AN3025: an immune-stimulatory anti-human TNFR2 antibody with strong anti-tumor activity

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ABSTRACT

Background:

The unique biology and expression pattern of tumor necrosis factor receptor-2 (TNFR2) make it an attractive therapeutic target for immuno-oncology. TNFR2 highly expresses on a subset of regulatory T cells (Tregs) and MDSCs within tumor microenvironment where it activates these cells through nuclear factor kappa B (NF-kB) pathway. TNFR2$^+$ Treg has been shown to be most suppressive among all Treg populations in tumor. In addition, TNFR2 is also abundantly expressed on the surface of many human tumors. TNFR2 blocking antibody is expected to relieve TNFR2-mediated immunosuppression and inhibit TNFR2-expressing tumor cell survival. AN3025 is a novel humanized IgG1 (variant) anti-hTNFR2 antibody that is currently under preclinical development. The immunomodulatory and anti-tumor activity of AN3025 were evaluated both *in vitro* and *in vivo*.

Materials and methods:

AN3025 was generated through rabbit immunization followed by phage display, then it was humanized by CDR grafting. The binding affinity and specificity were studied by ELISA and FACS. The ability of AN3025 to mitigate TNF/TNFR2 signaling pathway was characterized using hTNFR2 overexpressing Jurkat cell line in vitro. The in vivo anti-tumor activity was evaluated in hTNFR2 humanized mouse model bearing MC38 tumor. The tumor samples from control and AN3025 treated mice were taken for further FACS and RNA seq analysis.

Results:

AN3025 binds to the extracellular domain of human TNFR2 with sub-nanomolar affinity and specificity. It cross-reacts with cynomolgus TNFR2 with similar affinity, but not with mouse or rat TNFR2. Mechanistically, AN3025 blocks the binding of TNFα to TNFR2 and inhibits TNFα induced hTNFR2 overexpressing Jurkat cell death, whereas it lacks agonist activity towards TNFR2 even in the presence of hFC crosslinking. In vivo AN3025 significantly inhibits MC38 tumor growth as a monotherapy in hTNFR2 mouse model, while has no impact on body weight. Subsequent FACS analysis suggests a decrease in Tregs% in AN3025 treated tumor. RNA seq suggests immune activation such as increased IFN-gamma and Granzyme expression. In addition, AN3025 enhances anti-tumor efficacy of mPD-1 antibody in a combination study.

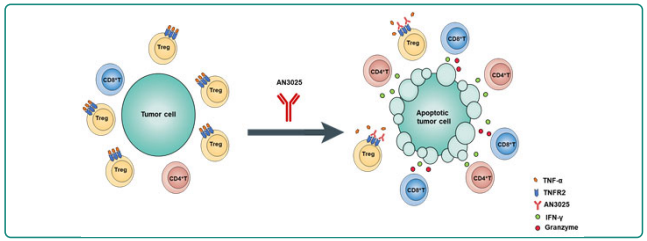
Conclusions:

AN3025 is an anti-hTNFR2 antibody and demonstrates immunomodulatory activity and potent in-vivo anti-tumor efficacy, supporting its further clinical development for the treatment of human cancers.

Reference:

- Chen X, Subleski JJ, Kopf H, Howard OM, Mannel DN, Oppenheim JJ. Cutting edge: expression of TNFR2 defines a maximally suppressive subset of mouse CD4$^+$CD25$^+$FoxP3$^+$ T regulatory cells: applicability to tumor-infiltrating T regulatory cells. *J Immunol.* (2008) 180:6467–71. doi: 10.4049/jimmunol.180.10.6467.
- Chen X, Wu X, Zhou Q, Howard OM, Netea MG, Oppenheim JJ. TNFR2 is critical for the stabilization of the CD4$^+$Foxp3$^+$ regulatory T. cell phenotype in the inflammatory environment. *J Immunol.* (2013) 190:1076–84. doi: 10.4049/jimmunol.1202659.
- Nie Y, He J, Shirota H, Trivett AL, Yang K, Linman DM, et al. Blockade of TNFR2 signaling enhances the immunotherapeutic effect of CpG ODN in a mouse model of colon cancer. *Sci Signal.* (2018) 11:790. doi: 10.1126/scisignal.aan0790.
- Chen X, Oppenheim JJ. Targeting TNFR2, an immune checkpoint stimulator and oncoprotein, is a promising treatment for cancer. *Sci Signal.* (2017) 10:2328. doi: 10.1126/scisignal.aal2328.

RATIONALE FOR ANTI-TUMOR ACTIVITY OF AN3025



RESULTS and DISCUSSION

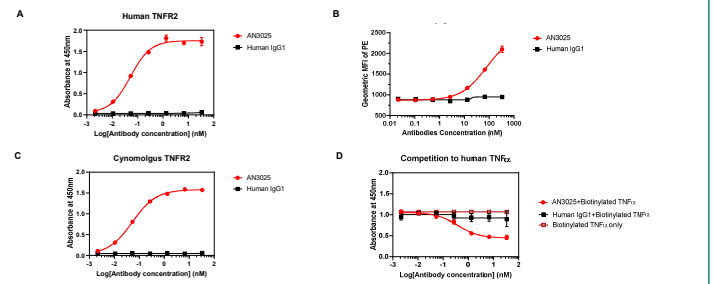


Figure 1. AN3025 binds to human TNFR2 and blocks its binding to TNFα. (A) ELISA Binding of AN3025 to human TNFR2 on plate (EC_{50}=0.052nM).(B) Cellular binding of PE-Conjugated AN3025 to human TNFR2 over-expressed Jurkat cells. (C) ELISA binding of AN3025 to cynomolgus TNFR2 on plate (EC50=0.053nM). (D) Competition ELISA binding of AN3025 with biotinylated human TNFα to human TNFR2 on plate. AN3025 does not recognize human TNFR1, mouse TNFR2 or rat TNFR2 (data not shown).

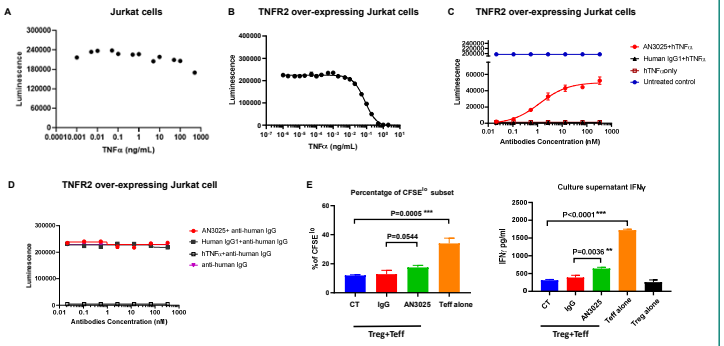


Figure 2. AN3025 inhibits TNFα-TNFR2 mediated Jukat cell death and enhances IFNγ production in Treg/Teff co-culture assay. (A) Human TNFα didn't affect parental Jurkat cell viability. (B) Human TNFα induced dose-dependent cell death of TNFR2 over-expressing Jurkat cells. (C) AN3025 limited human TNFα induced cell death of TNFR2 over-expressing Jurkat cells. (D) AN3025 lacked agonist activity towards TNFR2 even in the presence of hFC crosslinking. (A-D) CellTiter-Glo™ luminescent cell viability assays were performed on Jurkat cells or TNFR2 over-expressing Jurkat cells with various treatments for 24 hours. (E) AN3025 increased IFNγ production by Teff cells in Treg/Teff co-culture assay. Human Tregs were co-cultured with CFSE-labeled auto-CD4$^+$ Teff cells for 4 days. IFNγ quantification in the supernatant were detected by ELISA assay. CFSE^{lo} Teff cells were quantified by flow cytometry.

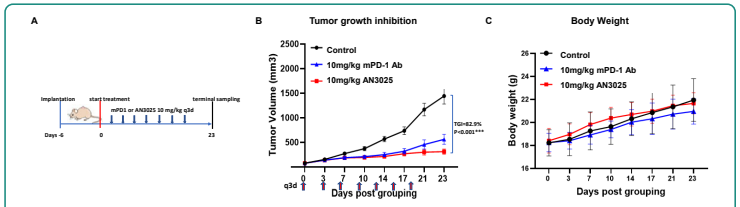


Figure 3. AN3025 significantly inhibits MC38 tumor growth as a monotherapy in hTNFR2 mouse model. (A) Schematic diagram of experimental design. Murine colon cancer MC38 cells (5E5) were implanted subcutaneously into homozygous humanized TNFR2 mice (female, n=8 each group). Mice were grouped when tumor volume reached approximately 100 mm^3. Then they were treated with 10 mg/kg AN3025 or mouse PD-1 antibody every 3 days intraperitoneally for 7 doses in total. (B) Tumor volume measurement during the treatment. AN3025 significantly limited MC38 tumor growth as a monotherapy in hTNFR2 mouse. (C) Body weight record during the treatment. AN3025 treatments did not change mice body weight. Values were expressed as mean ± SEM. ***p<0.001.

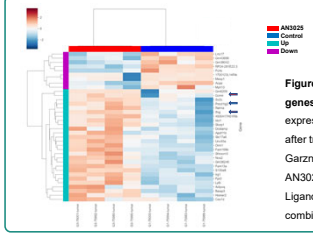


Figure 4. AN3025 increases expression of immune activation genes in the MC38 tumor tissue. RNA seq analysis of gene expression in the MC38 tumor tissues on humanized TNFR2 mice after treatment. Immune activation genes such as *Gzmk* (gene for Garznyme K) and *Ifng* (gene for IFN-γ) were upregulated by AN3025 treatment. The upregulation of *pdcd1 lg2* (gene for PD1 Ligand 2) suggested better efficacy might be achieved by combination treatment of AN3025 and PD1 antibody.

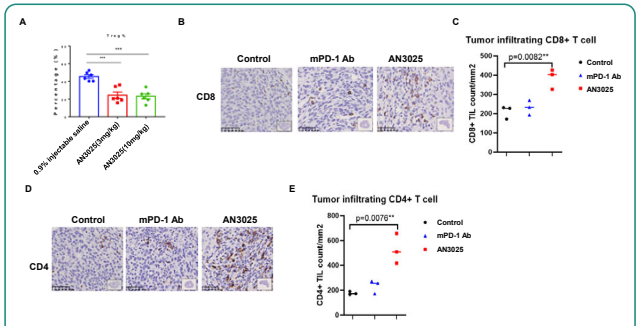


Figure 5. AN3025 decreases Treg cell number or frequency in the MC38 tumors and increases tumor infiltrating CD4$^+$ T cells and CD8$^+$ T cells. (A) AN3025 decreased Treg (CD45$^+$CD3$^+$CD4$^+$Foxp3$^+$) number or frequency in the total tumor-infiltrating CD4$^+$ T cells in MC38 tumors. Treg cell number or frequency in total CD4$^+$ T cells were quantified by flow cytometry (n=6 each group). (B) Representative immunohistochemistry of CD8 staining on MC38 tumors. (C) AN3025 increased CD8$^+$ T cells infiltration in the MC38 tumors. CD8$^+$ T cells were quantified by immunohistochemistry (n=3 each group). (D) Representative immunohistochemistry of CD4 staining on MC38 tumors. (E) AN3025 increased CD4$^+$ T cells infiltration in the MC38 tumors. CD4$^+$ T cells were quantified by immunohistochemistry (n=3 each group). ***P<0.001.

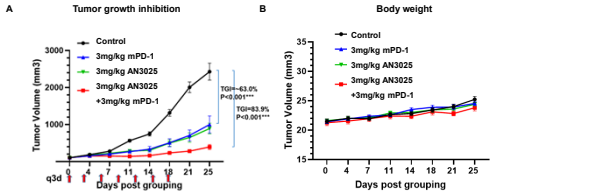


Figure 6. AN3025 enhances anti-tumor efficacy of mPD-1 antibody in combination study. Murine colon cancer MC38 cells (5E5) were implanted subcutaneously into homozygous humanized TNFR2 mice (female, n=8 each group). Mice were grouped when tumor volume reached approximately 100 mm^3. Then they were treated with 3mg/kg AN3025 or mouse PD-1 antibody or combination of AN3025 and mouse PD-1 antibody every 3 days intraperitoneally for 7 doses in total. (A) Tumor volume measurement during the treatment. The combination treatment of AN3025 and mouse PD-1 antibody decreased MC38 tumor growth with better efficacy than either monotherapies. (B) Body weight record during the treatment. Neither monotherapies nor the combination therapy changed mice body weight. Values were expressed as mean ± SEM. ***P<0.001.

CONCLUSION

We have developed an anti-TNFR2 antibody program (AN3025) with excellent antitumor activity.

- AN3025 binds human and cynomolgus TNFR2 with sub-nanomolar affinity.
- AN3025 blocks the binding of TNFα to TNFR2.
- AN3025 significantly inhibits MC38 tumor growth as a monotherapy in hTNFR2 mouse model.
- AN3025 promotes immune activation with reduced Treg but increased Teff tumor infiltration.
- Combination therapy of AN3025 and PD-1 antibody exhibits improved anti-tumor efficacy than respective monotherapies.