to C1861 PDL1+ melanoma cells. Peptide-DM1 potency was determined in-vitro using a calcine-AM and propidium iodine live/dead cell double staining.

**Results** Antibody-Binding Peptide Linker (APL) was developed from a series of space filling amino acid substitutions at key residues on an 18-mer peptide derived from a hydrophobic pocket on human albumin (figure 1a). A lysine containing tail was added to the C-terminus for conjugation to small molecule therapeutics through amine coupling. APL has nanomolar binding affinity for the fab region of IgG1 antibodies (KD= 1.85 × 10⁻⁸), bevacizumab (KD= 5.2 × 10⁻⁸), trastuzumab (KD= 8.87 × 10⁻⁸), and atezolizumab (KD= 3.78 × 10⁻⁸) (figure 1b). Kinetic binding models, performed by Biacore surface plasmon resonance, showed a 2:1 association of peptide to antibody. All four antibodies retained their antigen affinity when bound by APL (figure 2a). Labeling of APL with an alexafluor showed delivery to PDL1+ melanoma cells when given bound to the anti-PDL1 antibody atezolizumab (figure 2b). Conjugation of APL with the tubulin inhibitor DM1 (figure 2c) resulted in a drug conjugated peptide that retained the potency of the drug itself (figure 2d).

**Conclusions** Antibody-Binding Peptide Linker (APL) non-covalently binds clinical IgG1 antibodies at a fixed two to one ratio without affecting antigen affinity. Conjugation of APL with a drug of choice provides a modular Antibody-Drug Conjugate platform where both the antibody and drug can be substituted with ease.

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**REFERENCES**

618 VEDOTIN ADCS INDUCE ER STRESS AND ELICIT HALLMARKS OF ICD ACROSS MULTIPLE CANCER INDICATIONS

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Background Effective cancer treatment requires durable elimination of malignant cells. Cytotoxic chemotherapeutic agents used to treat cancer often show initial anti-tumor efficacy, but fail to produce long-term durable responses in patients. The elicitation of durable responses and improved survival in response to cytotoxic agents may be associated with the induction of innate and adaptive immune response to the cancer. For example, tumor cells undergoing apoptosis following exposure to some cytotoxic agents emit immunostimulatory damage-associated molecular patterns (DAMPs), this form of cell death is termed immunogenic cell death (ICD). ICD can promote the recruitment and activation of both the innate and adaptive immune systems, providing an additional mechanism to drive an anti-tumor response.

Methods Vedotin-based antibody drug conjugates (ADCs) drive cytotoxicity in tumor cells by engaging tumor antigens on the cell surface, internalizing with the cell surface antigen, and delivering monomethyl auristatin E (MMAE) payload. Following intracellular delivery, MMAE induces mitotic arrest, as well as an endoplasmic reticulum (ER) stress response resulting from microtubule disruption. Following tumor cell treatment, indicators of the ER stress response are observed with vedotin-based ADCs including induction of phospho-JNK and CHOP. This mechanism of MMAE induced ER stress results in emission of hallmark ICD DAMPs including cell-surface calreticulin, extracellular release of HMGBl and ATP. In this presentation we highlight the ability of MMAE to induce the hallmarks of ICD in multiple cancers across different tissue origins using distinct valine-citrulline-MMAE (vedotin)-based ADCs.

Results The culmination of these ICD hallmarks resulted in innate immune cell activation in vitro and in vivo in mouse xenograft models. Tumor bearing mice treated with vedotin-based ADCs resulted in the promotion of immune cell recruitment and activation in tumors. Analysis of immune activation by vedotin-based ADCs included production of innate cytokines and upregulation of HLA/MHC-Class II expression, which supports a role in activating both the innate and adaptive immune response. To further our understanding of the potent and broad ability of vedotin ADCs to induce ICD, we have also begun to examine the ICD potential of different classes of ADC payloads including other microtubule inhibitors (auristatins and maytansines), and DNA damaging agents (DNA alkylators or topoisomerase inhibitors). Initial data indicate differences in ICD induction by these agents.

Conclusions These results help build the rationale for vedotin-based ADCs as preferred partners for immune checkpoint blockade agents.

619 EVALUATING THE EFFECTIVENESS OF TARGETED ADC THERAPY IN A PATIENT-DERIVED EX VIVO TUMOROID MODEL, 3D-EX, FOR QUANTITATIVE TUMOR CELL KILLING

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Background Antibody drug conjugates (ADCs) are an effective tool for site directed delivery of cytotoxic agents to cancer cells. Tailoring of ADC-specificity to the uniqueness of a patient's tumor can aid in direct-targeting of tumor cells and potentially improve drug responsiveness. Here we evaluate the potential of using an ADC therapy for targeted tumor cell death and immune cell activation in combination with checkpoint inhibitors in 3D tumoroids.

Methods All human tumor samples were obtained with proper patient consent and IRB approval. Fresh patient tumor tissue of various histologic types including CRC and NSCLC were processed to generate uniform sized live 3D tumoroids measuring 150 μm in size. Treatment groups included a conjugated ADC therapeutic antibody alone or in combination with PD-1/PD-L1 inhibitors. Culture supernatants were collected for multiplex analysis of cytokine release in media. Additionally, flow cytometry was used to assess the activation profile of resident immune cells in combination with high-content confocal imaging to determine extent of tumor cell death in the intact tumor extracellular matrix.

Results Using fresh patient-derived tumoroids, we observed ADC-mediated cell death and activation of immune cells within the tumor microenvironment. Production of pro-inflammatory cytokines correlated with increased activation of tumor infiltrating immune cell populations. The improved immune response led to increased tumor cell killing within the 3D tumor microenvironment observed by high-content confocal imaging.

Conclusions In this study we demonstrate that our physiologically relevant 3D tumoroid model is an effective system to assess novel antibody drug conjugates and to develop rational drug combinations with other immuno-ontology agents. Furthermore, implementation of 3D-EX platform, in the clinical setting, may also allow for determination of the most effective combinatorial immuno-ontology treatment strategies for individualized patient care.

Ethics Approval The study was approved by Chesapeake IRB Pro0014313.