Background Bilirubin, a metabolite of the heme degrading enzyme heme oxygenase-1 (HO-1/HMOX1), is commonly believed to be a waste product. Studies in other diseases revealed that bilirubin can alter normal immune cell function including macrophage antigen presentation and phagocytosis. However, the effects of bilirubin on the tumor microenvironment (TME) remain unknown. We hypothesized that tumor cell-HO-1 activity and subsequent bilirubin secretion enhance triple-negative breast cancer (TNBC) metastasis by supporting immune suppressive, pro-tumor macrophage function.

Methods We tested the impact of tumor cell-HO-1 and bilirubin on macrophage immune suppression and efferocytic capacity (engulfment of dead tumor cells) using qRT-PCR, flow cytometry and live cell imaging. Human and mouse macrophages were analyzed after treatment with exogenous bilirubin or bilirubin-depleted conditioned medium collected from tumor cells treated with tin mesoporphyrin (SnMP), an FDA approved HO-1 enzymatic inhibitor. Primary tumor growth, lung metastatic burden and macrophages were observed in immunocompetent mice harboring 66Cl-4 mammary tumors without and with HO-1 genetic depletion (shHO1). Human TNBC specimens were analyzed via CIBERSORT to predict immune cell abundance in patients with high versus low levels of HMOX1.

Results Macrophages cultured with conditioned medium from tumor cells treated with the HO-1 inhibitor SnMP demonstrated a 35-65% decrease in immune suppressive genes (Arg1, Cd274, Tgfb1) and a 25% increase in the efferocytosis gene Mertk compared to those treated with control conditioned medium. This effect was rescued by exogenous treatment with 2.5 µM bilirubin. Direct bilirubin treatment enhanced macrophage PD-L1 mRNA and protein expression by over 6-fold. In contrast, bilirubin decreased expression of Mertk by at least 50% and nearly ablated macrophage efferocytic capacity. To test whether bilirubin supports tumor progression via modulation of macrophages, we evaluated tumor growth and metastasis after tumor cell-HO-1 genetic depletion. While mice with shHO1 tumors had enhanced primary tumor growth compared to shCnt tumors, HO-1 depletion decreased lung metastatic capacity. Flow cytometry revealed that macrophages from shHO1 primary tumors had decreased expression of suppressive markers including PD-L1 and CD206 than control tumors. In human TNBC specimens, CIBERSORT analysis revealed that tumors with high levels of HMOX1 have a significant increase in the abundance of suppressive M2-like macrophages compared to those with low HMOX1.

Conclusions Tumor cell-HO-1 may support immune cell suppression and dysfunction during TNBC metastasis via bilirubin. Since HO-1 inhibitors including SnMP are FDA approved for treatment of other diseases, these findings could rapidly be translated to provide an alternative or companion immunotherapy for metastatic TNBC.