P854 HEXABODY-CD38 INDUCES TROGOCYTOSIS AND EFFECTIVELY INDUCES COMPLEMENT-MEDIATED TUMOR CELL LYSIS AFTER TREATMENT WITH DARATUMUMAB OR ISATUXIMAB IN VITRO

**Topic:** 13. Myeloma and other monoclonal gammopathies - Biology & Translational Research

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**Background:**

HexaBody-CD38 (GEN3014) is a next-generation CD38-specific IgG1 antibody with a hexamerization-enhancing mutation. HexaBody-CD38 is designed to induce strong anti-tumor activity in patients with CD38-expressing hematological malignancies through potent complement-dependent cytotoxicity (CDC) and other Fc-mediated effector functions. The safety and preliminary efficacy of HexaBody-CD38 are currently being evaluated in a first-in-human trial in relapsed/refractory multiple myeloma (MM) patients (NCT04824794). Trogocytosis has been suggested as effector mechanism of daratumumab: reduction of CD38 expression on tumor and immune cells is thought to reduce local immunosuppression and contribute to improved adaptive immune responses against MM cells (Krejcik, 2018, Oncotarget 9, 33621).

**Aims:** The present study aimed to increase our understanding of the MoA of HexaBody-CD38, by studying its capacity to induce trogocytosis as well as its capacity to induce CDC in CD38+ tumor cells in the presence of daratumumab or isatuximab in vitro.

**Methods:**

Trogocytosis was evaluated as the amount of membrane transfer in absence of transfer of cytoplasm in a flow cytometry-based assay using Wien-133 cells as target cells and healthy donor monocytes as effector cells. Binding of HexaBody-CD38 to CD38+ cell lines SU-DHL-4, NCI-H929, and Wien-133 that were pre-treated with daratumumab or isatuximab was allowed for 15 min, 1 h, 4 h or 24 h, while daratumumab or isatuximab remained present at saturating concentrations. HexaBody-CD38 mediated CDC of daratumumab or isatuximab-opsonized Wien-133 cells, which were insensitive to CDC induction by daratumumab or isatuximab, was assessed by flow cytometry at 45 min, 4 h, or 24 h after adding human complement in the presence of daratumumab or isatuximab.

**Results:**

HexaBody-CD38 induced dose-dependent transfer of plasma membrane from CD38+ tumor cells to human monocytes (n=7). The mean EC50 for trogocytosis activity of HexaBody-CD38 was 8.79 ± 2.49 ng/mL (0.018 ± 0.014 nM). This was in the same range as EC50s of daratumumab and the HexaBody-CD38 parental antibody without E430G mutation, suggesting that the hexamerization mutation has limited impact on trogocytosis induction.

HexaBody-CD38 was found to compete with daratumumab and isatuximab for binding to CD38. Binding of HexaBody-CD38 to CD38+ cells increased in time and with increasing concentration, generally faster and more extensive in the presence of isatuximab compared to daratumumab. At equimolar concentrations, near-complete displacement of isatuximab and daratumumab was observed after incubations ≥1 h and ≥4 h, respectively. Accordingly, after 24 h HexaBody-CD38 induced comparable maximum CDC of CD38+ cells in presence or absence of daratumumab. Comparable CDC activity in the presence or absence of isatuximab was already observed after 4 h.
HexaBody-CD38 was shown to induce efficient monocyte-mediated trogocytosis of CD38+ tumor cells *in vitro*, suggesting HexaBody-CD38 may reduce CD38-associated immunosuppression in the tumor microenvironment. In addition, it has previously been reported that the main differentiating effector mechanism activity of HexaBody-CD38 compared to daratumumab and isatuximab is its increased capacity to induce CDC. Here we confirmed the CDC potency of HexaBody-CD38 even in the presence of saturating concentrations of daratumumab and isatuximab. This suggests that HexaBody-CD38 is capable of inducing CDC of CD38+ myeloma cells in patients who have received prior anti-CD38 mAb treatment with residual daratumumab or isatuximab present in their circulation.