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CHARACTERIZATION OF A NOVEL COMPOUND THAT INHIBITS PEROXYNITRITE GENERATION BY MYELOID DERIVED SUPPRESSOR CELLS

^{1,2}Gabriella Lapurga*, ²Steven Sun, ²Erick Carlson, ²Himanshu Savardekar, ²Kari Kendra, ²Blake Peterson, ²William Carson. ¹The Ohio State University Comprehensive Cancer Center, Chillicothe, OH, United States; ²The Ohio State University, Chillicothe, OH, United States

Background Myeloid-derived suppressor cells (MDSC) are immature immune cells that suppress immunity and mediate resistance to immune-based cancer therapies. MDSC exert their immunosuppressive effects partly through the production of reactive nitrogen and oxygen species, which combine to form peroxynitrite (PNT). PNT reacts with the tyrosine residues of key immune cell signaling proteins and inactivates them via nitration. Targeting MDSC via PNT inhibitors is an attractive avenue to improve the response to immunotherapy. The Peterson and Carson Labs have collaborated to develop a novel inhibitor of PNT and have explored its use in murine tumor models and human patients with cancer.

Methods Splenocytes (comprised of 12% MDSC) were isolated from mice bearing tumors derived from the EMT6 breast cancer cell line and cultured with 10 μ m beads labelled with polyclonal antibodies (immunoglobulin-G or IgG). Fluorescence emitted upon MDSC recognition and reaction with IgG was detected with a previously reported fluorescent sensor compound termed PS3. Cells were mixed with PS3 and IgG beads (or controls: IgG without beads and beads without IgG) and treated for 4 hours with the following agents: (1) BRP0112233, a novel biaryl furan discovered via high-throughput screening using PNT depletion as the readout (6 or 12 μ M); (2) Ibrutinib, an FDA-approved Bruton's tyrosine kinase inhibitor shown by the Carson Lab to inhibit the activity of nitric oxide synthase in MDSC, (2, 10 μ M); and (3) PBS control. Fluorescence produced by reaction of PS3 with PNT was measured in triplicate wells using a Clariostar plate reader.

Results Splenocytes from tumor-bearing mice produced significantly greater levels of PNT than normal splenocytes (24-fold vs 8-fold increase over plain beads, $p < 0.0001$). Differences in fluorescence were confirmed via confocal microscopy. BRP0112233 inhibited PNT levels by 40% and 85% for the 6 and 12 μ M doses, respectively. Ibrutinib inhibited PNT output by 90% and 100% at 2 and 10 μ M. Cell viability was $>90\%$ except for the higher BRP dose (60% viability). In humans, peripheral blood mononuclear cells (PBMC) isolated from patients with cancer produced more PNT than healthy donor PBMC.

Conclusions PNT output could be reproducibly quantified via this assay and BRP0112233 and ibrutinib greatly inhibited MDSC PNT production. Using the EMT6 model, these compounds are being tested in combination with anti-PD-1 antibodies approved for patients with cancer. This assay has shown similar results in human peripheral blood mononuclear cells isolated from patients with cancer.

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