INTRODUCTION
Many cancers suppress the natural immune response by activating inhibitory checkpoints between tumor and immune cells. Antibodies against checkpoint molecules such as PD-1 and PD-L1 have become a mainstay of immunotherapy, but not all patients have exhibited robust responses. TIGIT is an alternative checkpoint molecule that is active in non-small cell lung cancer (NSCLC). Antagonistic anti-TIGIT antibodies promote T-cell activation and NK-cell cytotoxicity, thereby activating the patient’s innate anti-tumor immunity. The present study aims to characterize a panel of anti-TIGIT antibodies for the development of an anti-TIGIT/PD-1 bispecific antibody, providing an improved treatment option for NSCLC.

METHODS
1. A series of anti-TIGIT antibodies were identified via phage panning of a naive human antibody library.
2. Preliminary binding screens were performed and positive clones were moved into mammalian expression vectors.
3. Antibodies were produced in Expi293 cells and purified via immune cells. Antibodies against checkpoint molecules have been run to test binding, kinetics, and competition with CD155.
4. All antibodies except TIG2 completely inhibited CD155 binding to TIGIT. TIG2 inhibited 50% of CD155 binding after saturation with the first antibody, indicating that it binds another epitope of TIGIT.
5. Though all antibodies bound TIGIT, kinetic comparison to the control antibodies indicated the need for further improvement.
6. The protein modeling data further identified that these antibodies target 2.5-3 epitopes of TIGIT. There are also two antibodies that directly compete with CD155's binding site, suggesting a direct method of blockade.
7. Corresponding paratopes of antibodies can be used as focus points for targeted mutagenesis to bring affinity up to clinically required levels.
8. The selected antibodies can then be further tested with in vitro biosays for future incorporation into therapeutics for NSCLC.

RESULTS

Figures 2 & 3: The binding curves demonstrate the binding of antibodies at various concentrations to TIGIT. Compared to the positive control antibodies (22G2 and 1SA6), TIG6, G1A7, and P6H12 demonstrated higher binding at lower concentrations. G2E8 demonstrated similar binding site sequence to Group 2, but the competition data indicated interaction with a similar TIGIT epitope as Group 1. The competition assay was conducted using the in-tandem method, which saturates the target (TIGIT) with the saturating antibody and tests binding of the competing antibody. The antibodies listed in the column represent the saturating antibodies and those listed across the top represent the competing antibodies. All antibodies except TIG2 completely inhibited CD155 binding to TIGIT, as demonstrated by the lack of CD155 binding after saturation. TIG2 inhibited 50% of CD155 binding after saturation with the first antibody, indicating that it binds another epitope of TIGIT.

Figures 4: The binding curves for anti-TIGIT scFv-Fc are shown.

Figures 5-7: 4 groups were identified based on binding site sequence similarity and competition data. Group 1: CD155, 22G2, TIG6, TIG6, P5610, P6H12, and G2E8. Group 2: TIG2, Group 3: P6G7 and 1GA7. Group 4: G1DH, G3P9, and 1SA6. Group 1’s binding site sequence had some overlap with the binding site sequences of Groups 2 and 3. Group 4’s binding site sequence was distinct from those of Groups 1, 2, and 3. Group 3 had a similar binding site sequence to Group 2, but the competition data indicated a similar TIGIT epitope as Group 1. The competition data also indicated that TIG2 interacts with a different TIGIT epitope than Group 1. This data supports the possibility that these antibodies target 2.5-3 epitopes of TIGIT.

REFERENCES

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Characterization of novel anti-TIGIT antibodies for potential anti-tumor activity
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