CXCR1 AND CXCR2 CHEMOKINE RECEPTOR AGONISTS PRODUCED BY TUMORS INDUCE NEUTROPHIL EXTRACELLULAR TRAPS THAT INTERFERE WITH IMMUNE CYTOTOXICITY

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Background Neutrophils are expanded and abundant in an important fraction (up to 35% of patients) in cancer-bearing hosts. When neutrophils are expanded, they usually promote exert immunomodulatory functions promoting tumor progression and the generation of metastases. Neutrophils can undergo a specialized form of cell death called NETosis that is characterized by the extrusion of their DNA to contain infections. In cancer NETs have been described to promote metastases in mouse models. IL-8, a CXCR1/2 ligand clinically targeted by blocking antibodies, has been described to induce NETosis and is upregulated in many cancer patients. Our hypothesis is that chemokines secreted by cancer cells can mediate NETosis in tumor associated neutrophils and that NETs can be one of the immunomodulatory mechanisms provided by tumor associated neutrophils.

Methods NETosis induction of peripheral neutrophils and granulocytic myeloid derived suppressor cells by different chemotactic stimuli, tumor cell supernatants and cocultures upon CXCR1/2 blockade. NET immunodetection in mouse models and xenograft tumors upon CXCR1/2 blockade. In vitro tumor cytotoxicity assays in the presence/absence of NETs, and videomicroscopy studies in vitro and by intravital imaging to test NETs inhibition of immune cytotoxicity by immune-cell/target-cell inhibition. Tumor growth studies and metastases models in the presence of NETosis inhibitors and in combination with checkpoint blockade in mouse cancer models.

Results Under the influence of CXCR1 and CXCR2 chemokine receptor agonists and other chemotactic factors produced by tumors, neutrophils, and granulocytic myeloid-derived suppressor cells (MDSCs) from cancer patients extrude their neutrophil extracellular traps (NETs). In our hands, CXCR1 and CXCR2 agonists proved to be the major mediators of cancer-promoted NETosis. NETs wrap and coat tumor cells and shield them from cytotoxicity, as mediated by CD8+ T cells and natural killer (NK) cells, by obstructing contact between immune cells and the surrounding target cells. Tumor cells protected from cytotoxicity by NETs underlie successful cancer metastases in mice and the immunotherapeutic synergy of protein arginine deiminase 4 (PAD4) inhibitors, which curtail NETosis with immune checkpoint inhibitors. Intravital microscopy provides evidence of neutrophil NETs interfering cytolytic cytotoxic T lymphocytes (CTLs) and NK cell contacts with tumor cells.

Conclusions CXCR1 and 2 are the main receptors mediating NETosis of tumor associated neutrophils in our in-vitro and in vivo systems expressing high levels of CXCR1 and 2 ligands. NETs limit cancer cell cytotoxicity by impeding contacts with cancer cells.