**Background**
Advanced biliary tract cancers (BTC) have a poor prognosis and low rates of response to immune checkpoint inhibition (CPI), with overall response rates ranging from 3-13%.1-3 The BTC tumor microenvironment contains immunosuppressive immune populations, desmoplastic stroma, and a paucity of tumor infiltrating effector T cells4,5, but the role of circulating immune cells in response and resistance to CPI is less well-characterized. We aimed to explore circulating immune cells from BTC patients treated with CPI to understand immunotherapy response and resistance.

**Methods**
We used multiplexed multi-omic single cell RNA sequencing and cell surface proteomics (CITEseq) to profile circulating immune cells from BTC patients treated with CPI to identify pharmacodynamic markers of immune response and targets for combination immunotherapy to overcome CPI resistance in BTC.

**Results**
Using CITEseq, we identified several unique CD14+ monocyte sub-populations in BTC patients’ circulation compared to healthy donors. In the circulation of patients with BTC tumors that are CPI-resistant, there was an increased frequency of “CD14CTX”, a monocyte sub-population expressing high levels of immunosuppressive cytokines and chemotactic molecules, following anti-PD-1 monotherapy. CD14CTX can directly suppress CD4+ T cells and induce SOCS3 expression in CD4+ T cells, rendering them functionally unresponsive. Analogous tumor-associated macrophage populations could also be found within tumor tissues. Consistent with the pleiotropic effects of GM-CSF, we saw changes in the frequency and genomic programs of multiple myeloid populations, including dendritic cells and monocytes, when GM-CSF was combined with anti-PD-1. Within one week of GM-CSF combination therapy, monocyte-derived dendritic cells were induced in the circulation of BTC patients and associated with pro-inflammatory signals, including upregulation of MHC I and II in monocytes and dendritic cells.

**Conclusions**
These results demonstrate the capacity of CPI plus GM-CSF combination therapy to induce monocyte states that both foster and inhibit adaptive immunity. Monocytes arising after anti-PD-1 treatment induced T cell paralysis in a subset of patients with CPI-resistant tumors, as a distinct mode of tumor-mediated immunosuppression. GM-CSF demonstrated the potential to enhance both pro-inflammatory and antigen-processing and presentation-related programs within myeloid cells. These results highlight the importance of identifying pharmacodynamic markers of immune response and targets for combination immunotherapy to overcome CPI resistance in BTC.

**Trial Registration**
NCT02703714

**References**