Background: Osteosarcoma is the most common bone tumor in children and adolescents. Outcomes for patients presenting with metastatic and recurrent disease are poor and have not improved over the past forty years; therefore, novel therapies are urgently needed. Immunotherapy has shown promise for some solid malignancies, however, durable responses are often rare and several challenges, including an immune-suppressive microenvironment, limit efficacy. We hypothesized that Genetically Engineered Myeloid cells (GEMys) could be used as a platform to locally deliver the anti-tumor cytokine IL12 to primary tumor and metastatic sites. We aimed to profile the tumor and pre-metastatic immune microenvironments in a syngeneic murine model of osteosarcoma and to evaluate the effect of IL12-GEMys on tumor microenvironment dynamics and survival.

Methods: We utilized a syngeneic orthotopic OS model (F40210 gifted by Dr. Yustein, Baylor College of Medicine) derived from a spontaneous tumor arising in Col2.3-Cre/p53fl/+ mice. C57BL/6 mice were inoculated with tumor cells via orthotopic intratibial injection. Immune populations in the primary tumor and pre-metastatic lung were profiled at various timepoints during tumor progression utilizing flow cytometry, immunofluorescence and RNAseq. We engineered myeloid cells with a lentiviral vector to express IL-12, a potent anti-tumor cytokine, and administered IL12-GEMys intravenously to mice inoculated with osteosarcoma tumors.

Results: Our characterization of the tumor and pre-metastatic microenvironment revealed a dense vascular and stromal matrix with steady enrichment of myeloid cells during cancer progression. Analysis of the immune microenvironment revealed an abundance of tumor-associated macrophages that develop an increasingly immune-suppressive phenotype during tumor progression. CD8+ T cells in the tumor were relatively rare and showed increased expression of activation markers PD-1 and CD44 during tumor progression. The presence of F42010 orthotopic tumors was associated with pre-metastatic niche formation in lungs of tumor-bearing mice, as evidenced by increased macrophage infiltration and upregulation of a myeloid-mediated immune suppressive gene signature. In the pre-metastatic lung, IL12-GEMy treatment increased T and NK cell activation and increased myeloid MHCII expression. IL12-GEMy administration resulted in enhanced T and NK cell infiltration into the primary tumor microenvironment. Finally, IL12-GEMy monotherapy reduced tumor growth and prolonged survival compared to vector control GEMy cell therapy.

Conclusions: These data provide insight into immune population dynamics and pre-metastatic niche formation in immune-competent murine model of osteosarcoma and show that IL12-GEMys represent a promising strategy to reverse or limit the immune-suppressive microenvironment in osteosarcoma.

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Ethics Approval: All animal studies were approved by the NCI Animal Care and Use Committee (Protocol PB-054) and were conducted in specific pathogen-free conditions at the NIH animal facility.