Ethics Approval The study was approved by NCI/NIH Institutional Review Board (#534360, 13C0016).

REFERENCES

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RNA-SEQUENCING REVEALS A UNIQUE IMMUNE TRANSCRIPTIONAL LANDSCAPE IN THE VACCINE SITES OF PATIENTS WITH CIRCULATING T-CELL RESPONSES TO CANCER IMMUNIZATION

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Background Vaccines are a promising therapeutic for patients with advanced cancer, but achieving robust T-cell responses remains a challenge. Melanoma-associated antigen-A3 (MAGE-A3) in combination with adjuvant AS15 (a formulation of

Abstract 611 Figure 1 Gene expression profiling of vaccine site samples from patients immunized with MAGE-A3/AS15. (A) Volcano plots showing the distribution of differentially expressed genes (DEGs) between immune responders (IR) and non-responders (NR), IR and control, and NR and control. (B) Heatmap of the top 500 most differentially expressed genes demonstrating hierarchical clustering of sequenced samples according to IR, NR, and control. (C) Table showing the 10 most highly up and down-regulated genes in IR compared to NR. 9 of the top 10 most highly up-regulated genes are related to the immune response. (D) Enrichment plots from a gene set enrichment analysis highlighting the upregulation of immune related pathways in IR compared to NR. Gene set enrichment data was generated from the Reactome gene set database and included all expressed genes. Significance was set at FDR p <0.01
Toll-Like-Receptor (TLR)-4 and 9 agonists and a saponin), induced systemic CD4+ T-cell responses in 50% of patients when given subcutaneously/intradermally. Little is known about the transcriptional landscape of the vaccine-site microenvironment (VSME) of patients with systemic T-cell responses versus those without. We hypothesized that patients with systemic T-cell responses to vaccination would exhibit increased immune activation in the VSME, higher dendritic cell (DC) activation/maturation, TLR-pathway activation, and enhanced Th1 signatures.

**Methods** Biopsies of the VSME were obtained from participants on the Mel55 clinical trial (NCT01425749) who were immunized with MAGE-A3/AS15. Biopsies were taken 8 days after immunization. T-cell response to MAGE-A3 was assessed in PBMC after in-vitro stimulation with recMAGE-A3, by IFNγ ELISPOT assay. Gene expression was assessed by

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**Abstract 611 Figure 2** Expression of T-cell markers in IR vs NR vs Control samples in the vaccine site microenvironment (VSME). (A) T-cell markers showing similar expression in IR vs NR but higher expression in IR vs control. (B) Markers of dendritic cell activation and maturation in the VSME which are higher in IR vs control but not IR vs NR. (B) Transcription factors and genes associated with Th1/Th2 responses within the VSME. (D) Genes associated with T-cell exhaustion at the VSME. (E) Expression of TLR pathway genes in the VSME. Expression data is provided in terms of normalized counts. Bars demonstrate median and interquartile range. N=9. IR = immune responder, NR = non-responder, TLR = Toll-like Receptor. * = <0.01, ** < 0.001, *** <0.0001, **** < 0.00001
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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 vs 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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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 polyconal 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 NY-ESO-1 expression at the vaccine site 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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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 XTENylated Protease-Activated bspecific 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