Digital Spatial Profiling of Molecular Responses to Nanoparticle STING Agonists Identify S100A9 and B7-H3 as Possible Escape Mechanisms

John T. Wilson¹, Daniel Shae¹, Paula I. Gonzalez-Ericsson², Violeta Sanchez², Jingjing Gong³, Yan Liang³, Doug Hinerfeld³, Joseph M. Beechem³, Justin M. Balko²

¹Vanderbilt University; Nashville, TN; ²Vanderbilt University Medical Center; ³NanoString Technologies, Seattle, WA

Introduction

- Cyclic dinucleotide (CDN) agonists of stimulator of interferon of genes (STING) are a promising class of immunotherapeutics.
- The efficacy of CDNs is limited by drug delivery barriers, including poor cellular targeting, rapid clearance, and inefficient cytosolic delivery.
- We have recently described STING-activating nanoparticles (STING-NPs) – endosomolytic polymersomes for enhanced cytosolic delivery of cGAMP, the endogenous ligand for STING.
- STING-NPs enhance biological potency and therapeutic activity of cGAMP in multiple murine tumor models via both intratumoral and intravenous administration routes (see Shae et al. Nature Nanotechnology, 14:269-278, 2019).
- Here, we describe the use of NanoString Digital Spatial Profiling (DSP) to identify potential resistance mechanisms to STING-NPs.

Enhanced Therapeutic Efficacy

Figure 5. In mice with two SC B16.F10 tumors, IT injection of STING-NP into one tumor, suppresses growth of a contralateral, distal tumor particularly when combined with systemic administration of a combination of CTLA-4 and PD-1 immune checkpoint blockade (ICB) antibodies.

STING-NP Formulation & Properties

Figure 2. Characterization of polymersomes. A/B) Representative transmission electron micrographs. C) Surface zeta potential of particles at pH 7.4. D) Dynamic light scattering analysis of particle size.

NanoString Digital Spatial Profiling

Figure 3. Optimization of endosomal delivery. A) Polymersome assembly, cGAMP loading, and vesicle crosslinking strategy. B) GPC analysis of polymers pre- and post-crosslinking. C) Effect of crosslinking on pH-dependent membrane destabilization as measured via hemolysis.

Increased STING Activation in vitro

Figure 4. Optimal crosslinked STING-NPs significantly enhance cGAMP potency in vitro. Relative IFN-γ response in THP-1 monocyte, RAW264.7 macrophage, and B16 melanoma iPS ISG reporter cells.

Figure 6. Systemically (intravenously) administered STING-NPs inhibit B16.F10 tumor growth, extend survival, and enhance the efficacy of anti-CTLA-4 and PD-1 immune checkpoint blockade (ICB), resulting in a 40% complete response rate.

Conclusions

- STING-NPs enhance the activity of CDNs and can be administered systematically via an IV route to inhibit tumor growth and increase responses to anti-CTLA-4/PD-1 immune checkpoint blockade.
- NanoString DSP analysis of T cell-rich regions of tumors and TDLN after STING-NP treatment reveals increased expression of B7-H3 and S100A9, which may act as resistance mechanisms to STING agonists.
- These findings motivate ongoing investigations combining STING-NPs and anti-B7-H3 antibodies and/or inhibitors of MDSC migration.

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