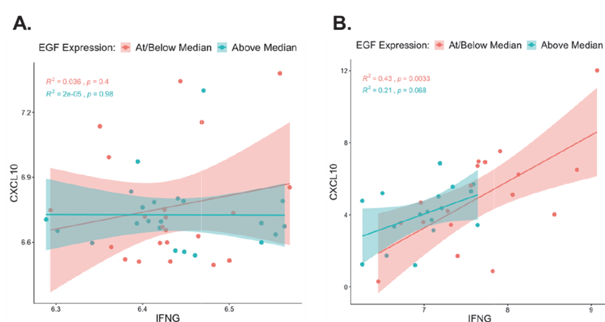

Abstract 606 Figure 3 Correlation between IFN-γ and CXCL10 expression stratified by EGF expression. A. Cutaneous squamous cell carcinoma cell lines (GSE98767, n=44). B. Cutaneous squamous and basal cell carcinoma tumor samples (GSE125285, n=35)

role of EGF in modulating inflammation, and to understand this process in the pathogenesis of EGF receptor inhibitor-induced cutaneous toxicities and skin cancers.

Acknowledgements Emory Integrated Genomics Core

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607 TJ210 (MOR210), A DIFFERENTIATED ANTI-C5AR ANTIBODY FOR ANTI-CANCER THERAPY

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Background Extensive investigations into the tumor microenvironment (TME) have uncovered molecular mechanisms linking aberrant complement activation and cancer progression. Specifically, C5a, as a highly potent chemoattractant, recruits immune suppressive myeloid derived suppressive cells (MDSCs), neutrophils and M2 macrophages into the tumor site and accelerates tumor progression. Blockade of C5a/C5aR (CD88) pathway has been identified as a promising target to control MDSCs and restore tumor-killing ability of T and NK cells. TJ210, in licensed from MorphoSys as MOR210, is a differentiated anti-C5aR monoclonal antibody with a unique binding epitope.

Methods Interaction of TJ210 with C5aR was assessed through binding of the recombinant antigen, Flp-In CHO cells expressing C5aR and primary neutrophils. In vitro blockade of C5a/C5aR pathway was tested by inhibition of CD11b upregulation on granulocytes and monocytes induced by C5a, as well as neutrophil migration towards C5a. The in vitro synergistic effect of TJ210 with anti-PD-1 antibody was assessed in a T cell and differentiated MDSC co-culture system. The in vivo anti-tumor effect was tested in the MC38 syngeneic mouse model, in which mice were treated with a TJ210 mouse surrogate antibody either alone or in combination with an anti-PD-1 antibody.

Results TJ210 bound to C5aR with high affinity and did not cross-react with other GPCR members including C5L2, ChemR23, FPR1 and C3aR. Unlike the reference antibody, TJ210 specifically interacted with the N-terminus of C5aR but not extracellular loops. TJ210 effectively inhibited CD11b upregulation on granulocytes and monocytes as well as neutrophil migration mediated by C5a. When compared with the reference antibody, TJ210 maintained potent antagonism at high ligand concentrations and over longer duration, properties that might translate into beneficial in vivo effects at pathophysiological conditions. In the in vitro co-culture system, presence of TJ210 and anti-PD-1 antibody enhanced IFN-γ release compared to either single agent, indicating a synergistic effect on T cells. In the in vivo syngeneic mouse model, combination treatment effectively inhibited tumor growth. Immune cell population analysis revealed significant elevation of CD8+ T cells and M1 macrophages compared to mono-treatment.

Conclusions This series of in vitro and in vivo data demonstrate that TJ210 is a differentiated anti-C5aR antibody with unique binding epitope exhibiting superior anti-tumor potential especially in combination with an anti-PD-1 antibody. These data support further clinical studies of TJ210 in patients with solid tumors.

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608 IMMUNODOMINANT LISTERIA EPITOPES COMPETE WITH VACCINE-DIRECTED CD8+ T-CELL RESPONSES RESCUED BY PEPTIDE-MHC STABILIZING MODIFICATIONS

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Background The Gram-positive bacterium Listeria monocytogenes (Lm) is a promising vector for cancer immunotherapy due to its ability to directly infect antigen-presenting cells, induce potent CD8+ T-cell immunity, and remodel immunosuppressive tumor microenvironments.[1] Recent clinical trials have demonstrated safety and immunogenicity of Lm-based cancer vaccines in lung, cervical, pancreatic, and other cancers. In colorectal cancer, the transmembrane receptor guanylyl cyclase C (GUCY2C) is an emerging target for immunotherapy.[2] Here, we examined the immunogenicity of a recombinant strain of Listeria monocytogenes secreting GUCY2C (Lm-GUCY2C). Surprisingly, Lm-GUCY2C vaccination induced

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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 class I complex stability was quantified by FACS-based surface peptide-MHC dissociation on the TAP-deficient cell line, RMA-S H-2Kd.[3]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.[2 4]

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 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.

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

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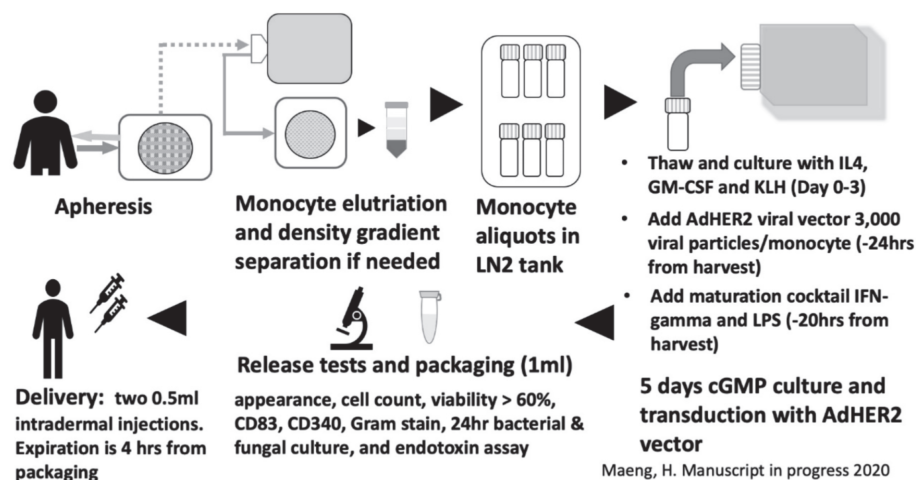
Background With the explosive growth of cancer immunotherapies, cancer vaccines have been in the spotlight for their ability to turn cold tumors hot. Particularly, dendritic cell vaccines (DCV) are capable of harnessing the immune system to recognize single or multiple epitopes as they are professional 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

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