Cellular selectivity of STING stimulation determines priming of anti-tumor T cell responses

Abstract #LB198

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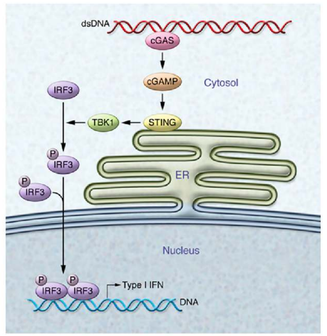
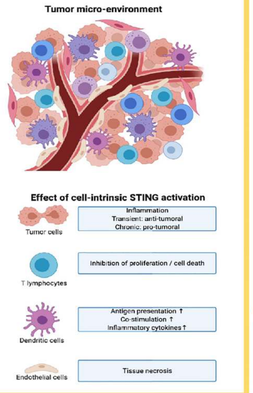
1- ABSTRACT

T cells that recognize tumor antigens are crucial for anti-tumor immune responses. Induction of anti-tumor T cells in immunogenic tumors depends on STING, the intracellular innate immune receptor for cyclic guanosine monophosphate-adenosine monophosphate (cGAMP) and related cyclic dinucleotides (CDN). However, the optimal way to leverage STING activation in non-immunogenic tumors is still unclear. Here, we show that cGAMP delivery by intra-tumoral injection of virus-like particles (cGAMP-VLP) leads to differentiation of tumor-specific T cells, decrease in tumor regulatory T cells (Tregs) and anti-tumoral responses that synergize with PD1 blockade. By contrast, intra-tumoral injection of synthetic CDN leads to tumor necrosis and systemic T cell activation but no differentiation of tumor-specific T cells, and a demise of immune cells in injected tumors. Analyses of cytokine responses and genetic models revealed that cGAMP-VLP preferentially targets STING in dendritic cells at a 1000-fold less dose than synthetic CDN. Sub-cutaneous administration of cGAMP-VLP showed synergy when combined with a tumor Treg-depleting antibody to elicit systemic tumor-specific T cells, leading to complete and lasting tumor eradication. These findings show that cell targeting of STING stimulation shapes the anti-tumor T cell response and reveal a therapeutic strategy with T cell modulators, which may address the current limitations of STING-based approaches in patients.

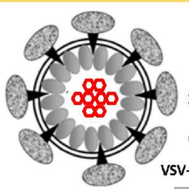
2- STING PATHWAY





Corrales et al, J.Clin. Invest., 2016
Corrales et al, Cell Rep, 2015

3- cGAMP-CONTAINING VIRUS-LIKE-PARTICULES

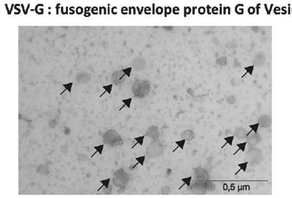


Gentili et al, Science, 2015
Birdgeman et al, Science, 2015

2'3' cGAMP : "non-canonical" *cyclic dinucleotide.*

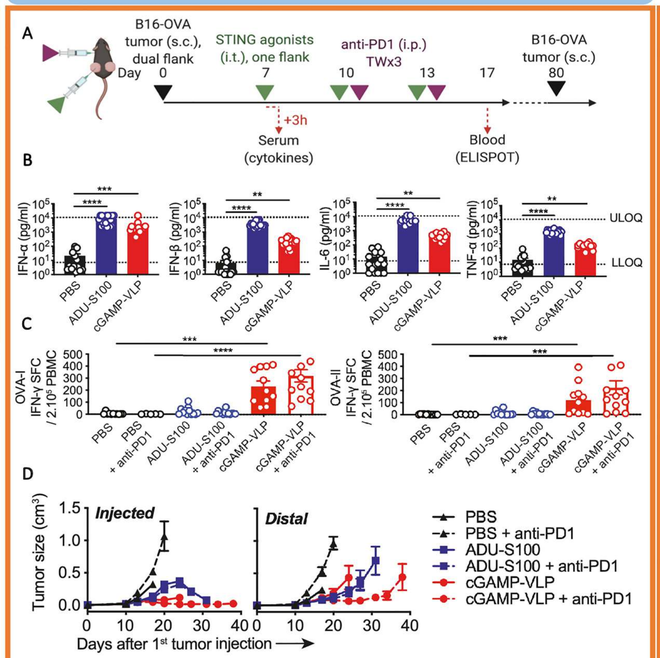
GAG : structural viral proteins of HIV.

VSV-G : fusogenic envelope protein G of Vesicular Stomatitis Virus.



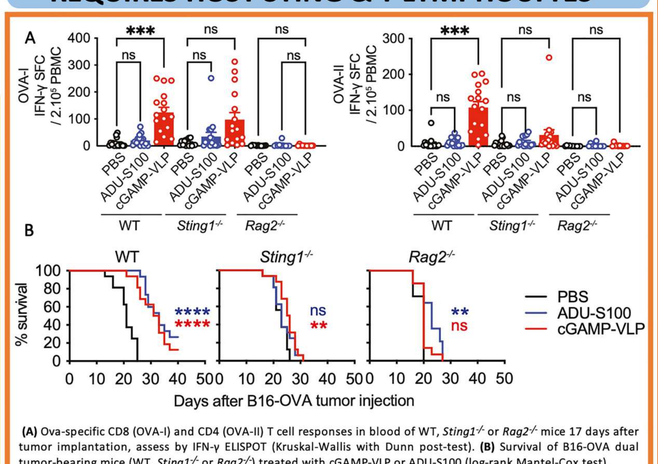
Electron microscopy image of purified cGAMP-VLPs. Scale bars at 0.5 µm. Arrows point to cGAMP-VLP.

4- SYSTEMIC ANTI-TUMOR ACTIVITY OF cGAMP-VLP & SYNERGY WITH ANTI-PD1



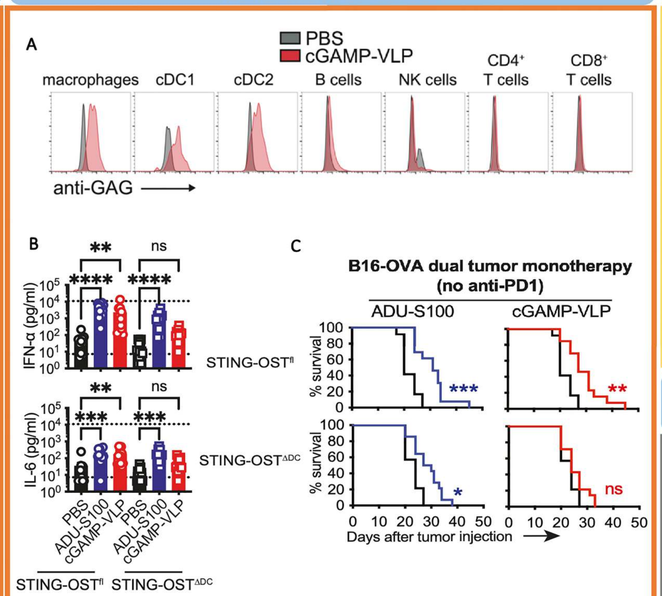
(A) Overview of the experimental design. Treatments were initiated on palpable tumors. **(B)** Concentrations of IFN-α, IFN-β, IL-6 and TNF-α in the serum of B16-OVA dual tumor-bearing mice 3 hours after treatment (Kruskal-Wallis with Dunn post-test, LLOQ = lower limit of quantification, ULOQ = upper limit of quantification). **(C)** Ova-specific CD8 (OVA-I) and CD4 (OVA-II) T cell responses in blood, assess by IFN-γ ELISPOT (Kruskal-Wallis with Dunn post-test). **(D)** Mean growth over time of B16-OVA injected and distal tumors treated as indicated.

5- ANTI-TUMOR EFFECT OF cGAMP-VLP REQUIRES HOST STING & T LYMPHOCYTES



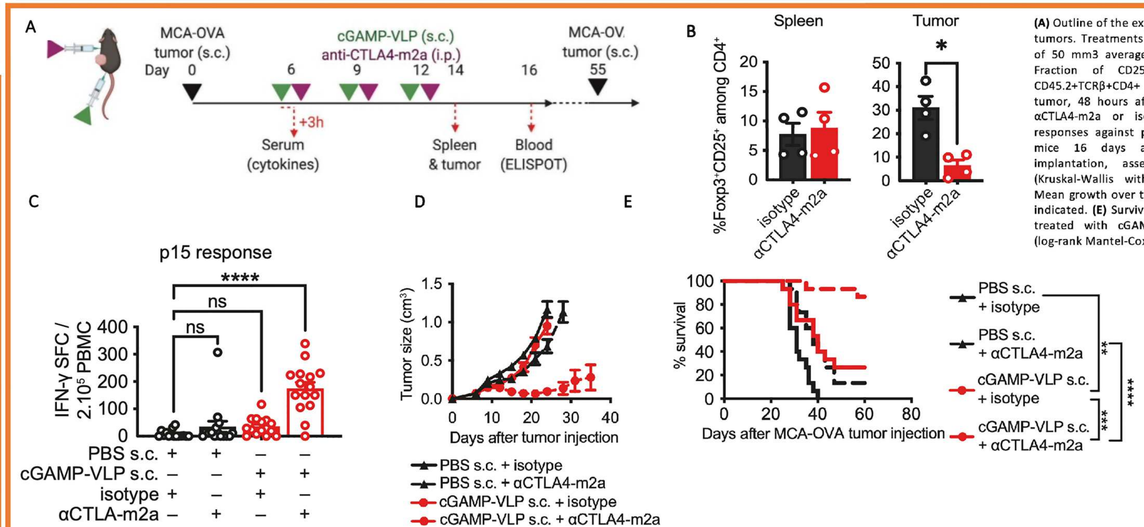
(A) Ova-specific CD8 (OVA-I) and CD4 (OVA-II) T cell responses in blood of WT, *Sting1*$^{-/-}$ or *Rag2*$^{-/-}$ mice 17 days after tumor implantation, assess by IFN-γ ELISPOT (Kruskal-Wallis with Dunn post-test). **(B)** Survival of B16-OVA dual tumor-bearing mice (WT, *Sting1*$^{-/-}$ or *Rag2*$^{-/-}$) treated with cGAMP-VLP or ADU-S100 (log-rank Mantel-Cox test).

6- cGAMP-VLP TARGETS PREFERENTIALLY DENDRITIC CELLS



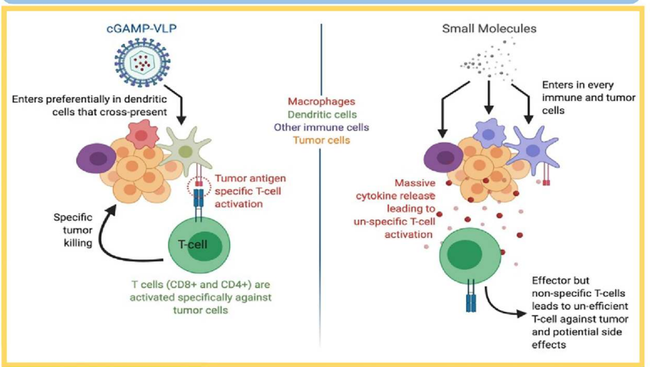
(A) Anti-GAG staining in the indicated immune cells from splenocytes treated with PBS or cGAMP-VLP. **(B)** Concentrations of IFN-α and IL-6 in the serum of B16-OVA dual tumor-bearing mice (WT or *Tmem173*$^{ltgax\text{-}cre}$) 3 hours after the first treatment by i.t. injection of PBS, 50 µg ADU-S100 or 50 ng cGAMP-VLP (Kruskal-Wallis with Dunn post-test, LLOQ = lower limit of quantification, ULOQ = upper limit of quantification). . **(C)** Survival of B16-OVA dual tumor-bearing WT or *Tmem173*$^{ltgax\text{-}cre}$ mice treated as indicated (log-rank Mantel-Cox test).
STING-OST^{fl} → *WT mice. STIING-OST*$^{\Delta DC}$ → *STING KO in dendritic cells mice.*

7- cGAMP-VLP SYNERGIZES WITH INTRATUMORAL T REG DEPLETION



(A) Outline of the experiment using MCA-OVA tumors. Treatments were started on tumors of 50 mm3 average volume per group. **(B)** Fraction of CD25+FoxP3+ Tregs within CD45.2+TCRβ+CD4+ cells in spleen and tumor, 48 hours after last i.p. injection of αCTLA4-m2a or isotype. **(C)** CD8 T cell responses against p15 antigen in blood of mice 16 days after MCA-OVA tumor implantation, assess by IFN-γ ELISPOT (Kruskal-Wallis with Dunn post-test). **(D)** Mean growth over time of tumors treated as indicated. **(E)** Survival of tumor-bearing mice treated with cGAMP-VLP +/- αCTLA4-m2a (log-rank Mantel-Cox test).

8- WORKING MODEL



9- CONCLUSIONS & PERSPECTIVES

Altogether, our results establish that :

i- Cell specific activation of STING is important to ensuring immune priming and activation.

ii- Synthetic STING agonists appear to induce promiscuous STING activation that does not necessarily entail priming of tumor-specific T cells.

iii- In contrast, cGAMP-VLP constitutes a biological product that activates STING preferentially in dendritic cells, leading to activation of tumor-specific T cells,

iv- Systemic cGAMP-VLP synergizes with ICB and Treg depletion.

Biological stimulation of STING with cGAMP-VLP has the potential, similar to other biological drugs such as antibodies and CAR-T cells, to contribute to a meaningful treatment regimen to induce anti-tumor immune responses in patients.