DUOBODY®-CD3x5T4 INDUCES EFFICIENT T-CELL ACTIVATION AND KILLING OF PATIENT-DERIVED HEAD AND NECK CANCER CELLS IN VITRO AND EX VIVO

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Background 5T4, also known as trophoblast glycoprotein, is expressed in many solid cancers, including non-small cell lung cancer, triple-negative breast cancer, bladder, esophageal, prostate, uterine and head and neck squamous cell carcinomas (HNSCCs). DuoBody-CD3x5T4 is a CD3 bispecific antibody that efficiently induces T-cell mediated cytotoxicity of 5T4-positive tumor cells. Currently, DuoBody-CD3x5T4 is being evaluated in a first-in-human clinical trial (NCT04424641) in solid cancers in partnership between Genmab and AbbVie. In this study we explored the preclinical mechanism-of-action of DuoBody-CD3x5T4 in vitro and ex vivo, using HNSCC as a case study.

Methods 5T4 protein expression in HNSCC tumor specimens was determined by immunohistochemistry (IHC) and flow cytometry. T-cell mediated cytotoxicity and T-cell activation induced by DuoBody-CD3x5T4 were studied in co-cultures of healthy donor T cells and patient-derived HNSCC cell lines in vitro. Lastly, the capacity of DuoBody-CD3x5T4 to activate tumor-infiltrating lymphocytes (TILs) was analyzed in freshly dissociated 5T4-expressing HNSCC tumor specimens ex vivo.

Results IHC analysis confirmed expression of 5T4 in HNSCC oral biopsies, including specimens from primary tumors, recurrent tumors and lymph node metastases. Patient-derived HNSCC cell lines (n=22) demonstrated 5T4 expression on the plasma membrane, ranging from 10,000 - 61,000 5T4 molecules per cell. Moreover, 5T4 expression was evident on EGFR+CD45- tumor cells in single-cell suspensions from freshly dissociated HNSCC biopsies, independent of the tumor site. DuoBody-CD3x5T4 demonstrated potent, target-dependent cytotoxicity in vitro in co-cultures of healthy donor T cells and patient-derived HNSCC cell lines across the range of 5T4 expression levels tested. Tumor cell kill was associated with CD4+ and CD8+ T-cell activation and granzyme B secretion. Importantly, DuoBody-CD3x5T4 induced potent activation of autologous TILs in single-cell suspensions from freshly dissociated HNSCC biopsies. Notably, T-cell activation (as assessed by expression of CD69, CD25 and CD137) was also observed in PD-1+ TILs, suggesting that DuoBody-CD3x5T4 was able to engage antigen-experienced T cells in the tumor microenvironment. In this autologous assay, preliminary data showed that 5T4-expressing HNSCC tumor cells were specifically eradicated.

Conclusions 5T4 was broadly expressed in HNSCC cell lines, tumor biopsies and primary tumor cell suspensions. DuoBody-CD3x5T4 activated healthy donor T cells in co-cultures with patient-derived HNSCC cell lines, resulting in secretion of granzyme B and efficient tumor cell kill. In single-cell suspensions from freshly dissociated 5T4+ HNSCC biopsies, DuoBody-CD3x5T4 activated autologous CD4+ and CD8+ TILs, including PD-1+ TILs. This dataset adds to the preclinical evidence for targeting 5T4-expressing solid cancers with DuoBody-CD3x5T4.

Ethics Approval Written informed consent was obtained from all patients from whom fresh tumor biopsies were used for research, as part of the HNcol protocol at the Department of Otolaryngology|Head and Neck Surgery of Amsterdam UMC (VUmc) as approved by the Institutional Review Board (2008.071|A2016.035). Archival FFPE specimens were used for scientific research in agreement with the medical ethical guidelines described in the Code of Conduct for Proper Secondary Use of Human Tissue of the Dutch Federation of Biomedical Scientific Societies (Federa) in accordance with the Declaration of Helsinki and after Biobank approval (BUP2019-74).

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