

CD3 and HER2. Attached to the core, two unstructured polypeptide masks (XTEN) sterically reduce target engagement and extend T1/2. Protease cleavage sites at the base of the XTEN masks enable proteolytic activation of XPATs in the tumor microenvironment, unleashing a potent TCE with short T1/2, further improving the TI. HER2-XPAT, a tumor protease-activatable prodrug with wide safety margins, can co-opt T-cells regardless of antigenic specificity to induce T-cell killing of HER2+ tumors.

**Methods** Preclinical studies were conducted to characterize the activity of HER2-XPAT, HER2-PAT (cleaved XPAT), and HER2-NonClv (a non-cleavable XPAT) for cytotoxicity in vitro, for anti-tumor efficacy in xenograft models, and for safety in NHPs.

**Results** HER2-PAT demonstrated potent in vitro T-cell cytotoxicity (EC50 1-2pM) and target-dependent T-cell activation and cytokine production by hPBMCs. HER2-XPAT provided up to 14,000-fold protection against killing of HER2 tumor cells and no cytotoxicity against cardiomyocytes up to 1uM. In vivo, HER2-XPAT induced complete tumor regressions in BT-474 tumors with equimolar dosing to HER2-PAT, whereas HER2-NonClv had no efficacy, supporting requirement of protease cleavage for T-cell activity. In NHP, HER2-XPAT has been dose-escalated safely up to 42mg/kg (MTD). HER2-XPAT demonstrated early T-cell margination at 2 mg/kg but largely spared CRS, cytokine production, and tissue toxicity up to 42 mg/kg. PK profiles of HER2-XPAT and HER2-NonClv were comparable, consistent with ex vivo stability for cleavage when incubated in cancer pts plasma for 7 days at 37°C. HER2-PAT by continuous infusion induced lethal CRS and cytokine spikes at 0.3 mg/kg/d but was tolerated at 0.25 mg/kg/d, providing HER2-XPAT with >1300-fold protection in tolerability vs. HER2-PAT, >4 logs over cytotoxicity EC50s for HER2 cell lines, and a 20-fold safety margin over the dose required for pharmacodynamic activity.

**Conclusions** HER2-XPAT is a potent prodrug TCE with no CRS and a wide TI based on NHPs. With XTEN's clinical data demonstrating low immunogenicity, the XPATs are a promising solution. IND studies are ongoing. Additional PK/PD, cytokines, safety, and efficacy data will be presented.

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614

#### CO-STIMULATION VIA PD1-41BB CHIMERIC SWITCH RECEPTOR ENHANCES FUNCTION OF TCR-T CELLS IN AN IMMUNE-SUPPRESSIVE MILIEU AND UNDER CHRONIC ANTIGEN STIMULATION

<sup>1</sup>Melanie Salvermoser, <sup>1</sup>Maria Gerget, <sup>1</sup>Franziska Hasselmann, <sup>2</sup>Elfriede Noessner, <sup>1</sup>Christian Ellinger, <sup>1</sup>Monika Braun, <sup>3</sup>Dolores Schendel, <sup>1</sup>Daniel Sommermeyer, <sup>1</sup>Nadja Sailer\*. <sup>1</sup>Medigene Immunotherapies GmbH, Planegg-Martinsried, Germany; <sup>2</sup>Helmholtz Zentrum München, Munich, Germany; <sup>3</sup>Medigene AG, Planegg-Martinsried, Germany

**Background** The immunosuppressive tumor microenvironment (TME) of solid tumors negatively influences the efficacy and fitness of tumor-specific T cells and can render them non-functional. In this repressive tumor milieu, expression of inhibitory immune checkpoint molecules and cytokines as well as deprivation of essential metabolic factors contribute to T cell exhaustion and reduced T cell infiltration. Due to these harsh conditions found in the TME of solid tumors, successful treatment of non-hematological cancer indications with T cell-based immunotherapies remains challenging. New strategies

are required to equip therapeutic tumor-specific T cells with the necessary traits to overcome inhibitory signals in the TME and increase T cell persistence in an environment lacking essential metabolic nutrients, like oxygen or glucose. To enhance the clinical efficacy of TCR-T cells in treatment of solid tumors, we generated a chimeric receptor that combines the co-stimulatory domain of 4-1BB with the extracellular domain of PD-1. Expression of this chimeric PD1-41BB switch receptor in TCR-T cells should reverse the inhibitory signal induced by the PD-1/PD-L1 interaction and provide additional co-stimulation to increase functionality and persistence.

**Methods** Using 2D and 3D in vitro model systems we mimic immunosuppressive conditions in the TME of solid tumors, including low glucose and high TGFbeta levels as well as repeated tumor cell challenge. We evaluate the ability of the chimeric PD1-41BB switch receptor to enhance TCR-T cell activity and functionality under these repressive conditions.

**Results** Our results demonstrate that TCR-T cells expressing the chimeric PD1-41BB switch receptor show an increased capacity to recognize and kill tumor cells during chronic stimulation with antigen. The enhanced functionality of PD1-41BB-TCR-T cells allows them to eradicate tumor cells even in the presence of additional immunosuppressive factors, including nutrient starvation and expression of inhibitory PD-L1 checkpoint molecules. Furthermore, PD1-41BB-expressing TCR-T cells show a higher persistency and proliferation rate in these challenging co-culture model systems.

**Conclusions** Equipping therapeutic T cells with the chimeric PD1-41BB switch receptor enhances T cell functionality under immunosuppressive conditions and counteracts checkpoint-mediated dysfunction. For the treatment of PD-L1-positive malignancies, expression of PD1-41BB by TCR-T cells has the potential to greatly improve the targeting of solid tumors using T cell-based immunotherapies. These preclinical studies support our approach to enhance the clinical efficacy of TCR-T therapies of solid tumors using the chimeric PD1-41BB switch receptor. Subsequent in vivo studies and safety evaluations will pave the way for clinical use of PD1-41BB in adoptive T cell therapy.

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615

#### TARGETED IMMUNE CELL ACTIVATION BY SYSTEMIC DELIVERY OF TOLL-LIKE RECEPTOR 9 AGONIST ANTIBODY CONJUGATES INDUCE POTENT ANTI-TUMOR IMMUNITY

<sup>1</sup>Ons Harrabi\*, <sup>1</sup>Amy Chen, <sup>1</sup>Emma Sangalang, <sup>1</sup>Laura Doyle, <sup>1</sup>Danielle Fontaine, <sup>1</sup>Bora Han, <sup>2</sup>Curt Bradshaw, <sup>3</sup>Jaume Pons, <sup>1</sup>Janet Sim, <sup>1</sup>Tracy Kuo, <sup>1</sup>Hong Wan. <sup>1</sup>Tallac Therapeutics, Burlingame, CA, USA; <sup>2</sup>Arrowhead Pharmaceuticals, Pasadena, CA, USA; <sup>3</sup>ALX Oncology, Inc., Burlingame, CA, USA

**Background** Toll-like receptor (TLR) pathways play a crucial role in mounting potent innate immune responses against invading pathogens, as well as the subsequent engagement of adaptive immunity. Innate immune activation via the TLR9 pathway has potential for treating cancer as demonstrated clinically with TLR9 agonists administered intra-tumorally in melanoma patients.<sup>1</sup> We developed a novel toll-like receptor agonist antibody conjugate (TRAAC) platform to systemically deliver a differentiated, targeted TLR9 agonist (T-CpG) for immune activation. The activation of TLR9 pathways can be directed systemically towards specific immune cell populations and tumor microenvironment via antibodies binding to various

immune cell receptors. Using multiple TRAACs targeting either immune cells including plasmacytoid DCs (pDCs), myeloid and B lymphocytes, or tumor specific antigens, we evaluated immune modulatory phenotypes, therapeutic potentials, as well as safety and tolerability of this platform in pre-clinical settings.

**Methods** TRAACs were generated using site-specific conjugation. In vitro activity of immune- and tumor-targeted antibody-CpG conjugates was evaluated using human PBMCs. Anti-tumor efficacy and mechanistic assessment of B lymphocyte and myeloid cell-targeted antibody-CpG conjugates were conducted in syngeneic tumor models. Pharmacokinetic (PK), pharmacodynamic and exploratory toxicity evaluations were performed in non-human primates (NHP).

**Results** T-CpG is comprised of monomeric CpG-containing oligonucleotides optimized for potency and stability as an antibody conjugate. TRAACs targeting immune cells enable directed TLR9 activation leading to potent cytokine production and cellular activation that is superior to free CpG. This targeted immune activation also elicits a cascade of downstream modulation of non-targeted immune cells. When administered systemically in multiple syngeneic models, murine TRAACs targeting either immune cells or tumor antigens exhibited potent, durable, and dose-dependent anti-tumor activity as a single agent and in combination with T-cell checkpoint inhibitors (CPIs). A single peripheral dose of either B lymphocyte or myeloid targeted-CpG evoked both innate and adaptive immune responses within the tumor microenvironment as demonstrated by NanoString analysis. The observed immunomodulatory phenotypes are consistent with those elicited by direct intra-tumoral CpG delivery. Following repeated intravenous doses in NHP, TRAACs demonstrated targeted receptor occupancy, antibody-like PK, and favorable tolerability profile.

**Conclusions** Pre-clinical evaluation of a novel platform comprised of antibodies conjugated to a differentiated TLR9 agonist demonstrated targeted immune activation, potent anti-tumor activity as single agent and in combination with CPIs and favorable tolerability profiles in NHPs. Such antibody-CpG conjugates have the potential for clinical development as systemically delivered therapeutics providing powerful innate and adaptive anti-tumor immunity across multiple tumor types.

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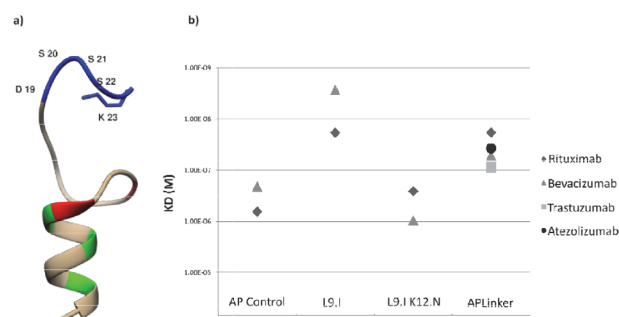
**616 A NOVEL NON-COVALENT LINKER PEPTIDE WITH NANOMOLAR AFFINITY FOR CLINICAL IGG1 ANTIBODIES PRESERVES ANTIBODY-ANTIGEN AFFINITY AND DRUG POTENCY AGAINST PDL1+ MELANOMA WHEN CONJUGATED WITH DM1**

John Butterfield\*, Shari Sutor, Wendy Nevala, Svetomir Markovic. *Mayo Clinic, Rochester, MN, USA*

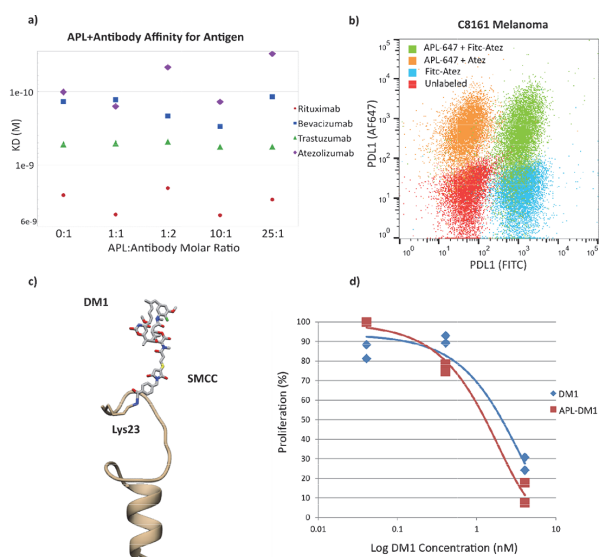
**Background** Antibody-drug conjugates (ADC) increase the efficacy of current chemotherapeutics, decrease off site toxicity, and pair drug function with immunomodulatory effects. Current ADC platforms depend on the use of covalent linker

molecules between the antibody and the drug of choice. This approach leads to significant variation in the number of drug molecules bound, the location of their binding, and inconsistency in maintaining the structure and antigen affinity of the antibody. Because of this, covalent-based ADC development requires extensive separation steps to isolate the ideal isotypes of the ADC. This testing and separation must be repeated for each antibody and each drug considered. Here we present a peptide that non-covalently binds multiple clinically relevant IgG1 antibodies at a controlled ratio and location, then demonstrate its use as a modular ADC linker platform.

**Methods** Peptide-antibody and antibody-antigen affinity were determined using Biacore surface plasmon resonance. Peptides conjugated with alexafluor or DM1 were purified using HPLC and structure was confirmed through mass spectrometry. Flow cytometry verified delivery of peptide-attezolizumab conjugates



**Abstract 616 Figure 1** a) APLinker peptide structure showing the hydrophobic side chains necessary for antibody binding (green), an isoleucine substitution to increase affinity (red), and the addition of a lysine residue to the C terminus for amine conjugation. b) Binding affinity of each peptide mutant to common therapeutic antibodies, determined using Biacore surface plasmon resonance



**Abstract 616 Figure 2** a) Affinity of clinical antibodies for their antigen when bound by APLinker peptide at different molar ratios. b) Labeling of PDL1+ C8161 melanoma cells with atezolizumab bound by AF647 conjugated APLinker. c) Structure of APLinker conjugated with the chemotherapeutic DM1 onto the C-terminus lysine using an SMCC crosslinker. d) In-vitro proliferation assay of DM1 alone and APL-DM1 conjugate using A-375 melanoma cells.