NON-REDUNDANT MECHANISMS OF RESISTANCE TO IMMUNOTHERAPY AND RADIOTHERAPY CONVERGE ON INNATE IMMUNITY

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Background Although immune checkpoint inhibitors (ICIs) have demonstrated activity in triple-negative breast cancer (TNBC), it is unclear how to best treat patients who progress on ICI or are ineligible for treatment. Given clinical evidence of synergy between ICI and radiotherapy (RT), we used single-cell RNA sequencing (scRNA-seq) to understand how ICI reprograms the immune response to RT and to identify novel pathways of immune resistance that restrain the local and systemic efficacy of combination therapy.

Methods C57BL/6 mice bearing orthotopic EO771 tumors, a syngeneic model for TNBC, were treated with ICI (anti-PD-1/CD47) or neutralizing antibodies (anti-Ly6G/Gr-1) by intraperitoneal injections. Tumors were treated with ablative RT (16 Gy x 1 fraction) using the X-RAD SmART platform with CT image-guidance. scRNA-seq and CITE-seq analyses of the immune microenvironment were performed by Seurat and BBROWSER (v3.0, Bioturing).

Results Using scRNA-seq, we found that anti-PD-1 reprogrammed the immune response to RT by shifting TAMs from a lipid-associated phenotype (APOE, FABP5) to an M1-like interferon-primed state (ISG15, CXCL10). Notably, anti-PD-1 also promoted the late-stage recruitment of intratumoral neutrophils. Given that neutrophils promote resistance to RT in other models, we eliminated neutrophils from tumors using two separate antibodies, anti-Ly6G and anti-Gr-1. Compared to ICI-RT alone, neutrophil depletion improved local tumor control and overall survival in mice with advanced tumors (P<0.001). To avoid indiscriminate neutrophil depletion, we tested alternative immune targeting approaches. Driven by our observation that TAMs upregulated several innate immune checkpoints (e.g., SIRPα) following RT on scRNA-seq and flow cytometry, we demonstrated that disruption of CD47-SIRPα by anti-CD47 enhanced the antitumor response to ICI-RT by improving tumor control and survival (P<0.001). Using scRNA-seq, we found that anti-CD47 eliminated an entire cluster of chronically-inflamed TAMs expressing several NF-κB (IL1A, SOD2) genes and chemokines (CCL3, CXCL1). Anti-CD47 was also associated with increased recruitment of central memory TCF7+ T cells and CD19+ B cells. Lastly, anti-CD47 reduced intratumoral neutrophils and specifically eliminated a cluster of neutrophils enriched for markers of PMN-MDSCs (S100a8, WFDC17). Given that these PMN-MDSCs expressed several markers of ferroptosis (TFRC, PTGS2), we showed that small molecule inhibition of ferroptosis significantly enhanced the antitumor efficacy of ICI-RT (P<0.001).

Conclusions Collectively, our data indicate that innate immune cells, in particular neutrophils and chronically-inflamed TAMs, promote resistance to ICI-RT in the EO771 model of TNBC. Inhibition of CD47-SIRPα is a novel and promising therapeutics strategy to overcoming immune resistance by eliminating PMN-MDSCs and reprogramming TAMs.

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REFERENCES

Ethics Approval All animal experiments were conducted on a protocol approved by the Cedars-Sinai Medical Center Institutional Animal Use and Care Committee (Protocol number 8905).

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