CHARACTERIZATION AND VALIDATION OF A HUMANIZED LEAD ANTIBODY AGAINST OX003, A NOVEL IMMUNO-ONCOLOGY TARGET

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Background Immunotherapy, an approach to target or manipulate the immune system, has changed the field of oncology and cancer treatment. Antibodies targeting checkpoint proteins CTLA-4, PD-1, and PD-L1 have shown great success, at the same time some of these checkpoint inhibitors are known to eventually induce immunotherapy resistance in several cancers leading to poor treatment prognosis. At Oxford BioTherapeutics, using the proprietary OGAP database, novel targets on TILs (Tumor infiltrating Lymphocytes) have been identified that can lead to a breakthrough in the field of cancer immunotherapy. From proteomic analysis, OX003 is identified as a surface protein on T cells with a co-stimulatory function. OX003 is expressed on most lymphocytes with TILs showing the highest expression.

Methods Immunohistochemistry technique is used to evaluate the expression of OX003 in TILs and wide variety of solid tumors. In vitro evaluation of OX003 expression on T cells is performed using flow cytometry technique. A fully humanized agonistic mAb is developed against OX003 and its functional activity on T cells is evaluated in vitro. T cell proliferation measured by CFSE dye dilution, IFN-γ (interferon gamma) cytokine and Granzyme B secretion via ELISA, Perforin accumulation via flow cytometry, ELISpot assay, and 1-way Mixed lymphocyte reaction (MLR) assays are used for T cell functional activity.

Results Evaluation of OX003 by immunohistochemistry shows abundant expression on TILs, and in a variety of solid tumors. OX003 positive TILs are observed both intratumorally and in the stromal components of the TME (Tumor micro environment). In vitro evaluation of OX003 expression on T cells shows upregulation upon activation of naïve T cells which persists between activated and exhausted phenotypes and decreases upon resting, closely following the time course of activation and exhaustion markers. This humanized antibody against OX003 shows a strong agonistic effect in a dose-dependent manner on CD8+ T cells promoting proliferation, IFN-γ cytokine secretion, Granzyme B release and Perforin accumulation indicating a strong cytolytic ability. In an allogenic one-way MLR setup, it enhances functional activity of T cells in co-culture with PBMC as stimulators, showing dose-dependent increase in IFN-γ release compared to an isotype control. Ex vivo assays conducted with the antibody on fresh tumor samples showed increased IFN-γ release as compared to Isotype as measured by the ELISpot assay.

Conclusions This data show that OX003 is a novel immunostimulatory receptor on CD8+ T cells and the lead agonistic antibody targeting OX003 enhances T cell functional activity critical for successful immunotherapy response.

REFERENCES