RNAseq using DESeq2. Comparisons were made between immune-responders (IR), non-responders (NR), and normal skin controls. FDR $p<0.01$ was considered significant.

Results Four IR, four NR, and three controls were evaluated. The 500 most variable genes were used for principal component analysis (PCA). Two IR samples were identified as outliers on PCA and excluded from further analysis. There were 882 differentially expressed genes (DEGs) in the IR group vs the NR group (figure 1A). Unsupervised clustering of the top 500 DEGs revealed clustering according to the experimental groups (figure 1B). Of the 10 most highly upregulated DEGs, 9 were immune-related (figure 1C). Gene-set enrichment analysis revealed that immune-related pathways were highly enriched in IRs vs NRs (figure 1D). CD4 and CD8 expression did not differ between IR and NR (figure 2A), though both were higher in IR compared to control. Markers of DC activation/maturation were higher in IR vs NR (figure 2B), as were several Th1 associated genes (figure 2C). Interestingly, markers of exhaustion were higher in IR v NR (figure 2D). Expression of numerous TLR-pathway genes was higher in IR vs NR, including MYD88, but not TICAM1 (figure 2E).

Conclusions These findings suggest a unique immune-transcriptional landscape in the VSME is associated with circulating T-cell responses to immunization, with differences in DC activation/maturation, Th1 response, and TLR signaling. Thus, immunologic changes in the VSME are useful predictors of systemic immune response, and host factors that modulate immune-related signaling at the vaccine site may have concordant systemic effects on promoting or limiting immune responses to vaccines.

Trial Registration Samples for this work were collected from patients enrolled on the Mel55 clinical trial NCT01425749.

Ethics Approval This work was completed after approval from the UVA institutional review board IRB-HSR# 15398.

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612 HUMAN CLEC9A ANTIBODIES DELIVER NY-ESO-1 ANTIGEN TO CD141+ DENDRITIC CELLS TO ACTIVATE NAÏVE AND MEMORY NY-ESO-1-SPECIFIC CD8+ T CELLS

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Background Dendritic cells (DC) are crucial for the efficacy of cancer vaccines, but current vaccines do not harness the key cDC1 subtype required for effective CD8+ T cell mediated tumor immune responses. Vaccine immunogenicity could be enhanced by specific delivery of immunogenic tumor antigens to CD141+ DC, the human cDC1 equivalent. CD141+ DC exclusively express the C-type-lectin-like receptor CLEC9A, which is important for the regulation of CD8+ T cell responses. This study developed a new vaccine that harnesses a human anti-CLEC9A antibody to specifically deliver the immunogenic tumor antigen, NY-ESO-1 to human CD141+ DC. The ability of the CLEC9A-NY-ESO-1 antibody to activate NY-ESO-1 specific naïve and memory CD8+ T cells was examined and compared to a vaccine comprised of a human DEC-205-NY-ESO-1 antibody that targets all human DC.

Methods Human anti-CLEC9A, anti-DEC-205 and isotype control IgG4 antibodies were genetically fused to NY-ESO-1 polypeptide. Cross-presentation to NY-ESO-1- epitope specific CD8+ T cells and reactivity of T cell responses in melanoma patients was assessed by IFNγ production following incubation of CD141+ DC and patient peripheral blood mononuclear cells with targeting antibodies. Humanized mice containing human DC subsets and a repertoire of naïve NY-ESO-1-specific CD8+ T cells were used to investigate naïve T cell priming. T cell effector function was measured by expression of IFNγ, MIP-1β, TNF and CD107a and by lysis of target tumor cells.

Results CLEC9A-NY-ESO-1 Ab were effective at mediating delivery and cross-presentation of multiple NY-ESO-1 epitopes by CD141+ DC for activation of NY-ESO-1-specific CD8+ T cells. When benchmarked to NY-ESO-1 conjugated to an untargeted control antibody or to anti-human DEC-205, CLEC9A-NY-ESO-1 was superior at ex vivo reactivation of NY-ESO-1-specific T cell responses in melanoma patients. Moreover, CLEC9A-NY-ESO-1 induced priming of naïve NY-ESO-1-specific CD8+ T cells with polyclonal effector function and potent tumor killing capacity in vitro.

Conclusions These data advocate human CLEC9A-NY-ESO-1 antibody as an attractive strategy for specific targeting of CD141+ DC to enhance tumour immunogenicity in NY-ESO-1-expressing malignancies.

Ethics Approval Written informed consent was obtained for human sample acquisition in line with standards established by the Declaration of Helsinki. Study approval was granted by the Mater Human Research Ethics Committee (HREC13/MHS/83 and HREC13/MHS/86) and The U.S. Army Medical Research and Materiel Command (USAMRMC) Office of Research Protections, Human Research Protection Office (HRPO; A-18738.1, A-18738.2, A-18738.3). All animal experiments were approved by the University of Queensland Animal Ethics Committee and conducted in accordance with the Australian Code for the Care and Use of Animals for Scientific Purposes in addition to the laws of the United States and regulations of the Department of Agriculture.

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Immuno-conjugates and chimeric molecules

613 HER2-XPAT, A NOVEL PROTEASE-ACTIVATABLE PRODRUG T CELL ENGAGER (TCE), WITH POTENT T-CELL ACTIVATION AND EFFICACY IN SOLID TUMORS AND LARGE PREDICTED SAFETY MARGINS IN NON-HUMAN PRIMATE (NHP)

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Background TCEs are effective in leukemias but have been challenging in solid tumors due to on-target, off-tumor toxicity. Attempts to circumvent CRS include step-up dosing and/or complex designs but are unsuccessful due to toxicity and/or enhanced immunogenicity. HER2-**XPAT**, or **X**TENylated **P**rotease-**A**ctivated bispecific **T**-Cell Engager, is a prodrug TCE that exploits the protease activity present in tumors vs. healthy tissue to expand the therapeutic index (TI). The core of the HER2-XPAT (PAT) consists of 2 tandem scFvs targeting

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

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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-poitive 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

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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.[1] 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