Combination Immunotherapies

DECOSTAR™, A NOVEL PLATFORM FOR TNF RECEPTOR SUPERFAMILY AGONISM

Dulce Alvarado*, Wojciech Bartkowski, Rakesh Verma, Mickey Pentecost. *Diadem Biotherapeutics, Torrance, CA, USA*

Background Immune checkpoint inhibitors (ICIs) such as Pembrolizumab and Atezolizumab have been approved as first-line treatment for some advanced solid tumors.[1][2] However, ICIs have failed to demonstrate an overall survival benefit among patients with advanced solid tumors that are traditionally ineligible for clinical trials.[3] For example, there are no effective immunotherapies for patients with advanced metastatic colorectal cancer, which ranks second as the most lethal cancer and the third most prevalent malignant tumor in the world.[4] This emphasizes an unmet need for immunotherapies beyond immune checkpoint blockade. Diadem Biotherapeutics has developed Decorated Extracellular Vesicles for CO-Stimulatory Activation or Repression – DECOSTAR™, a novel platform that enables cell-free activation of members of the tumor necrosis factor receptor superfamily (TNFRSF).

Methods We expressed extracellular vesicle (EV)-targeted chimeric proteins containing 4-1BBL, GITRL or OX40L in 293H cells. We established robust and scalable protocols for downstream purification of modified EVs. Nanoparticle tracking analysis (NTA, Nanosight) was used to quantify EV size and concentration. Antibody-conjugated bead capture was used for semiquantitative analysis of EV surface markers by flow cytometry (Miltenyi MACSPlex Exosome Kit). Ligand expression was measured by ELISA. Single particle interferometry and immunofluorescence (ExoView, Unchained Labs) was used to demonstrate ligand expression on individual EVs. Agonist signaling of EVs was assessed using Jurkat signaling bioassays (Promega). Stimulation of antigen specific $CD8^+$ T cell proliferation was determined using a CMV recall antigen assay. Real-time cell analysis (xCELLigence) was used to measure cytotoxic activity of purified T cells against target human cancer cell lines MCF-7, Hs578T, SKOV3, and OVCAR3. We evaluated the efficacy of intratumorally administered DB202 and Urelumab against subcutaneous MC38 tumors in h4-1BB transgenic mice.

Results We established 160-fold greater EV expression of chimeric ligands compared to overexpression of the native ligands, resulting in at least 20 ligand trimers per EV. Using the DECOSTAR™ platform, we demonstrate specific agonism of 4-1BB, GITR or OX40 that surpasses clinical stage agonist antibodies both in terms of ligand EC_{50} and E_{max}. To further validate the platform, we present compelling evidence that 4-1BB agonist DB202 enhances the proliferation and cell cytotoxicity of $CD8^+$ T cells *ex vivo*. When administered IT, DB202 more effectively inhibits the growth of subcutaneous (SQ) MC38 tumors in h4-1BB transgenic mice compared to Urelumab, without evidence of enhanced liver toxicity.

Conclusions In summary, DECOSTAR™ can facilitate higher-order receptor clustering, potentially expanding the therapeutic window for agonist immunotherapy when compared to monoclonal antibodies.

REFERENCES

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