Blockade of the inhibitory collagen receptor LAIR-1 with NC410, a LAIR2-Fc fusion protein, enhances anti-tumor activity of the bifunctional fusion protein bintrafusp alfa

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ABSTRACT

Background: Leukocyte-associated immunoglobulin-like receptor 1 (LAIR-1) is an immune inhibitory receptor that binds collagen-like domains commonly found in extracellular matrix (ECM) collagens and complement component C1q. LAIR-1 is expressed on several immune cell types including activated T cells, B cells, NK cells, dendritic cells, and macrophages. Numerous cancer types including gastric, colon, ovarian, bladder, and others, upregulate collagens which enhances tumor growth, metastases, and invasion while actively suppressing antitumor immunity. While a soluble decoy, LAIR-2, is expressed in humans and competes with LAIR-1 for binding of collagen domains, excess LAIR ligands in the tumor often result in an immune suppressive environment. Here, we report on a novel immunotherapy approach combining NC410, a novel fusion protein consisting of two LAIR-2 molecules grafted on to an IgG1 antibody backbone, capable of targeting the tumor ECM and blocking LAIR-1 signaling; and bintrafusp alfa, a first-in-class bifunctional fusion protein composed of the extracellular domain of the human transforming growth factor β receptor II (TGF-βRII or TGF-β "trap") fused via a flexible linker to the C-terminus of each heavy chain of an IgG1 antibody blocking programmed death ligand 1 (anti–PD-L1). We have demonstrated that the combination of NC410 and bintrafusp alfa more effectively controls in vivo tumor growth of the collagen rich MC38 colon and EMT6 mammary carcinomas compared to either monotherapy. We demonstrate that this potent anti-tumor immune response is propagated through the synergy of activated tumor infiltrating lymphocytes and a repolarization of myeloid cells in the tumor microenvironment. MC38 tumors treated with the combination of NC410 plus bintrafusp alfa contained higher numbers of infiltrating T cells, NK cells, and M1 polarized macrophages. This study highlights the synergy of reshaping the large suppressive myeloid cell populations often present in tumors with activation of adaptive T-cell immune responses dampened by checkpoint inhibition. The results also provide the rationale for the future evaluation of this combination therapy in the clinic.

BACKGROUND

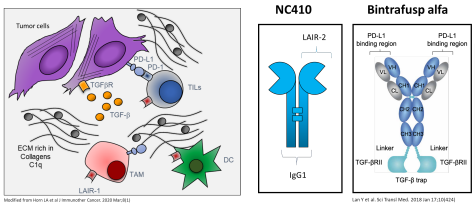

Figure 1. Tumor cells, immune cells, stroma, and extracellular matrix components continually shape the tumor microenvironment. Soluble TGF-β, PD-L1 binding to PD-1 on TIL, extracellular matrix collagens inhibiting T cells and promoting alternative macrophage polarization through LAIR-1, and other factors actively suppress anti-tumor immunity and promote tumor progression. Reagents Used: (1) NC410 (NextCure) is a LAIR-2-Fc fusion protein that binds to collagens domains acting as a decoy for LAIR-1. (2) Bintrafusp alfa (EMD Serono) is a bifunctional fusion protein combining an anti-PD-L1 monoclonal antibody and the extracellular domain of human TGF-βRII. These studies were conducted at the National Cancer Institute under Cooperative Research and Development Agreements (CRADAs) with NextCure and EMD Serono.

RESULTS

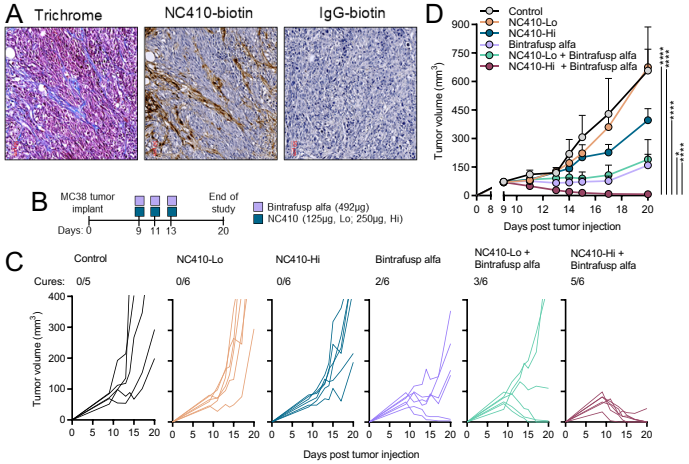

Figure 2. NC410 and bintrafusp alfa synergize for effective tumor control. (A) Representative images of MC38 tumors analyzed for collagen (trichrome staining), NC410-biotin and control IgG-biotin staining. (B) Treatment schedule for mice bearing MC38 tumors. Individual tumor growth and number of cures (C) and average tumor growth (D) are shown; n= 5 mice/group (control) or n=6 (all other groups). Error bars indicate SEM of biological replicates. *p ≤ 0.05; **p ≤ 0.01; **** p ≤ 0.0001 for two-way ANOVA in (D).

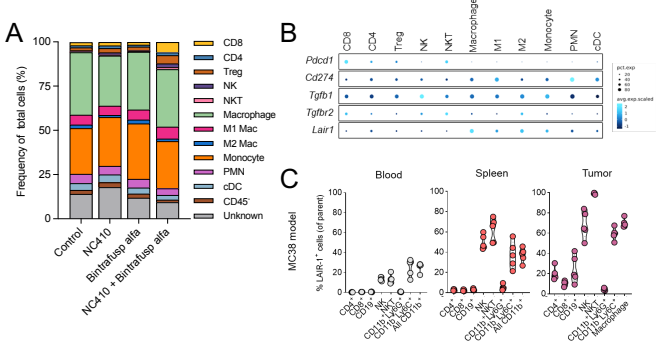

Figure 3. Expression of target molecules and treatment effect on tumor immune infiltrates. (A) MC38 tumor bearing mice were left untreated or treated on days 9 and 11 post tumor implantation with 250μg NC410, 250μg of bintrafusp alfa, or a combination of both agents. On day 12 tumors were collected, and single-cell RNAseq profiling of tumor infiltrating CD45⁺ cells was performed. Frequency of selected immune cell subsets identified by scRNAseq analysis were graphed. (B) Expression of selected genes by scRNAseq in bubble plot representation across selected immune cell subset clusters. Bubble size shows percentage of cells expressing the indicated gene; color intensity represents scaled expression levels. (C) Flow cytometry analysis of LAIR-1 expression on indicated immune cell subsets in the blood, spleen, and tumors from MC38 tumor-bearing mice; n= 5 mice/group.

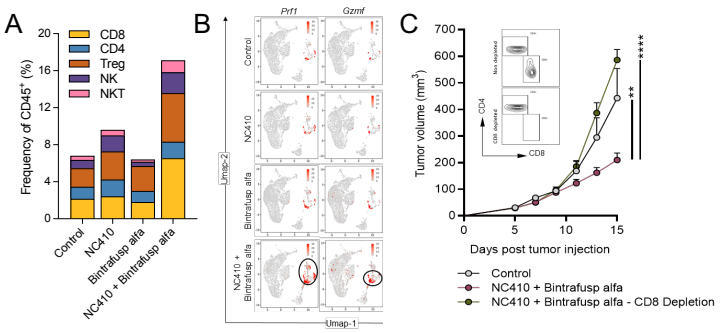

Figure 4. NC410 plus bintrafusp alfa increases tumor infiltration with activated CD8+ T cells which mediate effective tumor control. (A) Frequency of immune effector CD4⁺, CD8⁺, T regulatory (Treg) cells, NK and NKT cells as a percentage of total CD45⁺ cells as identified by scRNAseq analysis from MC38 tumors from Figure 3. (B) UMAP plots showing expression of selected genes by scRNAseq analysis from MC38 tumors in each group. (C) Average tumor growth of MC38 tumor-bearing mice untreated or treated with NC410 plus bintrafusp alfa with or without depleting antibodies for CD8⁺ T cells; n=8 (Control and NC410 + bintrafusp alfa with CD8 depletion) or n=9 (NC410 + bintrafusp alfa). Scatter plots demonstrate CD8⁺ T-cell depletion efficiency in blood of representative mice. Error bars indicate SEM of biological replicates. ** p ≤ 0.01; **** p ≤ 0.0001 for two-way ANOVA in (C).

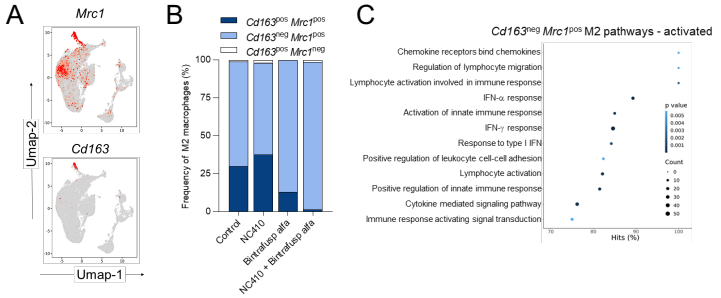

Figure 5. NC410 plus bintrafusp alfa reduces tumor infiltration with M2 tumor-associated macrophages. (A) UMAP plots showing expression of Mrc1 and Cd163 genes used to identify M2 cell clusters by scRNAseq across treatment groups. (B) Frequency of subpopulations of M2 macrophages according to their expression of *Cd163* and *Mrc1*. (C) Selected activated GO/REACTOME/KEGG/HALLMARK gene pathways in Cd163^{neg}Mrc1^{pos} M2 clusters identified by scRNAseq in the NC410 + bintrafusp alfa compared to the control group.

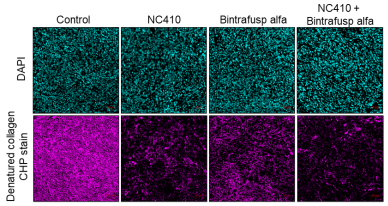

Figure 6. Remodeling of collagen in tumors treated with NC410 plus bintrafusp alfa therapy. Representative images of immunofluorescence-based staining of denatured collagen utilizing a linearized collagen hybridizing peptide (CHP, magenta) in MC38 tumors collected as indicated in Figure 3. DAPI staining of nuclei (cyan) is shown in top panel.

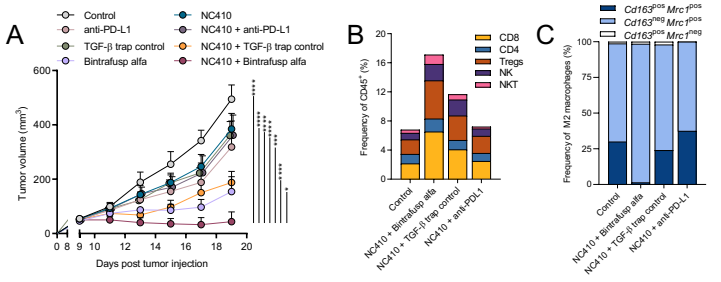

Figure 7. Inhibition of TGF-β and PD-L1 are both required for optimal tumor control in combination with NC410. (A) MC38 tumor-bearing mice were administered NC410, bintrafusp alfa, anti-PDL1, or TGF-β trap control on days 9, 11, and 13 post-tumor injection. Graph shows average tumor growth; n=6 mice/group (bintrafusp alfa, NC410 + bintrafusp alfa) or n=7 (control, anti-PD-L1, TGF-β trap control, NC410, NC410 + anti-PD-L1, NC410 + TGF-β trap control). Error bars indicate SEM of biological replicates. * p ≤ 0.05; *** p ≤ 0.001; **** p ≤ 0.0001 for two-way ANOVA. (B) Frequency of effector CD4⁺, CD8⁺, T regulatory (Treg) cells, NK and NKT cell clusters as determined by scRNAseq, shown as a percentage of total CD45⁺ cells. (C) Frequency of subpopulations of M2 macrophages according to their expression of *Cd163* and *Mrc1*.

CONCLUSIONS

This work describes a combinatorial immunotherapy approach consisting of neutralization of PD-L1 and TGF-β with blockade of collagen-LAIR-1 signaling. This combination enhances tumor recruitment and activation of CD8⁺ T cells, reduces M2 macrophage populations, and remodels collagens in the TME resulting in effective tumor control in murine models, which was not achievable with the individual components of the combination.