mRNA Delivered TIGIT-Fc-LIGHT Potentiates Anti-Tumor Activity in the Setting PD-1 Acquired Resistance #502

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Abstract

Background: Previously, we characterized the bispecific Fc fusion protein (TIGIT-Fc-LIGHT) that blocks the PVR checkpoint axis and agonizes HVEM and LTβR through LIGHT. In a preclinical tumor model of acquired resistance to PD-1 blockade (CT26/AR), TIGIT-Fc-LIGHT promoted antitumor activity, but the mechanism was not elucidated. LIGHT can promote vascular remodeling through LTβR/HVEM and linking LIGHT to a vascular targeting peptide (VTP) is one strategy to enrich HEV/TLS formation in the TME. TNFRs (e.g. HVEM/LTβR) are kinetically regulated and localizing co-stimulation to a tissue may not be sufficient for maximum efficacy in the absence of durable exposure to the TNF-ligand that aligns with the expression pattern of the target receptor. We hypothesized that tumor checkpoint receptor engagement via TIGIT could also localize TIGIT-Fc-LIGHT to the TME and TIGIT-Fc-LIGHT delivered as an mRNA/LNP, could prolong therapeutic exposures to match the expression of the cognate TNF-receptors; to promote combined HEV/TLS formation, checkpoint blockade, and anti-tumor immune responses.

Results: *In vivo* delivery of mRNA/LNP encoding TIGIT-Fc-LIGHT led to rapid production of functional hexameric fusion protein, increasing overall exposure (AUC) relative to the recombinant protein by ~28-fold over 96 hours in serum and ~135-fold higher within tumors. This resulted in significant tumor growth delay, which improved in combination with anti-PD(L)1. This anti-tumor activity was associated with increased serum innate and adaptive immune cytokines and tumor infiltration of antigen-specific CD8+ T cells. In the RIP-Tag pancreatic neuroendocrine model, TIGIT-Fc-LIGHT increased intratumoral MECA79 staining (HEV), TLS formation, and infiltration of cytotoxic T cells and macrophages.

Conclusions: Our findings underscore the potential of delivering biologics as lipid-encapsulated mRNAs. The delivery of TIGIT-Fc-LIGHT mRNA resulted in high expression in the serum and tumor following a single administration. TIGIT-Fc-LIGHT exposure was associated with tumor vasculature remodeling, promoting immune cell infiltration and anti-tumor efficacy. The versatility of mRNA therapeutics and the ability to fine-tune expression/exposure could mark a significant advancement in immunotherapy.

Ethics Approval: All murine animal studies have been conducted by and with the approval of an Institutional Animal Care and Use Committee (IACUC) and reviewed and approved by a licensed veterinarian.

TNF-Receptor Expression Kinetics

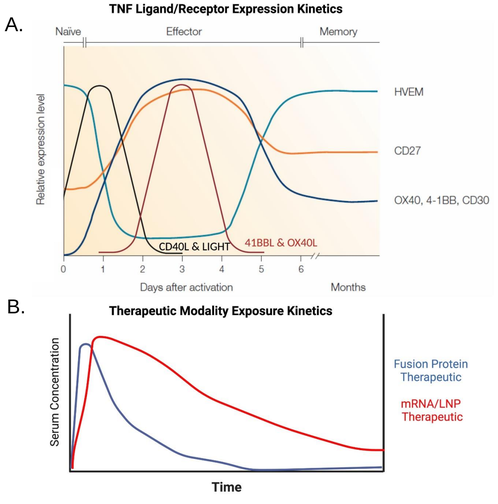

Figure 1. Challenge in therapeutically targeting TNF receptors. (A) As immune cells become activated and differentiate, TNF receptors are expressed with varying kinetics during this time. (B) Therapeutically targeting these receptors effectively is a challenge since the expression kinetics of the therapeutic being delivered may not match the expression kinetics of the native receptors. mRNA/LNP formulations which express the payload protein with extended kinetics, may present an opportunity to activate multiple TNF receptors during this immune activation / differentiation process. Shown are approximate expression patterns of TNF superfamily ligands/receptors and theoretical serum kinetics of fusion protein or mRNA/LNP therapeutics.

Proof of Concept Achieved with Hexameric Bifunctional Fusion Protein

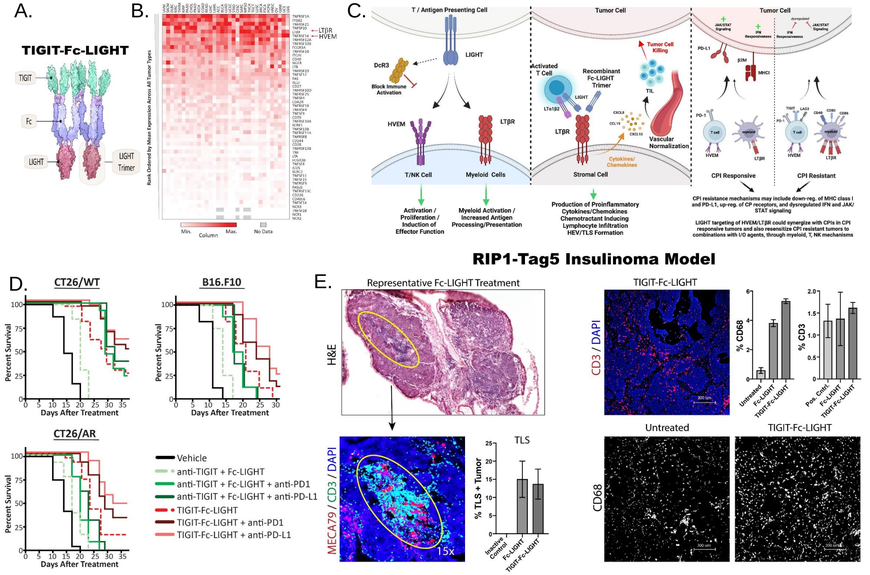

Figure 2. The generation of hexameric TIGIT-Fc-LIGHT and the evaluation of efficacy in a range of preclinical models. (A) TIGIT was selected as a representative checkpoint and (B) LIGHT was selected as an immune agonist, due to the high expression of its cognate receptors (HVEM and LTβR) in cancer (TCGA). (C) LIGHT plays a broad immune stimulatory role, including T/myeloid cell activation, induction of proinflammatory cytokines/chemokines, and promotion of tertiary lymphoid structures (TLS) and high-endothelial venules (HEV) within a tumor. TIGIT-Fc-LIGHT is hexameric due to a combination of Fc-mediated dimerization and native LIGHT trimerization. (D) TIGIT-Fc-LIGHT is efficacious as both monotherapy and in combination with anti-PD(L)1 in the CPI responsive colorectal CT26, the Shattuck-generated CPI acquired resistance version of CT26, and the CPI primary resistant B16.F10 melanoma models. (E) TIGIT-Fc-LIGHT induces TLS/HEV (by H&E and confirmed using MECA79/CD3) formation and increased tumor infiltration of CD3+ T cells and CD68+ macrophages (% by surface area) in the RIP1-Tag5 pancreatic insulinoma model.

Mechanistic Differentiation of mRNA Delivery

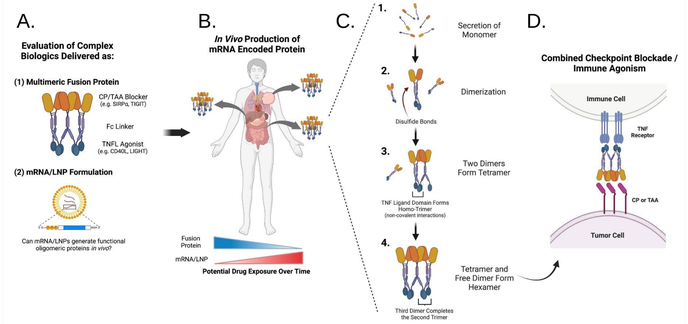

Figure 3. Encoding complex fusion proteins with mRNA/LNP. (A) mRNA/LNP can induce *in vivo* production of complex biologics with (B) favorable expression and exposure kinetics compared to the delivery of a recombinant protein. (C) Self-assembly of subunits could facilitate increased intra-tumoral delivery of the hexameric protein through increased serum and (D) tumor exposure.

mRNA Increased Drug Exposure and Efficacy over Fusion Protein

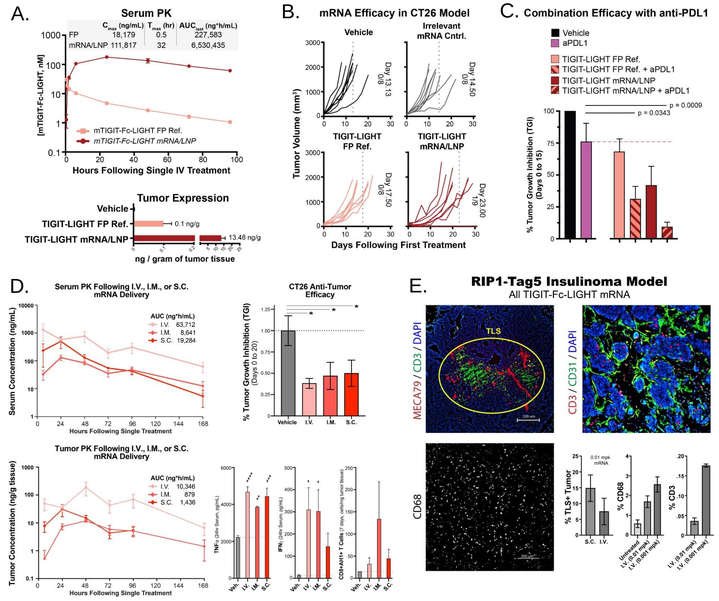

Figure 6. mRNA delivery of TIGIT-Fc-LIGHT significantly increased serum/tumor exposure and anti-tumor efficacy. (A) TIGIT-Fc-LIGHT mRNA serum PK (top) and expression within CT26 tumors (bottom) 24 hrs after delivery (0.3 mpk, I.V.). (B) mRNA delayed tumor growth at a higher rate than the fusion protein (5 mpk, I.V.)(shown: average days each group reached tumor burden, group size, and # of animals rejecting the tumor). (C) mRNA delivered TIGIT-Fc-LIGHT synergized with anti-PDL1 (2.5 mpk, I.P.). (D) I.V., I.M., and S.C. delivered TIGIT-Fc-LIGHT mRNA (0.1 mpk) all resulted in significant exposures in serum (top) and tumor (bottom) detected at least 1 week following a single injection, resulting in delayed growth of CT26 tumors, increased serum IFNγ and TNFα, and increased tumor infiltration of antigen-specific AH1-tetramer+ CD8 T cells (right). (E) TIGIT-Fc-LIGHT mRNA induced TLS formation and increased tumor infiltration of CD3+ T cells and CD68+ macrophages in the RIP1-TAG5 pancreatic insulinoma model.

Conclusions

- TIGIT-Fc-LIGHT induced TLS/HEV formation within tumors, and significantly extended survival in mouse models of checkpoint-responsive, checkpoint-primary resistant, and checkpoint-acquired resistance tumors.
- mRNA/LNP formulations of TIGIT-Fc-LIGHT were shown to encode functionally active, hexameric proteins *in vivo*.
- TIGIT-Fc-LIGHT mRNA induced intra-tumoral TLS/HEV, increased the infiltration of T cells and macrophages, delayed tumor growth, and outperformed the corresponding fusion protein.
- mRNA therapeutics are an exciting approach in oncology and other indications, where increased expression and exposure of the payload differentiates from the delivery of a recombinant protein, as higher mRNA encoded protein exposure may align better with target ligands and receptors that are dynamically regulated.

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