Background. Costimulatory molecules, such as CD137 (4-1BB), have emerged as promising targets for cancer immunotherapy. Despite promising results in animal tumor models, clinical trials with CD137 agonists have had limited success due to both dependency on FcgR-mediated clustering and dose limiting hepatotoxicity. Merus has developed an Fc-silenced Biclonics® CD137×PD-L1 antibody, MCLA-145, whose stimulatory activity is correlated with PD-L1-mediated CD137 clustering which is designed to be preferentially confined to the PD-L1 expressing tumor microenvironment. The aim of this study was to characterize the mechanism of action of MCLA-145 in resected hepatocellular carcinoma (HCC), a heterogenous solid tumor with limited therapeutic options. We studied target expression and ex vivo responses in human HCC tumor-infiltrating lymphocytes (TIL).

Methods. Lymphocytes and myeloid cells isolated from resected HCC (n=10) tumors (TIL), paired tissues adjacent to tumor (TFL) and peripheral blood were characterized for CD137, PD-1 and PD-L1 expression by flow cytometry. Additionally, TIL proliferation upon either anti-CD3/CD28 stimulation or autologous B cell blasts electroporated with tumor-associated antigens (TAA) glypican-3 (GPC3) and/or melanoma-associated antigen (MAGE)-C2 stimulation was assessed ex vivo in presence of MCLA-145, its bivalent monospecific parental mAbs, the bivalent mAbs urelumab (anti-CD137) or atezolizumab (anti-PD-L1) or isotype control.

Results. Compared to lymphocytes derived from adjacent tissues and blood, CD137 and PD-1 expression was found to be highest in TIL, and specifically on activated regulatory T cells. CD137 was also found to be expressed on myeloid cells such as tissue resident Kupffer cells and granulocytes. PD-L1 was mainly found to be expressed on CD3+ cells. Treatment with MCLA-145 led to increased proliferation, as measured by Ki67 staining, of HCC-derived CD8+ TILs, compared to isotype control treatment. Expression of CD137 was significantly increased on these proliferating CD8 T cells upon the treatment with MCLA-145. Additionally, MCLA-145 enhanced CD8+ TIL proliferation more potently compared to its bivalent monospecific parent mAbs or Urelumab.

Conclusions. Biclonics® antibody MCLA-145 has the capacity to enhance activation and proliferation of tumor-derived CD8+ T cells in the PD-L1 expressing tumor microenvironment of HCC ex vivo, warranting further evaluation in HCC.

Ethics Approval. Ethical approval (METC) for this study has been obtained.