anti-rHER2 ISAC, and IHC at day 6 showed a 5-fold increase in CD11c+ cells. Control-treated tumors had sparse CD8+ T cells, whereas rHER2-targeted ISAC treatment led to ~3.5-fold increase in T cell frequency that shifted the tumor microenvironment from immunologically cold to hot. The recruitment of both phagocytes and CD8+ T cells was consequential, as depletion of either abrogated anti-tumor efficacy of the rHER2-targeted ISAC. Systemically delivered ISACs were well-tolerated.

Conclusions In contrast to other immune therapies, such as anti-PDL1/PD1 and anti-CD40, systemically administered ISACs locally engage both the innate and adaptive arms of the immune system to eradicate tumors. The molecular and cellular phenotype associated with ISAC-mediated activation is being evaluated in the on-going BDC-1001 Phase I/II clinical trial.[2]

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606 EPIDERMAL GROWTH FACTOR MODULATION OF CXCL10 IN KERATINOCYTES AND CUTANEOUS CANCERS

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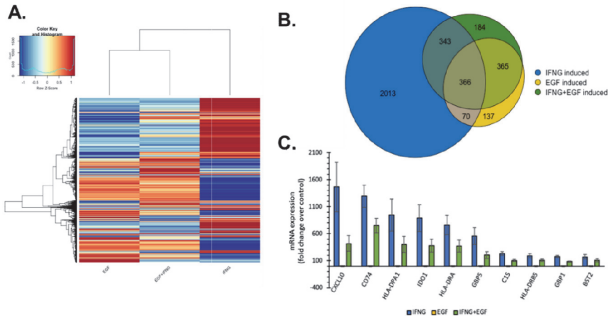
Background Epidermal growth factor (EGF) signaling has well-established roles in cellular proliferation in normal tissue homeostasis and tumorigenesis. EGF receptor inhibitor therapy is associated with the development of a papulopustular rash and other cutaneous inflammatory effects.[1] [2] These dose-dependent toxicities are linked to treatment response and survival, and may reflect the interplay between EGF and the immune response.[3] [4] However, the effects of EGF signaling on inflammation in the skin and elsewhere are not entirely understood.[5] [6] In this study, we aimed to elucidate the immunomodulatory role of EGF in human keratinocytes exposed to the proinflammatory cytokine interferon-γ (IFN-γ).

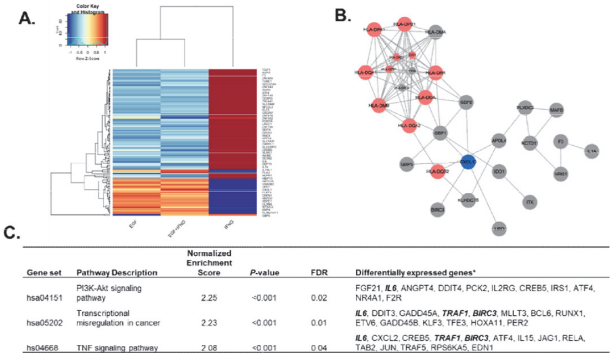
Methods Human keratinocyte cell lines (HaCaT) were exposed to IFN-γ, EGF, or both (48 hours). Differential gene expression analyses of RNA expression was performed using DESeq2.[7] Fold change in gene expression on the log2 scale were calculated for each experimental treatment group relative to control. Web Gestalt was used to identify differentially expressed biologic pathways and gene networks, and further investigated in publicly available cutaneous squamous cell (cSCC) cell lines (GSE98767) and cSCC and basal cell carcinoma (BCC) tumor samples (GSE125285).[8]

Results As compared to untreated control cells, 2792 genes were differentially expressed following IFN-γ treatment, 938 following EGF treatment, and 1248 following the combination of IFN-γ and EGF (figure 1). To assess the impact of EGF on the cellular response to IFN-g, we identified IFN-g-induced genes whose expression was significantly altered by EGF (figure 2). We found that the induction of CXCL10 by IFN-g was among those significantly attenuated in the presence of EGF (padjusted= 0.01) and selected CXCL10 as a model to further define the impact of EGF on immune gene expression. We found that in cutaneous SCC (cSCC) cell lines as well as cSCC and basal cell carcinoma tumor samples, the correlation between IFN-γ and CXCL10 expression was abrogated in samples with higher EGF expression (figure 3).

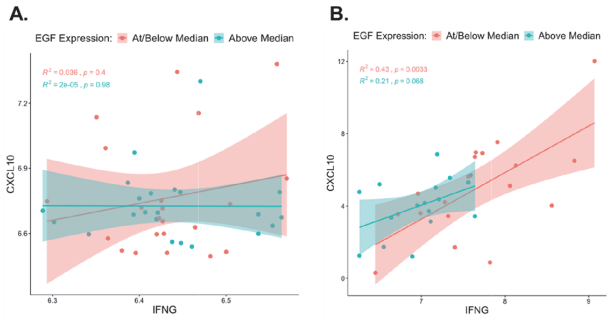
Conclusions EGF has pleotropic roles in cancer including immunologic effects relevant to anti-tumor immunity. These studies demonstrate that EGF alters the transcriptional response to IFN-g including the induction of CXCL10 by IFN-g. Moreover, these studies suggest that in the setting of high EGF levels, there is a modulation of IFN-g-regulated chemokine expression. Further research is needed to clarify the



Abstract 606 Figure 1 EGF modulates IFN-γ-induced gene expression in human keratinocytes. A. Heatmap showing differentially expressed genes (Padjusted <0.01) induced by IFN-γ alone, EGF alone, or IFN-γ plus EGF (excluding genes that were not differentially expressed in any treatment group relative to control). B. Venn-diagram showing differentially expressed genes (Padjusted < 0.01) induced by IFN-γ and/or EGF. C. Fold change of the top 10 genes induced after treated with IFN-γ alone. The top 10 genes which were induced by IFN-γ include CXCL10, CD74, several HLA-D genes, IDO1, GBP5, C1S, and BST2



Abstract 606 Figure 2 Pathway analysis of genes induced by IFN-γ then differentially regulated by EGF. A. Heatmap showing log2fold change in gene expression of top IFN-γ-regulated genes whose expression was significantly dampened or augmented by EGF (Pinteraction < 0.05). The EGF* IFN-γ interaction fold-change (far left) column indicates the excess fold change due to interaction between EGF and IFN-γ. Within this column, blue and red shading indicates dampening and augmentation of IFN-γ-induced gene expression by EGF, respectively. B. Sub-network graph from Network Topology Analysis (NTA) of IFN-γ-regulated genes of which expression was either 2-fold higher or lower when EGF was added; genes in the top enriched GO Biological Process category are highlighted in red (GO:0019886 [antigen processing and presentation of exogenous peptide antigen via MHC class II]; Padjusted = 2.65 x 10^{-7}); blue shading of CXCL10 denoting it as the most strongly upregulated gene by IFN-γ in this gene set to be dampened by EGF treatment. C. IFN-γ-induced genes attenuated by EGF, clustered according to significantly enriched KEGG pathways. Differentially expressed genes are listed in order of their score within the gene set enrichment analyses. Bolded italics type indicates common genes in multiple enriched pathways



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.

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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