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Investigating the PK-PD Relationship Governing STING Agonist Nanoparticle Efficacy Upon Systemic Administration

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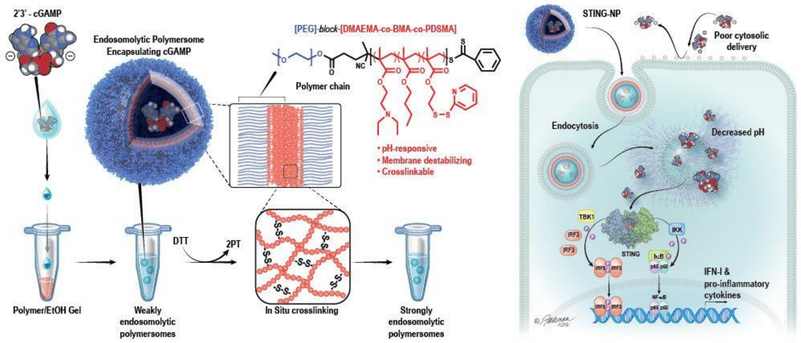
Introduction

- The **St**imulator of **I**nterferon **G**ene (STING) pathway has shown great promise for cancer immunotherapy.
- Cyclic dinucleotides (ex: 2'3'-cGAMP) are the natural binding ligand of STING protein. These compounds are rapidly metabolized and are poorly membrane permeable limiting cytosolic delivery.
- Delivery challenges can be overcome by nanoparticle encapsulation in a endosomolytic polymerosome but little is known as to what design criteria constitute a "optimized" delivery platform.
- This work investigates the pharmacokinetic-pharmacodynamic relationship of STING Activating Nanoparticles (STING-NP) to better understand potential barriers limiting translation.

STING Activating Nanoparticles (STING-NP)



- STING-NPs are formulated using a polymer thin film hydration approach. These particles are capable of cytosolic delivery of cGAMP and subsequent activation of STING protein.

STING-NP Toxicity

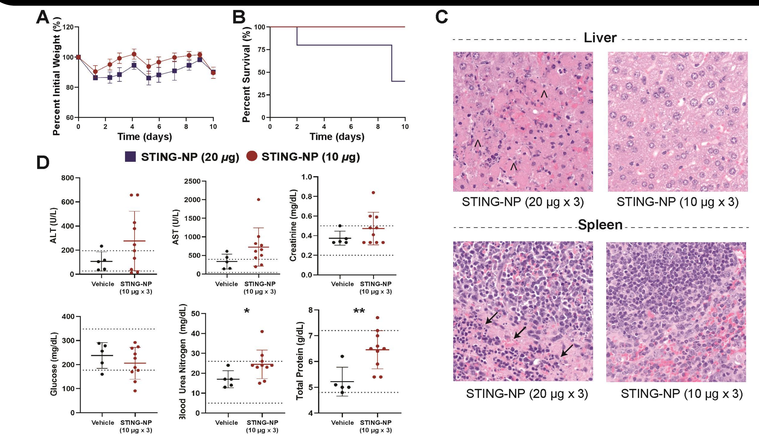


Figure 1: Determination of maximum tolerated dose for STING-NP. C57BL/6 mice were treated with Vehicle or STING-NPs at 10 or 20 μg per mouse intravenously every 3 days for a total of 3 injections. Mice were weighed and monitored for 10 days; the percent initial body weight **(A)** and survival **(B)** were plotted. On day 10, mice were euthanized and the organs were formalin fixed for gross pathology. **(C)** H&E staining of liver and spleen images showing necrosis (Λ) in the liver and apoptosis (→) in the spleen. **(D)** Blood chemistry of mice treated with STING-NPs at 24 hours after final injection. *P*<0.05, ** *P*<0.01, *** *P*<0.001, **** *P*<0.0001, indicate a statistically significant difference relative to vehicle (PBS).

Pharmacokinetic–Pharmacodynamic Profiles

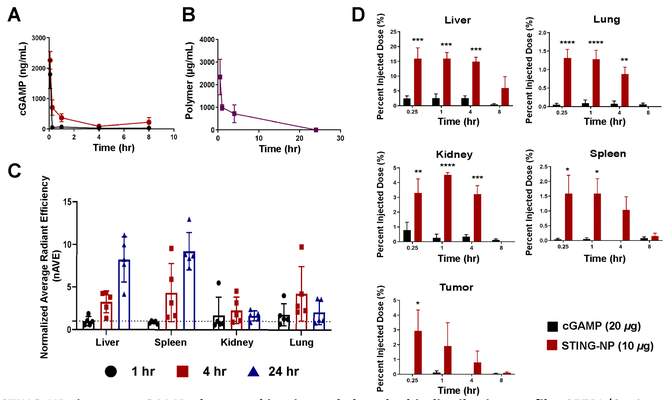


Figure 2: STING-NPs improve cGAMP pharmacokinetics and alter the biodistribution profile. C57BL/6 mice were inoculated with B16-F10 tumor cells and treated with cGAMP (1 μCi per mouse, 20 μg of cGAMP) or STING-NP (1 μCi per mouse, 10 μg cGAMP), at indicated timepoints mice were euthanized. (**A**) Plasma cGAMP concentrations as a function of time. (**B**) Plasma polymer concentration as a function of time. (**C**) Organ polymer distribution using IVIS imagine at indicated timepoints from whole organs. (**D**) Organ cGAMP distribution as a function of time plotted as percent injected dose.

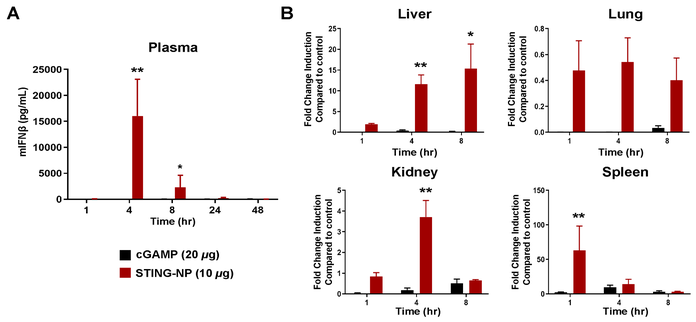


Figure 3: STING-NPs activate a type-I interferon response in blood and clearance organs. C57BL/6 mice were treated with vehicle, cGAMP (20 μg per mouse) or STING-NP (10 μg cGAMP per mouse) and euthanized at indicated timepoints. (**A**) Plasma mouse interferon beta was measured by ELISA. (**B**) qRT-PCR was used to measure *Ifnb1* transcript levels in mouse tissue.

STING-NP Efficacy

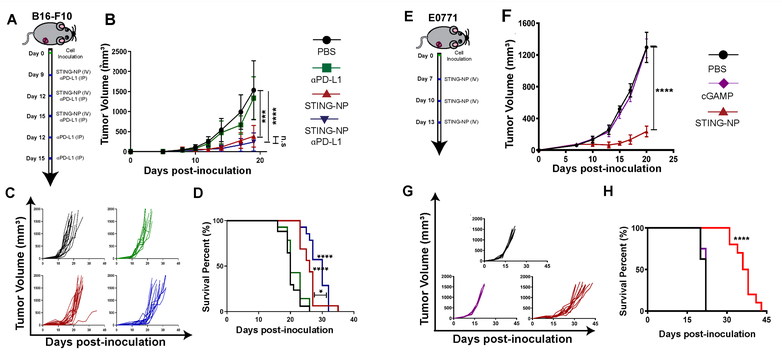


Figure 5: STING-NP efficacy in melanoma and breast cancer models. (**A**) Schematic summary of treatment for mice with B16-F10 tumors. (**B**) Average tumor volume and (**C**) spider plots for treated and untreated tumors. (**D**) Kaplan-Meier survival curves of mice treated with indicated formulation. **(E)** Schematic representation of study design and treatment regimen for E0771 breast cancer model. (**F**) Average tumor volume, (**G**) spider plots, and (**H**) Kaplan-Meier survival curves for mice injected intravenously with vehicle or STING-NP. Mice were treated with indicated formulation using a total tumor volume >1500 mm^3 as endpoint criteria. B16-F10 and E0771 tumor volumes were compared on day 19 using a one-way ANOVA. Kaplan–Meier survival analysis (two-tailed Mantel–Cox test).

Tumor Microenvironment

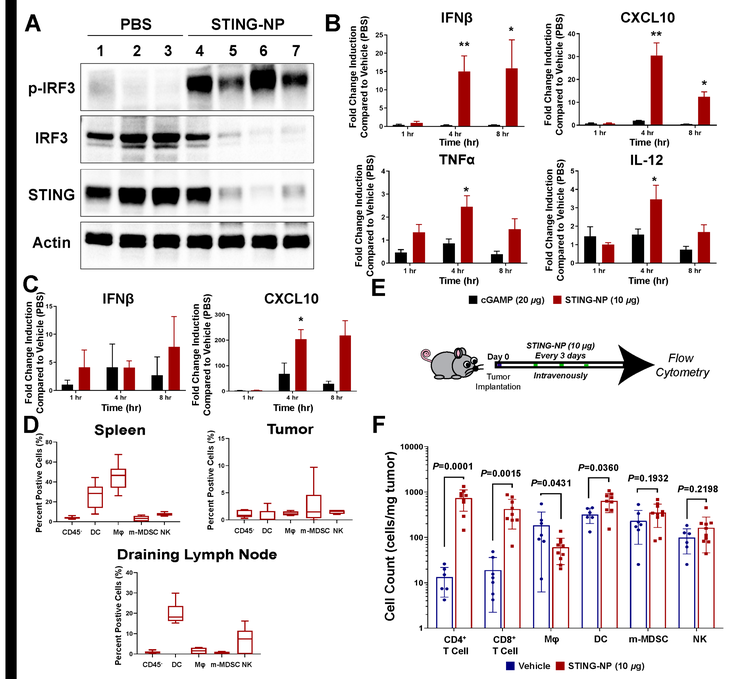


Figure 4: Systemic STING-NP treatment causes STING activation in tumors and remodeling of the TME. (**A**) C57BL/6 B16-F10 tumor bearing mice were treated with vehicle or STING-NP (10 μg cGAMP per mouse). Western blot analysis of tumors 24 hours after treatment examining STING and IRF3/p-IRF3 expression. (**B**) qRT-PCR was used to measure *Ifnb1*, *Cxcl10*, *Tnfa*, and *Il12* transcript levels in Vehicle (PBS), cGAMP or STING-NP treated tumor, data is shown as fold change over vehicle. (**C**) qRT-PCR was used to measure lymph node *Ifnb1* and *Cxcl10* transcript levels in Vehicle (PBS), cGAMP or STING-NP treated lymph node, data is shown as fold change over vehicle. (**D**) Mice were treated with Cy5-labelled polymersomes and euthanized at 24 h post-injection. (**E**) Mouse treatment scheme for flow cytometry study. (**F**) Immune cell infiltration into B16-F10 tumors obtained 24 hours after third vehicle/STING-NP treatment analyzed using flow cytometry.

Conclusions

Together, these data, which represent the first rigorous investigation into the PK-PD relationship of a STING-activating nanomedicine, demonstrate that STING-NPs open a therapeutic window for systemic administration of cyclic dinucleotides and provide insight into design criteria for engineering of optimized delivery platforms for systemic delivery of STING agonists.

Future Work

- Optimizing the STING-NP platform for systemic therapy of metastatic cancer
- Investigating drug combination synergy of STING agonists with other immunotherapies

Acknowledgments

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