

robust Lm-specific CD8<sup>+</sup> T-cell immunity but failed to prime GUCY2C-specific CD8<sup>+</sup> 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 class I complex stability was quantified by FACS-based surface peptide-MHC dissociation on the TAP-deficient cell line, RMA-S H-2Kd.<sup>3</sup> In vivo efficacy studies employed IFN $\gamma$ -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.<sup>2, 4</sup>

**Results** Lm-GUCY2C vaccination of BALB/c mice generated Lm-specific CD8<sup>+</sup> 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 anti-tumor 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.

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

## REFERENCES

1. Flickinger JC, Rodeck U, Snook AE. *Listeria monocytogenes* as a Vector for Cancer Immunotherapy: Current Understanding and Progress. *Vaccines* (Basel) 2018;6. doi:10.3390/vaccines6030048.
2. Snook AE, Baybutt TR, Xiang B, Abraham TS, Flickinger JC, Hyslop T, et al. Split tolerance permits safe Ad5-GUCY2C-PADRE vaccine-induced T-cell responses in colon cancer patients. *J Immunother Cancer* 2019;7:104. doi:10.1186/s40425-019-0576-2.
3. Müllbacher A, Lobigs M, Kos FJ, Langman R. Alloreactive cytotoxic T-cell function, peptide nonspecific. *Scand J Immunol* 1999;49:563–9.
4. Flickinger J. JC, Singh J, Carlson R, Leong E, Baybutt T, Barton J, et al. Chimeric Ad5.F35 vector evades anti-adenovirus serotype 5 neutralization opposing GUCY2C-targeted antitumor immunity. *J Immunother Cancer* 2020.

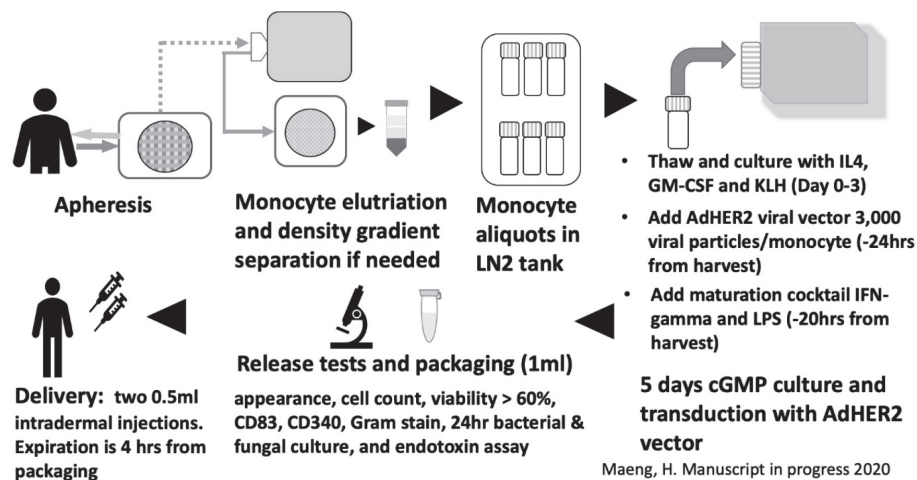
<http://dx.doi.org/10.1136/jitc-2020-SITC2020.0608>

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

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**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-



**Abstract 610 Figure 1** Autologous DC vaccine manufacturing at the NIH clinical center

Abstract 610 Table 1

Factors in Manufacturing	Complications & Deviations	Patients Affected n, (%) of total 33	Doses Affected n, (%) of total 134
Apheresis collection	Venous access issues*	8 (24)	
	Adverse reaction**	14 (42)	
	Technical/procedural***	10 (30)	
Monocyte enrichment	High neutrophil content	13 (39)	
	Poor density gradient separation†	2 (6)	
Cell culture	Poor morphology	5 (15)	5 (4)
	Insufficient cell growth	3 (9)	4 (3)
	Culture deviation†††	3 (9)	3 (2)
Product characterization	Appearance failure	1 (3)	1 (1)
	Viability failure	4 (12)	4 (3)
	Failed maturation (%CD83)	9 (27)	14 (10)
	Low transduction efficiency (%CD340)	8 (24)	10 (7)
Product sterility	Positive Gram stain‡	1 (3)	1 (1)
	Positive final sterility‡	2 (6)	2 (1)
Materials management	Testing reagent availability‡	1 (3)	1 (1)
	Reagent compromise‡	6 (18)	16 (12)
Facility	Environmental monitoring event‡	2 (6)	2 (1)
	Facility failure†††‡	1 (3)	1 (1)
Delivery	Patient ineligible for treatment	4 (12)	4 (3)
	Patient unavailability (no show or death)	3 (9)	3 (2)

\*Includes pain/discomfort at venous access site (Grade 1), bleeding (Grade 1), and repeated venipuncture.

\*\*Includes symptoms attributable to citrate toxicity (perioral numbness and tingling, Grade 2), pain/discomfort not attributable to venous access site, and other general patient complaints.

\*\*\*Includes poor flow rates, unstable collection interface, and high/low pressure alarms.

†There were cases requiring density gradient separation to remove neutrophils monocytes elutriation due to excess neutrophils (>15%).

††In the dose-level 5x10E6, issues related to heat-inactivated, filtered (HF) autologous plasma in manufacturing process including poor transduction efficiency that was not identified during the engineering runs with healthy volunteers were noted and allogeneic heat-inactivated, irradiated and filtered (HIF) AB plasma was used instead afterward [2].

†††Water damage to facility due to a sprinkler maloperation

‡ "Controllable", patient-unrelated events. The rests are considered patient variability related events.

No issues related to storage or transportation were identified. Toxicity was graded by CTCAE ver 4.0 where feasible.

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.<sup>1</sup> 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.

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

REFERENCES

1. Maeng, H.M., *et al.*, Preliminary results of a phase I clinical trial using an autologous dendritic cell cancer vaccine targeting HER2 in patients with metastatic cancer or operated high-risk bladder cancer (NCT01730118). *Journal of Clinical Oncology* 2019. **37**(15\_suppl): p. 2639–2639.
2. Jin, P., *et al.*, Plasma from some cancer patients inhibits adenoviral Ad5f35 vector transduction of dendritic cells. *Cytotherapy* 2018;**20**(5): p. 728–739.

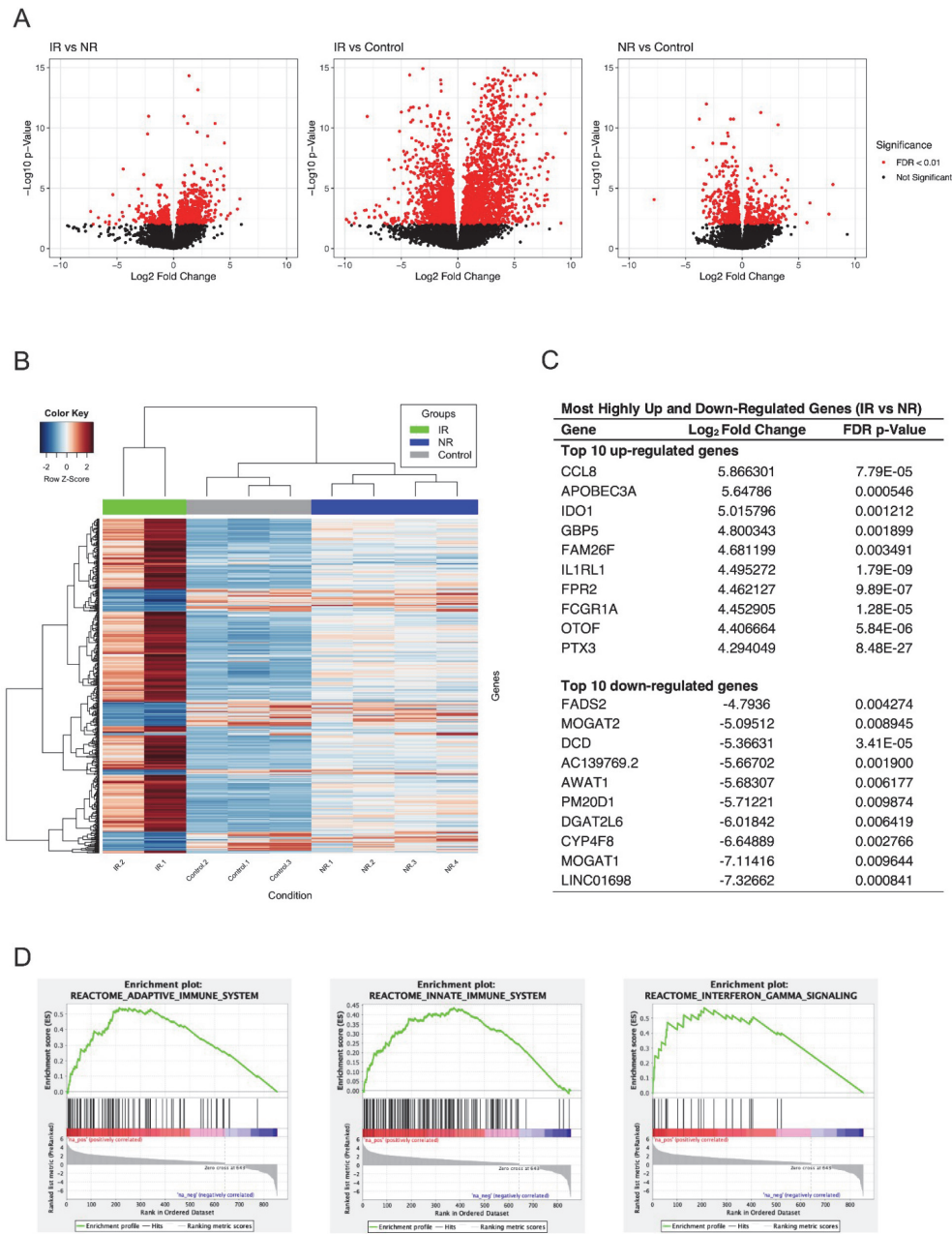
<http://dx.doi.org/10.1136/jitc-2020-SITC2020.0610>

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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  $p < 0.01$