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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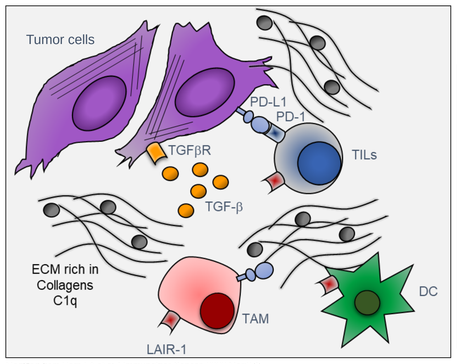
ABSTRACT

LAIR-1 is an immune inhibitory receptor expressed on several immune cell types including activated T cells, B cells, NK cells, macrophages, and dendritic cells. The ligands for LAIR-1 contain collagen-like domains which are commonly found in extracellular matrix collagens and complement component C1q. In numerous cancer types, including gastric, colon, ovarian, bladder, and others, upregulation of collagens has been shown to enhance tumor growth, metastases, and invasion while actively suppressing antitumor immunity. Although humans produce a natural, soluble decoy, LAIR-2, that 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 which combined NC410, a LAIR-2-Fc fusion protein capable of 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 demonstrate that the combination of NC410 and bintrafusp alfa more effectively controls in vivo tumor growth of the collagen rich MC38 colon carcinoma compared to either monotherapy. We hypothesize that this potent anti-tumor immune response is propagated through the synergy of activated tumor infiltrating lymphocytes and a repolarization of macrophages towards a tumoricidal phenotype. MC38 tumors treated with the combination of NC410/Bintrafusp alfa contained higher numbers of infiltrating CD4$^+$ and CD8$^+$ T cells and higher numbers of CD38$^+$ and MHCII$^+$ 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



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Lan Y et al. Sci Transl Med. 2018 Jan 17;10(424)

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.

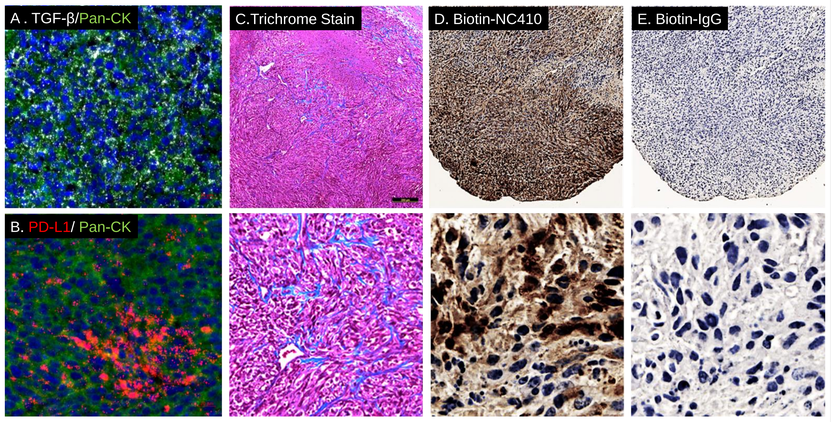
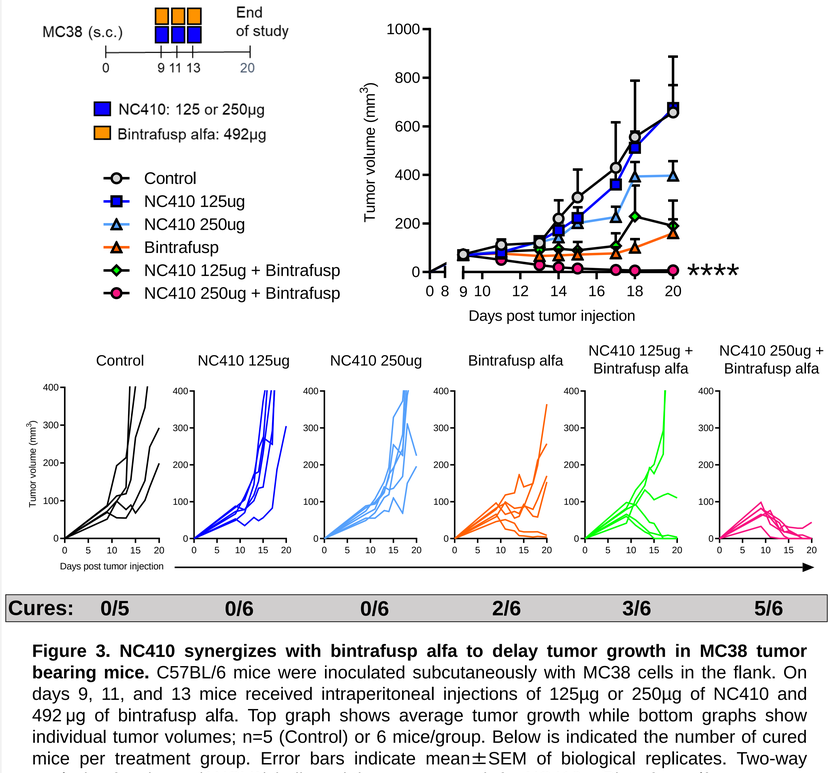


Figure 2. MC38-CEA tumors express high levels of TGF-β, PD-L1, and collagens and bind NC410. MC32a tumors were harvested from CEA.Tg mice and stained. A, B. Representative images of in situ hybridization for mRNA expression of TGFβ (white) and PD-L1 (red), simultaneously stained for pan-cytokeratin (Pan-CK, green) for tumor cells and DAPI (blue) for nuclei staining. C. Representative low (top) and high (bottom) magnification images of Masson's trichrome stain for collagen expression (blue). D, E. NC410 or control IgG were biotinylated and utilized in a chromogenic IHC type assay to determine binding of reagents to tumor tissue. Representative low (top) and high (bottom) magnification images for positive binding of reagent.

RESULTS



Cures:	0/5	0/6	0/6	2/6	3/6	5/6

Figure 3. NC410 synergizes with bintrafusp alfa to delay tumor growth in MC38 tumor bearing mice. C57BL/6 mice were inoculated subcutaneously with MC38 cells in the flank. On days 9, 11, and 13 mice received intraperitoneal injections of 125μg or 250μg of NC410 and 492 μg of bintrafusp alfa. Top graph shows average tumor growth while bottom graphs show individual tumor volumes; n=5 (Control) or 6 mice/group. Below is indicated the number of cured mice per treatment group. Error bars indicate mean±SEM of biological replicates. Two-way analysis of variance (ANOVA) indicated that tumor growth for NC410 + Bintrafusp alfa group was p ≤ 0.0001 (****) compared to all groups.

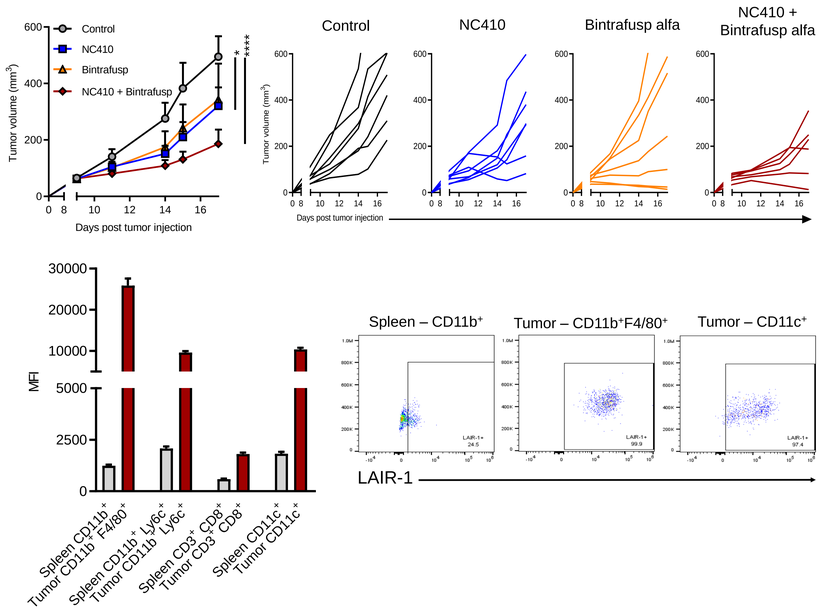


Figure 4. NC410 synergizes with lower dose bintrafusp alfa to delay tumor growth in MC38 tumor bearing mice. LAIR-1 is upregulated on immune cells in the TME. MC38 tumor bearing C57BL/6 mice received intraperitoneal injections of 250μg of NC410 and a lower dose (250 μg) of bintrafusp alfa on days 9, 11, and 13. Top left graph shows average tumor growth; top right graphs show individual tumor volumes; n=6 (NC410 + Bintrafusp alfa) or 7 mice/group. Error bars indicate mean±SEM of biological replicates. *p≤0.05; ****p≤0.0001 for two-way ANOVA. Flow cytometry analysis of mean fluorescence intensity (MFI) of LAIR-1 expression on immune cell subsets in the spleen compared to tumor tissue on day 17 in Control mice. Error bars indicate mean±SEM. Scatter plots show representative percentages of LAIR-1$^+$ immune cells in the spleen and tumor.

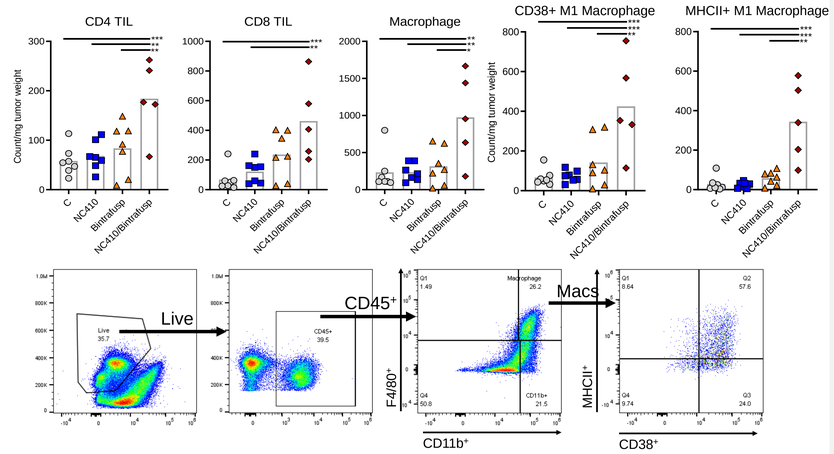
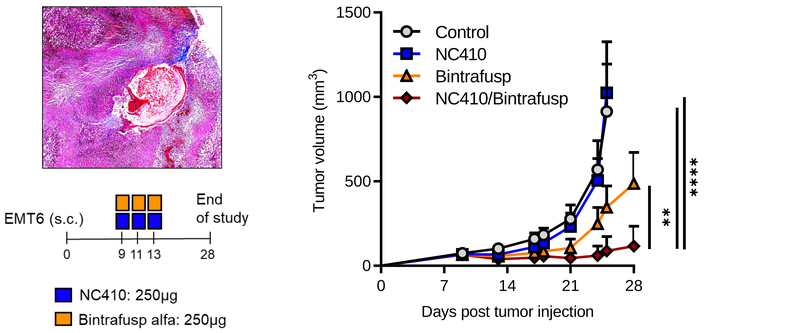


Figure 5. NC410 plus bintrafusp alfa combination therapy increases numbers of T cells and M1 macrophages in the tumor microenvironment. Flow cytometry analysis of tumors from Figure 4 shown as immune cell numbers per tumor weight on day 17. Individual points represent data from one tumor. *p≤0.05; **p≤0.01; ***p≤0.001; for one-way ANOVA followed by Tukey's post hoc test. Representative scatter plots show gating strategy for determination of CD38$^+$ and MHCII$^+$ M1 macrophages in MC38 tumors.



Cures:	0/9	1/9	2/9	8/9

Figure 6. NC410 synergizes with bintrafusp alfa to delay tumor growth in EMT6 tumor bearing mice. Representative image of Masson's trichrome stain of high collagen expression (blue) in EMT6 tumors. BALB/c mice were inoculated subcutaneously with EMT6. On days 9, 11, and 13 mice received intraperitoneal injections of 250ug of NC410 and 250 μg of bintrafusp alfa. Top graph shows average tumor growth while bottom graph shows individual tumor volumes; n=8 (Control) or 9 mice/group. Below is indicated the number of cured mice per treatment group. Error bars indicate mean±SEM of biological replicates. Two-way ANOVA indicated that tumor growth for NC410 + Bintrafusp alfa group was significantly delayed compared to all groups (**p≤0.01; ****p≤0.0001)

Conclusions

- Immune suppressive collagens are highly expressed in numerous murine models of cancer.
- LAIR-1 is an immune inhibitory receptor expressed on T cells and myeloid cells in the tumor microenvironment.
- NC410 synergizes with bintrafusp alfa to delay tumor growth in MC38 and EMT6 tumor bearing mice.
- NC410 plus bintrafusp alfa combination therapy increases numbers of T cells and M1 macrophages in the tumor microenvironment.