Dual function of CpG-STAT3 antisense oligonucleotides is essential for the induction of antitumor immunity against genetically distinct castration-resistant prostate cancers

Dayson Moreira[1], Tomasz Adamus[1], Xingli Zhao[1], Yu-Lin Su[1], Zhuoran Zhang[1], Seok Voon White[1], Piotr Swiderski[2], Xin Lu[5], Ronald A. DePinho[6], Sumanta K. Pal[3], **Marcin Kortylewski[1,4]**

[1]Department of Immuno-Oncology; [2]DNA/RNA Synthesis Core Laboratory; [3]Medical Oncology and Experimental Therapeutics; [4]Center for Gene Therapy; Beckman Research Institute at City of Hope Comprehensive Cancer Center, Duarte, CA, USA. [5]Department of Biological Sciences, University of Notre Dame, Notre Dame, IN, USA. [6]Department of Cancer Biology, Texas MD Anderson Cancer Center, Houston, TX, USA.

Prostate cancers show remarkable resistance to emerging immunotherapies, partly due to tolerogenic STAT3 signaling in tumor-associated myeloid cells. Here, we describe a novel strategy combining STAT3 inhibition with Toll-like Receptor-9 (TLR9) stimulation to unleash immune response against prostate cancers regardless of the genetic background.

We developed and validated a conjugate of the STAT3 antisense oligonucleotide (ASO) tethered to immunostimulatory TLR9 agonist (CpG oligonucleotide) to improve targeting of human and mouse prostate cancer and myeloid immune cells, such as myeloid-derived suppressor cells (MDSCs). CpG-STAT3ASO conjugates showed improved biodistribution and potency of STAT3 knockdown in target cells *in vitro* and *in vivo*. Systemic administration of CpG-STAT3ASO (5mg/kg) eradicated bone-localized, Ras-/Myc-driven and *Pten*$^{pc-/-}$*Smad4*$^{pc-/-}$*Trp53*$^{c-/-}$ prostate tumors in the majority of treated mice. These antitumor effects were primarily immune-mediated and correlated with an increased ratio of $CD8^+$ to regulatory T-cells and reduced $pSTAT3^+$/$PD\text{-}L1^+$ MDSCs. Both innate and adaptive immunity contributed to systemic antitumor responses as verified by the depletion of $Gr1^+$ myeloid cells and $CD8^+$, $CD4^+$ T-cells, respectively. Importantly, only the bi-functional CpG-STAT3ASO, but not the control CpG oligonucleotides, the STAT3ASO alone nor the co-injection of both oligonucleotides, succeeded in recruiting neutrophils and $CD8^+$ T-cells into tumors. Thus, the concurrence of TLR9 activation with STAT3 inhibition in the same cellular compartment is indispensable for overcoming tumor immune tolerance and effective antitumor immunity against prostate cancer.

The bi-functional, immunostimulatory and tolerance-breaking design of CpG-STAT3ASO offers a blueprint for the development of effective and safer oligonucleotide strategies for treatment of immunologically "cold" human cancers.

M.K., P.S. and D.M. are inventors on patent application that covers the design of oligonucleotides presented in this report. All other authors declare no potential conflict of interest.

This work was supported by the Department of Defense grant number W81XWH-16-1-0499, Prostate Cancer Foundation and Israel Cancer Research Fund. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.