Background Metastatic breast cancer (mBC) patients exhibit a 5-year survival rate of only 29% and metastasis most commonly occurs in the bone.1 2 Myeloid progenitors in the bone marrow can undergo trained innate immune responses, resulting in long-lasting inflammatory myeloid memory.3 4 Myeloid memory has been shown to promote anti-tumorigenic myeloid functions in the tumor microenvironment (TME).5 6 The TME in bone includes unique myeloid cells which are dynamically regulated by bone morphogenetic proteins (BMPs).7 8 We investigated myeloid memory in the context of BMP signaling in the mBC bone TME. Identifying the driver of myeloid functions distinct to mBC bone metastases will advance the understanding of potential immunotherapies to overcome incurable mBC bone lesions.

Methods A cohort of human mBC bone metastases and matched patient primary tumors and bone metastases were assembled and profiled. Gene expression analysis was performed on patient bone metastases and matched primary tumors using nCounter immune-ontology gene expression probes. Clinical bone metastasis regional protein expression was analyzed with GeoMx Digital Spatial Profiling (DSP). Single cell protein expression and spatial analysis was measured in matched patient primary tumors and bone metastases with Akoya Polaris panels. Syngeneic mouse models of MMTV-PyMT orthotopic tumors and intratibial bone metastases were treated with beta-glucan to induce trained innate immunity and/or LDN-193189-2HCl to inhibit BMP signaling. Mouse model readouts included tumor measurements, BMP expression by histology and circulating blood analysis, and myeloid memory function by circulating blood and Akoya Polaris analysis.

Results Differential nCounter gene expression analysis of clinical mBC bone metastases revealed a subset of patient samples with a high myeloid gene signature. High myeloid gene signature patients exhibited enhanced inflammatory gene pathways and BMP signaling. Regional DSP and single cell Polaris protein expression analysis of this patient cohort showed elevated myeloid cell infiltration and heterogeneity as well as myeloid memory phenotypes in the high myeloid gene signature samples. Analysis of matched patient primary tumor and bone metastases with nCounter gene expression panels exhibited enhanced BMP signaling and myeloid cell infiltration in bone samples compared to primary tumors. Syngeneic mouse models of mBC showed beta-glucan induced myeloid memory restricted primary tumor growth. mBC mouse models of bone metastases treated with beta-glucan and LDN-193189-2HCl revealed BMP signaling is required for myeloid memory anti-tumor functions in bone metastases.

Conclusions Distinct bone metastasis patients exhibited myeloid memory and BMP signaling which could allow for precision immunotherapy treatments to prevent myeloid suppression in mBC.

Acknowledgements We would like to thank Sabrina Wright-Hobart for her collaboration and metastatic breast cancer patient advocacy. We thank Ryan Orbis, Cheryl Tan, Liang Zhang, Yan Liang, Wenjie Xu, and Doug Hinerfeld of NanoString Inc. and Jeremy Rahkola of Department of Veterans Affairs for technical assistance. We would like to thank the University of Colorado Cancer Center Tissue Biobanking and Histology Shared Resource (P30CA046934) as well as the U.S. Department of Veterans Affairs Shared Equipment Evaluation Program (S1BXX033572) for experimental support. We also thank our funding sources NIH T01TS TL1TR002533 (CLI) and VA 1KBX0002929 (PO).