robust Lm-specific CD8+ T-cell immunity but failed to prime GUCY2C-specific CD8+ T-cell responses. These studies explore the hypothesis that immunodominant Lm antigens suppress primary immunity to subdominant GUCY2C epitopes in Lm-GUCY2C.

**Methods** Lm-GUCY2C expresses the extracellular domain of mouse GUCY2C23-429 downstream of an ActA promoter integrated into the genome of the live, attenuated delta actA delta inlB Lm strain. Altered peptide ligands were designed based on NetMHCpan 4.0 peptide-MHC binding algorithms and similarly cloned into Lm. Peptide-MHC stability was quantified by FACS-based surface peptide-MHC dissociation on the TAP-deficient cell line, RMA-S H-2Kd. In vivo efficacy studies employed IFNγ-ELISpot quantification of T-cell responses and tumor challenge studies with the CT26 colorectal cancer cell line. Adenovirus expressing GUCY2C was used as a positive control.

**Results** Lm-GUCY2C vaccination of BALB/c mice generated Lm-specific CD8+ T-cell responses but an absence of GUCY2C-specific immunity. Peptide-MHC stability studies revealed poor stability of the dominant GUCY2C254-262 epitope complexed with H-2Kd compared to H-2Kd-restricted Lm epitopes derived from the LLO and p60 Lm antigens. Mutation of the GUCY2C254-262 peptide at critical anchoring residues for binding H-2Kd revealed that the altered peptide ligand with an F255Y mutation significantly improved the stability of the GUCY2C254-262-H-2Kd complex. Similarly, vaccination of mice with recombinant Lm-GUCY2C expressing the altered peptide ligand (Lm-GUCY2CF255Y) restored GUCY2C immunogenicity and antitumor immunity.

**Conclusions** Immunodominant Lm antigens may interfere with immune responses directed to the vaccine target antigen GUCY2C by competing with GUCY2C epitope for MHC class I binding and presentation. Moreover, use of a substituted GUCY2C-peptide ligand with enhanced peptide-MHC class I stability restored GUCY2C-specific immunity in the context of Lm-GUCY2C, an approach that can be translated to patients. Importantly, these studies also suggest that ongoing Lm-based vaccine development programs targeting a variety of antigens in other cancer types may be similarly limited by the immunodominance of Lm epitopes.

**Acknowledgements** The authors thank Dr. Peter Lauer for providing the pPL2 integration vector used in cloning Lm-GUCY2C and Dr. Sean Murphy for providing the RMA-S H-2Kd cell line.

**Ethics Approval** Studies were approved by the Thomas Jefferson University IACUC (Protocol # 01956).

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http://dx.doi.org/10.1136/jitc-2020-SITC2020.0608

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**Abstract 610**

**Figure 1** Autologous DC vaccine manufacturing at the NIH clinical center.

**TECHNICAL CHALLENGES IN MONOCYTE-DERIVED DENDRITIC CELL VACCINE MANUFACTURING; A QI PROJECT**

**Background** With the explosive growth of cancer immunotherapies, cancer vaccines have been in the spotlight for their ability to turn cold tumors hot. Particularly, dendritic cell vaccines (DCV) are capable of harnessing the immune system to recognize single or multiple epitopes as they are professional antigen presenting cells. However, DCVs have not been recognized as the platform of choice in many studies due to relatively high cost, difficulty in standardizing manufacturing methods and risk of product inconsistency. We have been using monocyte-derived DCs transduced with an adenovirus vector expressing HER2/neu in a clinical trial to treat HER2-expressing cancers. The vaccine was administered on weeks 0, 4, 8, 16 and 24 at 4 different dose-
levels; $5 \times 10^6$, $10 \times 10^6$, $20 \times 10^6$ and $40 \times 10^6$ viable cells. The clinical outcome of the study is under analysis. To further optimize the safety and consistency of DCV, we reviewed the issues encountered in a first-in-human clinical trial during the manufacture of these cells at the NIH Clinical Center.

**Methods** Manufacturing records of NCT01730118 A Phase I Study of an Autologous DCV Targeting HER2 in Solid Tumors were reviewed to identify any complications or deviations encountered during manufacturing from apheresis to delivery of the fresh DCVs (figure 1).

**Results** Between April 2013 and October 2019, 134 vaccines were manufactured for 33 patients. A total of 113 (84%) DCVs were administered, with 103 (91%) of those meeting release criteria, and the remaining administered under authorized medical exception (AME). All patients underwent a single apheresis collection with 18 (median, range 15–20) liters processed and a goal of 6 aliquots ($333 \times 10^6$ monocytes/vial). Dual lumen catheterization was required in 23 (70%) patients, and all procedural reactions required no or minimal intervention. Summaries enumerate aberrancies encountered during the manufacturing process (table 1). Overall, interpatient variabilities may have contributed to 92 (78%) events, while 26 (22%) events arose in a ‘controllable’, patient-unrelated environment.

**Conclusions** In spite of the variable events encountered during the manufacturing process, the majority of products were administered successfully. Patient-related variabilities were linked to most of the events. Future studies should be designed to minimize the impact of such variabilities on DCVs to provide high-quality personalized therapies. Manufacturing one large lot of DCs and cryopreserving enough aliquots for the entire study and the incorporation of an automated, closed cell culture system may reduce the aforementioned incidents and improve product quality.

**Acknowledgements** This study was supported by the NIH Clinical Center and Center for Cancer Research, the National Cancer Institute. The authors are indebted to the staffs at NIH Clinical Center and the patients.
Ethics Approval  The study was approved by NCI/NIH Institutional Review Board (#534360, 13C0016).

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http://dx.doi.org/10.1136/jitc-2020-SITC2020.0610

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 upregulated 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

Abstract: 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...