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Spatiotemporal tumor immune modulation by localized delivery of cancer therapeutics using an implantable microdevice

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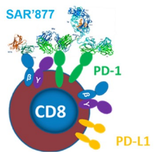
INTRODUCTION

Mechanism of action studies are imperative to translating oncology therapeutics into the clinic informing testable hypotheses and potential combination partners. This often involves large and time-consuming mouse studies to test a small number of compounds to show statistically significant benefit. To reduce the time and cost for mechanism of action studies of our cytokine portfolio, we applied the implantable microdevice (IMD) NanoNail™ developed by KiburMed to measure intra-tumoral drug responses and to differentiate the mechanism of action of multiple cytokine agents in parallel in the MC38 mouse model. With the NanoNail (IMD) device, we will compare murine surrogates of SAR445877 (mKD050) - a fusion of anti-PD1 to IL15-IL15Ra mutein - to anti-PD1 and combination of KD050 + anti-PDL1 in mouse tumors.



TECHNICAL APPROACH

C57BL/6 mice (3 groups x 11 mice per group) were subcutaneously inoculated on the flank with MC38 tumors. After sizable tumor growth, the IMD device containing 9 different therapeutic candidates was implanted. MC38-bearing mice were taken down at 24 hour, 4 days, and 7 days post IMD implantation to assess response. Pharmacodynamic response to treatments was measured by two readouts: Cyclic Immunofluorescence with 22 markers and whole transcriptome Nanostring GeoMx on regions of interest proximal and distal to tumor defined by PanCK and CD45. Among the candidate murine surrogate compounds in the device, KD050 was tested as a monotherapy, as a combination partner with anti-PDL1 and compared against anti-PD1 monotherapy.

Figure 1: Application of NanoNail™ with Cytokine Agonists for Deep Cellular Profiling

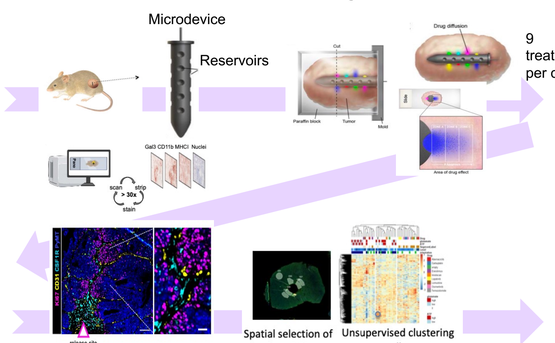


Figure 1: Mouse MC38 tumors were grown at Charles River Labs until tumors reached an average of 350 to 500mm^3 . Each reservoir was filled with liquid nanodoses based on known clinical activity and implanted into each mouse tumor. Potency at nanodose level was confirmed prior to start of study. At each time point, the tumors were excised, placed in 10% formalin and transferred to 70% ethanol 24 hours post collection. The recovered IMDs were embedded in a total of 5 parrafin blocks (up to 6 IMDs per blocks). Nine thin sections were cut from each block for a total of 54 thin sections and subsequently stained for cyclic immunofluorescence and or IF markers for whole transcriptome GeoMx DSP.

RESULTS

- Combination KD050 + anti-PDL1 increases T cells (CD3, CD4, CD8) and B cells (CD20 and CD27) both by Cyclic IF and GeoMx Nanostring whole transcriptome compared to mKD050 or anti-PD1 alone.
- Combination mKD050 + anti-PDL1 also increases NK cells and Cytotoxic Lymphocytes observed by Nanostring GeoMx whole transcriptome and MCP counter signatures.
- Most significant cellular changes were observed in proximal regions than in distal regions to IMD device.
- Differential expression and pathway enrichment results are still ongoing.

Figure 2: Analytical Methods for Quantification of Drug Effects by Cyclic IF

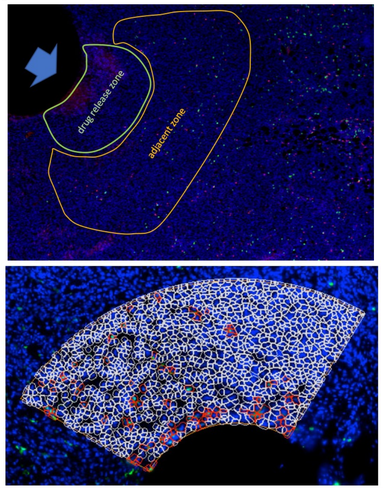
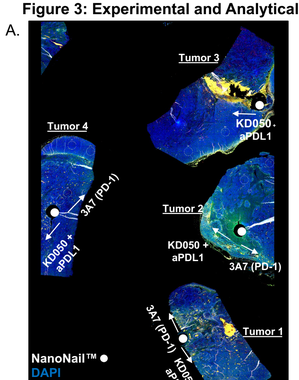


Figure 2
A) Quantification of Cyclic IF Image data. IF image is split by channel and each marker is quantified within region of drug distribution and adjacent zone. B) Automated quantification pipeline counts positive cells for each marker within a given zone[2].

Figure 3: Experimental and Analytical Design of Spatial GeoMx DSP experiment



B.

Data Points	Day 1	Day 4	Day 7
peg-IL2	5	7	5
3A7	4	6	7
mKD050	6	6	9
mKD050 + aPDL1	3	6	6

C.

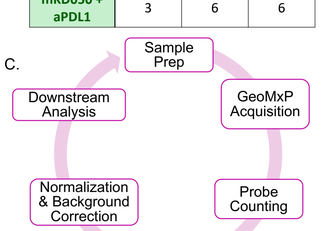


Figure 3: A) Each slide representation contains four to five IMD containing tissues. Each IMD flank tissue contains tumor (white circle) and adjacent tissue. Direction of compound release depicted with arrows and markers for ROI include Dapi nuclei, PanCK for tumor, and CD45 for immune cells. Regions of interest chosen in proximal vs adjacent regions to IMD device. B) Table of tumor cores per treatment per time point. C) GeoMx whole transcriptome analytical workflow.

Figure 4. Nanodose Pharmacological Effects of KD50 + anti-PDL1 combination

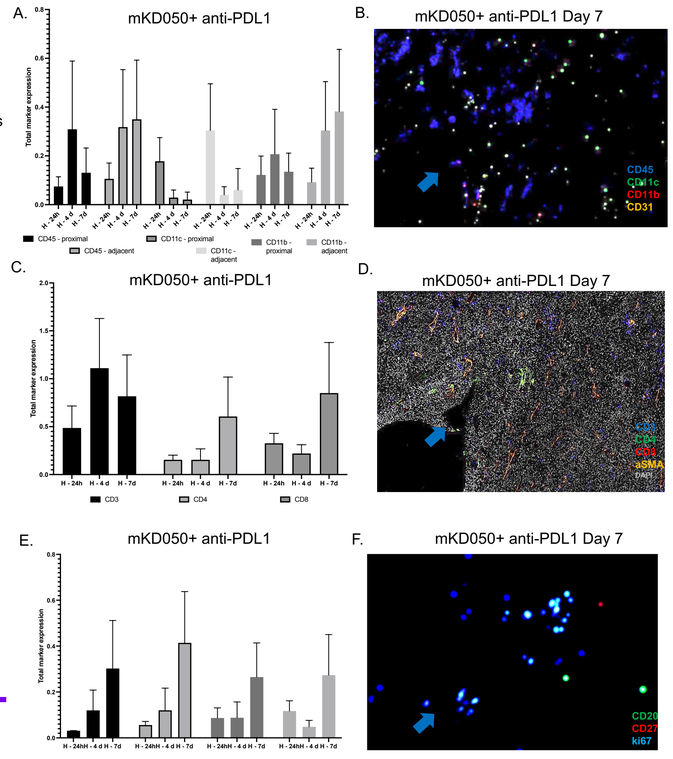
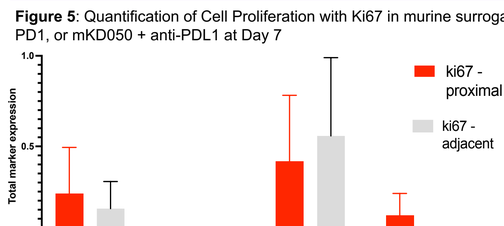


Figure 4: Quantification of Drug Release Proximal and Adjacent Zones for Compounds. Twenty-two markers were quantified in each zone for murine surrogates mKD050 + anti-PDL1 as well as monotherapies. Results shown are from combination therapy. A) Quantification of CD45, CD11 B and CD11C for proximal and adjacent zone B) Cyclic IF image of mKD50 + anti-PDL1 CD45, CD11c, CD11b, and CD31. C) Quantification of CD3, CD4 and CD8 proximal and adjacent zones. D) Cycle IF of CD3, CD4 and CD8 proximal and adjacent zones. E) Quantification of IF imaging of CD20, CD27, and ki67 of proximal and adjacent zones. F) Cyclic IF of CD20, CD27, and ki67

Figure 5: Quantification of Cell Proliferation with Ki67 in murine surrogates in mKD050, PD1, or mKD050 + anti-PDL1 at Day 7



Combination of mKD050 + anti-PDL1 shows more cell proliferation expression than either compound alone. Other timepoints also quantified but data not shown.

Figure 5: Increases in NK/B/CTL cells in Proximal and Distal Tumor Regions by GeoMx Nanostring

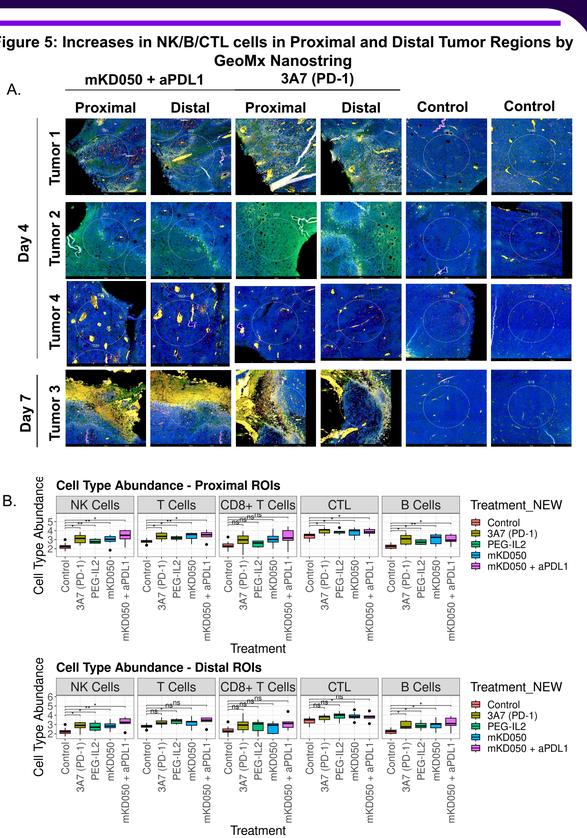


Figure 6: Whole Transcriptome of Drug Release Zones with Nanostring GeoMx DSP. Regions of interest chosen proximal and distal to IMD device. A) Fluorescent images of one slide with four tumor sections, four regions of interest (proximal IMD, distal IMD, control, control) and two time points. B) Quantification of NK cells, T cells, CD8+ T cells, Cytotoxic lymphocytes, and B cells in the proximal and distal regions of drug release zone.

CONCLUSIONS

Application of the NanoNail device to in-vivo mouse pharmacology work revealed an ability to study multiple compounds in parallel to understand drug response and differentiate the mechanism of action between those compounds in a spatiotemporal manner. We were able to directly visualize cell infiltration by Cyclic Immuno-fluorescence or GeoMx whole transcriptome either proximal and distal to implantation device.

- Murine surrogate for SAR445877 in combination with anti-PDL1 increases immune cell populations more than either single agent alone.
- Increases in NK cells, Cytotoxic Lymphocytes, and B cells were significantly increased in SAR'877 murine surrogate + anti-PDL1 compared to SAR'877 alone or anti-PD1 alone.
- T cells could infiltrate at a distance away from treatment release region, well into tumor core.
- Other significant changes in immune cell markers include increases in CD45+, CD11b, CD11c, CD20 and CD27 and decreases in FoxP3.

ACKNOWLEDGMENTS:
Thank you to Charles River Laboratories for the mouse work for these experiments.
In addition, thank you to Valeria Fantin for her support in thiswork.

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2. An Interactive Pipeline for Quantitative Histopathological Analysis of Spatially Defined Drug Effects in Tumors. Sebastian Ahn; Benjamin Ferland; Oliver Jonas. *Journal of Pathology Informatics*. 2021.