VB6-485d Tumor Cell Killing Elicits Immune Features of Tumor Immunogenic Cell Death

ABSTRACT

VB6-485d is a Targeted Protein Therapeutic (TPT) that comprises a Fab fragment specific for the Epithelial Cell Adhesion Molecule (EpCAM) genetically fused to deBougainville via a twin processive linker. DeBougainville (deB) is a de-immunized variant of the anti-Bouganville (Bouganville) anti-HER2 monoclonal antibody that would induce HER2 internalizing protein (IP) that, when internalized, blocked Her2 signaling thereby leading to cell death. While all antibodies: mechanochemical linkers, pathogen-derived poisons, and/or immunomodulatory cargos are attractive strategies for inducing immunogenic cell death (ICD) ICD is characterized by the collective appearance of distinct cellular immune targets forming DAMPs ( Danger-Associated Molecular Patterns, or DAMPs), which play a role in immune cell evolution by triggering immunostimulatory responses. Here we demonstrated that the anti-HER2 antibody VB6-485d (Taxol) and its conjugate VB6-485d (Duo) conjugated to the immunoconjugate compound DM1 or MMAE (MethylMannosyl Lysine-epothilone) as well as the 2-in-1 conjugate (Duo), all of which could be used for intracellular delivery of an immunostimulatory cargo (DM1 or MMAE). Additionally, VB6-485d was used to mediate target cell killing by an ICD pathway. The potential cross-priming effect induced by VB6-485d-induced ICD, suggests the use of VB6-485d in combination with immune-checkpoint inhibitors may enhance their effectiveness in EpiCam-positive epithelial cancers.

BACKGROUND

DeBougainville (deB) is a de-immunized variant of the bougainville, a type 1 fibroblast-targeting protein (DB) isolated from deimmunized bougainville epithelial cell line (bougainville). DeBougainville comprises 485 amino acids, including 19 conserved cysteine residues that can be used to engineer cysteine-to-serine (C->S) mutants that are more stable. The corresponding intracellular protein engineering (IPE) domains (used for protein synthesis inside cells) was shown to be secreted into cell culture media. In vivo studies have previously demonstrated that deBougainville is internalized by metastatic breast cancer cell lines. It is a surfactant that elicits cytokine production, which modulates the immune system in cancer treatment. The deBougainville antibody can thus be used to treat cancer and improve therapeutic outcomes.

METHODS

DeBougainville (deB) is a de-immunized variant of the deBougainville (B) monoclonal antibody (mAb) isolated from the deimmunized deBougainville cell line (deB). The antibodies target the HER2 receptor, which is overexpressed in breast cancer cells. deBougainville (deB) was conjugated to the immunoconjugate compound DM1 or MMAE (MethylMannosyl Lysine-epothilone) as well as the 2-in-1 conjugate (Duo). The conjugates were used for intracellular delivery of an immunostimulatory cargo (DM1 or MMAE) to induce immunogenic cell death. The conjugates were intravenously administered to tumor-bearing mice, and the immune responses in the tumor microenvironment were evaluated. The results showed that the conjugates induced an immunogenic cell death phenotype, which was associated with the expression of immune-stimulatory molecules such as CD47 and PD-L1. The conjugates also induced a CD47 and PD-L1 positive cells response, which was associated with the induction of an immunogenic cell death phenotype.

RESULTS

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CONCLUSIONS

Cell surface translocation of calicheamicin and extracellular release of HMGB1 and ATP suggested that target debougainville killing induces immunogenic cell death. Targeted debougainville conjugation may facilitate and augment checkpoint inhibitor anti-tumor activity. Data support the concept that debougainville mediated killing of tumor cells could facilitate and augment checkpoint inhibitor anti-tumor activity.

REFERENCES