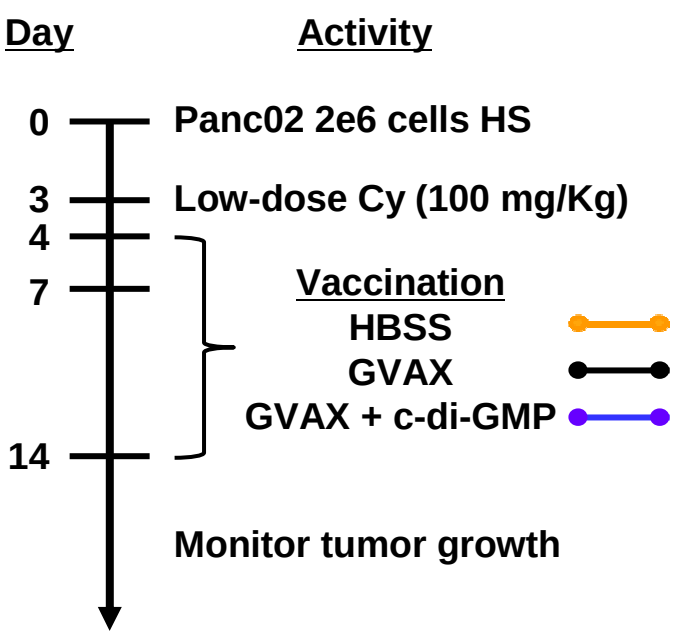


Synergistic MOA

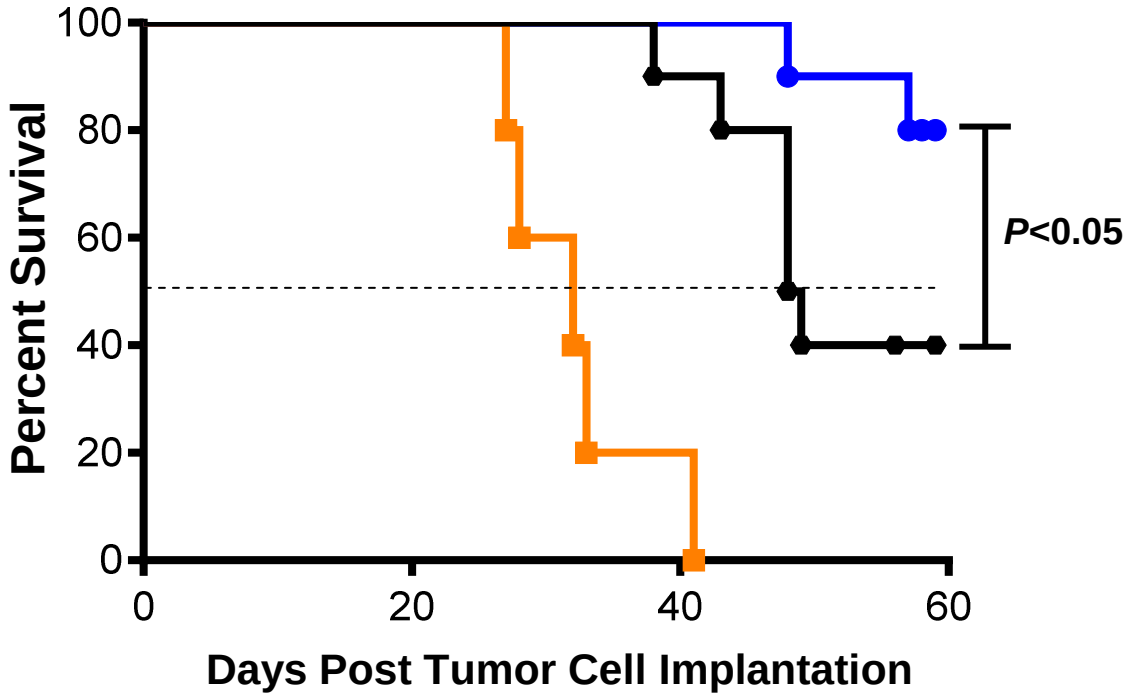
1. **GVAX Provides Multiple Tumor Antigens**
2. **GM-CSF Attracts Dendritic Cells**
3. **CDN Activates Dendritic Cells**
4. **Antigen-loaded Activated Dendritic Cells Induce an Effective Antitumor Response**

STINGVAX POC: Panc02 Hemi-Spleen Model

Schema

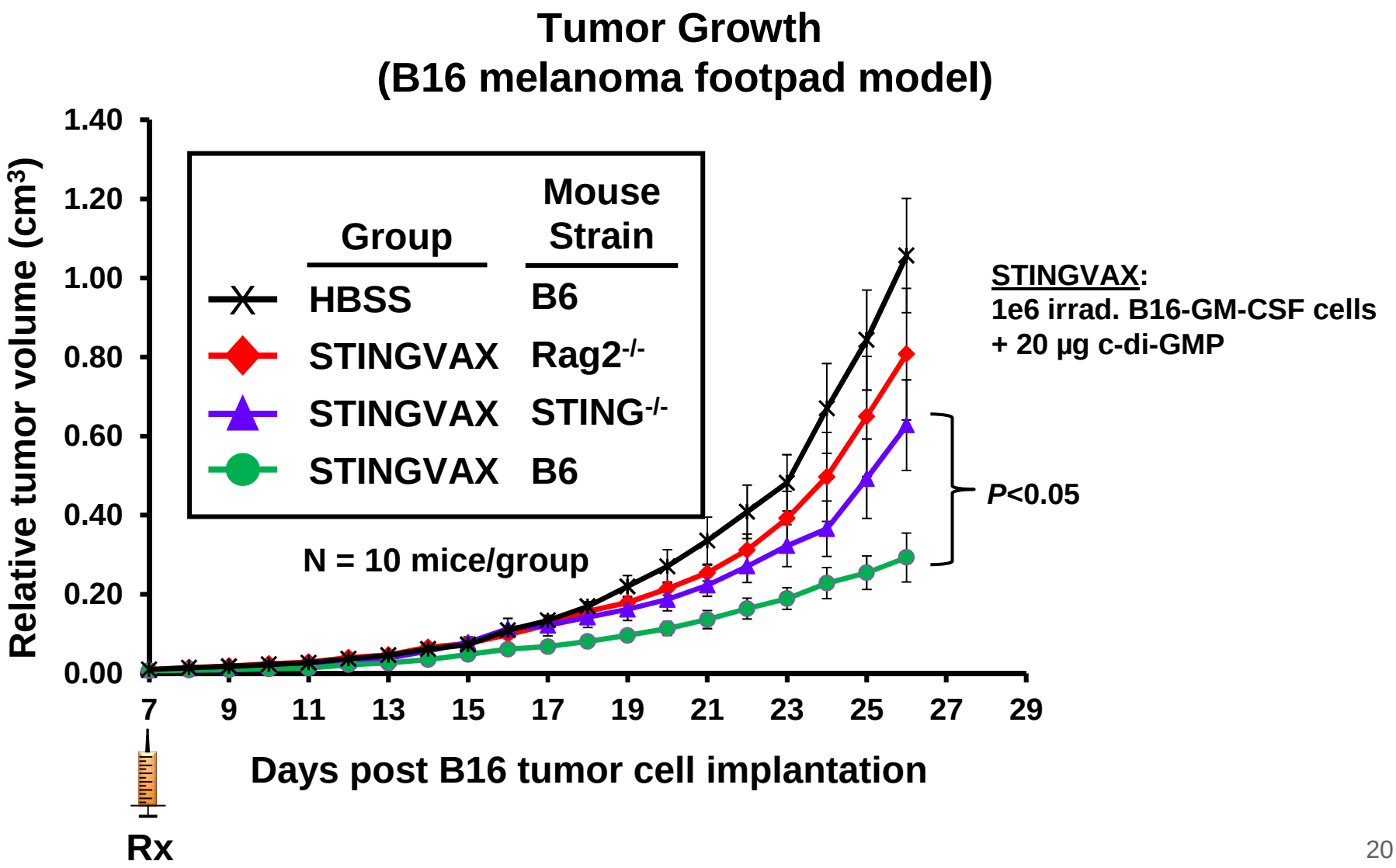


Survival



Group	N
HBSS	5
GVAX	10
GVAX+c-di-GMP	10

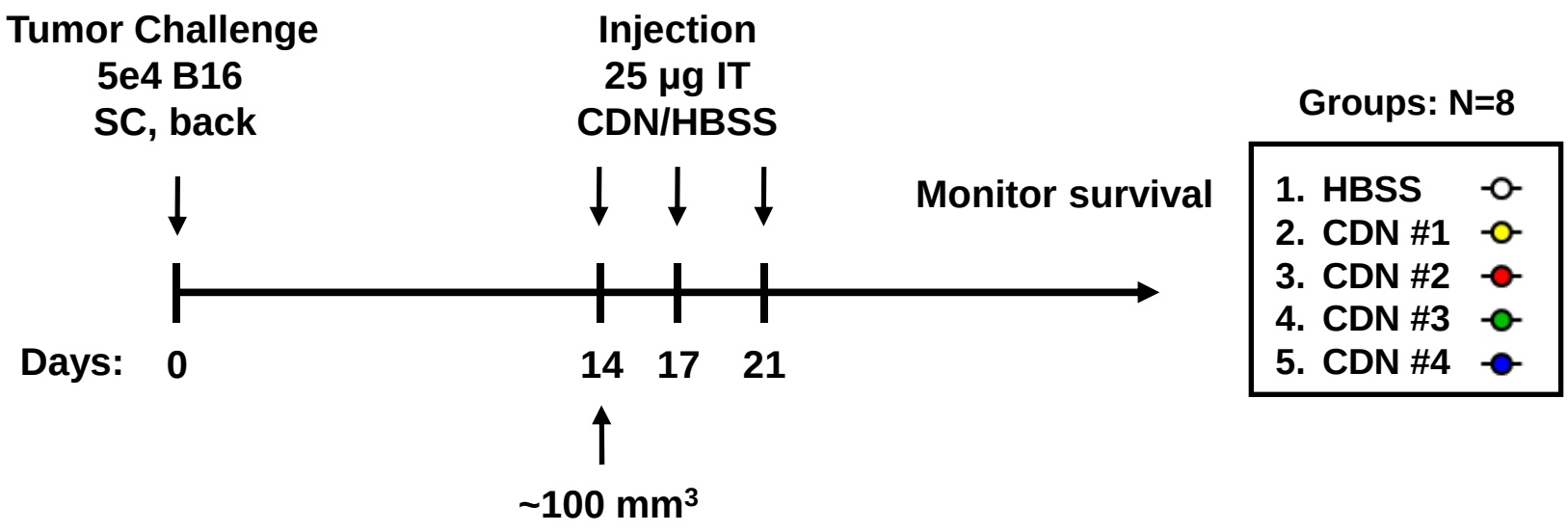
STINGVAX Efficacy is Dependent Upon STING Signaling and Adaptive Immunity



Single Agent CDN Therapeutic Anti-Tumor Efficacy

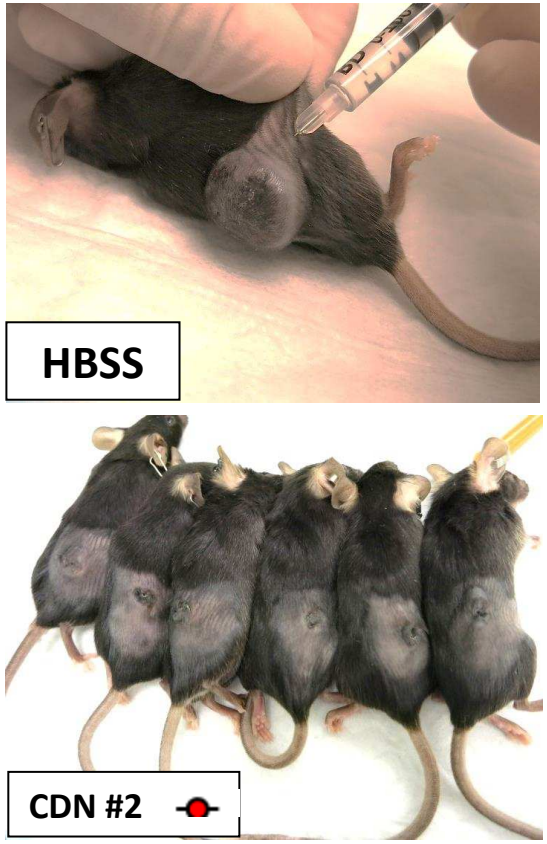
Hypothesis: Activation of tumor-resident CD8α$^+$ dendritic cells through the STING pathway will lead to significant anti-tumor efficacy

Study Design:

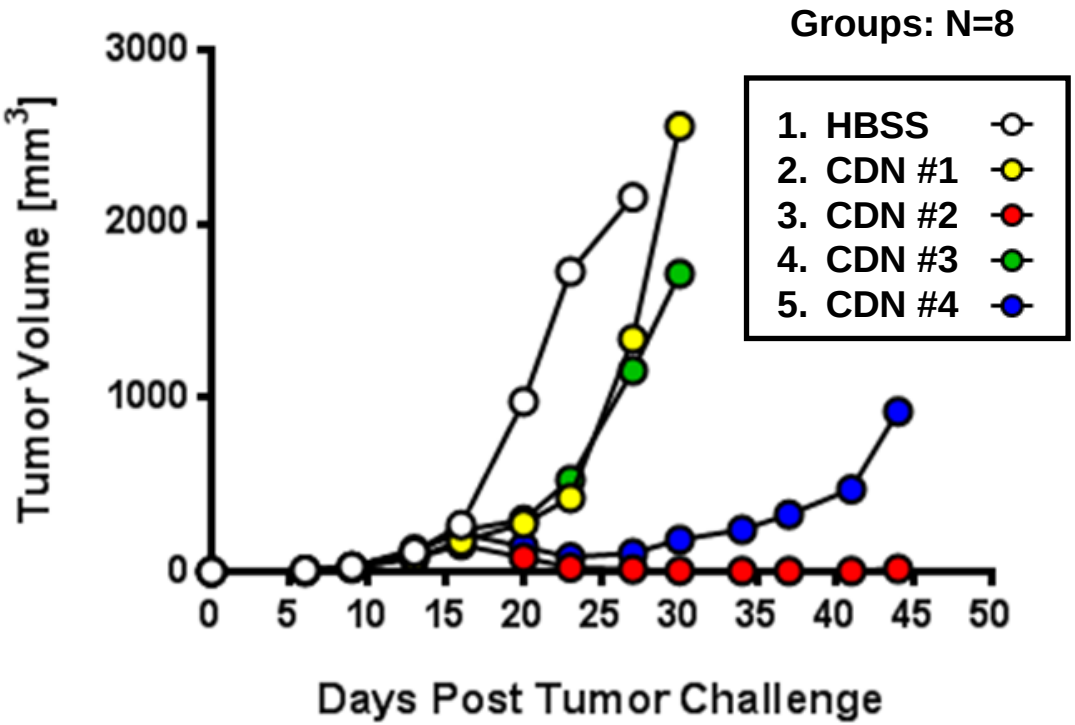


Therapeutic Efficacy of Single Agent CDN in Established B16 Melanoma

Tumor growth, Day 17



Tumor growth



Summary

- Cyclic-di-nucleotides are potent activators of innate immunity
  - Activate innate immunity through STING, a cytosolic PRR
  - Enhance priming of Ag-specific CD4 and CD8 T cells specific for co-formulated protein vaccines
  - Induce Th1-biased humoral immunity
  - Induce protective T cell-mediated immunity against intracellular pathogens
- The immunologic activity of CDNs can be enhanced by targeted modification of native molecules
- Two near-term applications in oncology with proprietary CDNs
  - STINGVAX
  - Single agent: IT or SC injection

Acknowledgements

Aduro BioTech

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U.C. Berkeley

Dan Portnoy

Russell Vance

•Elie Diner

•Dara Burdette

Univ Washington

Josh Woodward

Johns Hopkins University

Drew Pardoll

Elizabeth Jaffee

•Lei Zheng

•Kevin Soares

Young Kim

•Juan Fu