Targeting TNFR2 for Cancer Immunotherapy - Ligand blocking depletors versus receptor agonists

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1. Abstract

Despite the successes of current checkpoint inhibitors in cancer treatment, additional treatments are needed to help a larger fraction of patients. The therapeutic potential of targeting TNFR2 for cancer treatment has been previously indicated but the mechanism-of-action (MoA) of these reagents remains unclear, with conflicting data reported by different investigators.

We have identified and characterized a wide panel of human and mouse TNFR2-specific antibodies, generated from the n-CoDeR® F.I.R.S.T™ phage display platform. Based on their ability to block TNF-α:TNFR2 binding and to agonize or antagonize TNFR2 signaling, parallel human and mouse TNFR2-specific antibodies were identified. Two antibody variants with distinctly opposing in vitro activities were expressed in various IgG formats preferentially engaging activating FcγR (mIgG2a), inhibitory FcγR (mIgG1), or no FcγR (N297A Fc-mutated) and screened for in vivo antitumor activity. Both anti-TNFR2 antibody clones displayed anti-tumor efficacy but showed strikingly different FcγR-dependence. Further characterization demonstrated potent anti-tumor efficacy across several syngeneic in vivo cancer models (CT26, MC38 and B16), both as single agents, and when combined with anti-PD-1. In vivo mode-of-action studies indicated that the antagonist/blocking antibody caused intra-tumoral T reg depletion, while the agonist dramatic increased CD8⁺ T cell infiltration. Over time, both antibodies induce an increase in antigen specific effector T cells at the tumor site, improved CD8/T reg ratios, and tumor regression. In addition, the two antibodies similarly modulated the tumor myeloid content.

Two human lead candidate anti-TNFR2 antibodies are being developed for treatment of solid cancer; BI-1808, a ligand-blocking T reg depleting antibody and BI-1910, a TNFR2 agonist. BI-1808 is scheduled to enter Ph I in late 2020.

2. F.I.R.S.T™ - Phenotypic discovery of targets and human mAbs

Clinically relevant targets & mAbs

2. mAb generation
- αHuman αMouse
- Predefined receptors
- Target agnostic (TILs vs normal)

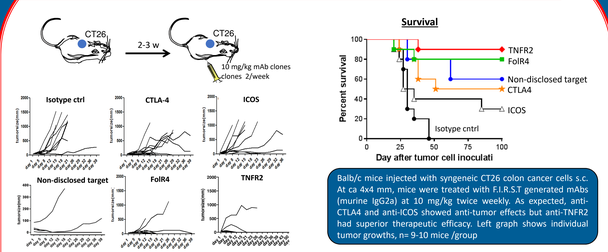
3. Functional Screening
- In vitro, ex vivo
- In vivo

4. Target deconvolution

1. Immune profiling
- Treg CD4⁺ CD8⁺ MΦ
- Phenotypic (FACS, RNA seq)
- Function (in vitro, vivo)

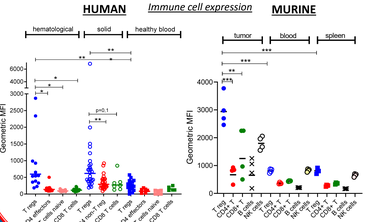
Output
1. Validation & quality of TILs for use in target agnostic cell-based functional discovery
2. Pre-defined candidate receptors

3. F.I.R.S.T™- Identifies TNFR2 & α-TNFR2 for Cancer Immunotherapy



Balb/c mice injected with syngeneic CT26 colon cancer cells s.c. At ca 4x4 mm, mice were treated with F.I.R.S.T generated mAbs (murine IgG2a) at 10 mg/kg twice weekly. As expected, anti-CTLA4 and anti-ICOS showed anti-tumor effects but anti-TNFR2 had superior therapeutic efficacy. Left graph shows individual tumor growths, n= 9-10 mice /group

Immune cell expression



TNFR2 is highly and specifically upregulated on T regs in all tumors investigated - both solid and lymphoma as well as human and murine.

The hematological cancer is diffuse large B cell lymphoma and mantle cell lymphoma patients. The solid cancer is ovarian cancer. Each dot is one patient. Similar data is observed in lower numbers of samples from in kidney, colorectal, lung, melanoma, breast, HNSCC and gastric cancer with no difference in between indications (data not shown).

Equivalent data is observed in CT26 tumor bearing mice.

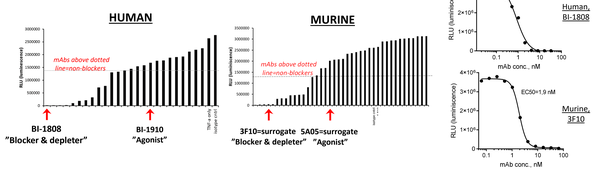
***= p<0,001, *=p<0,01 and *=p<0,05 as calculated using one-way ANOVA

4. mAb characterization matched human candidates and murine surrogates

Ligand-blocking assay

TNFR2 protein was coated in ELISA wells followed by incubation of nCoDeR mAbs specific for TNFR2. Soluble biotinylated TNF-α ligand and streptavidin-HRP was then added. Hence, a blocking antibody diminishes the HRP signal. TNF-α only or together with an isotype control antibody were used as negative controls.

Both ligand blocking and non-blocking human and murine TNFR2 specific antibodies generated



Agonism/antagonism assay #1

NK cells were isolated from human PBMCs and cultured with IL-2, IL-12. Concentration of IFN-γ was measured as a sign of activation and TNF-α ligand and isotype ctrl mAb were used as controls

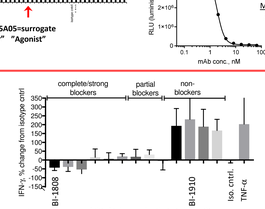


Figure shows mean +/- SD of 4 donors, all mAbs tested as hIgG1

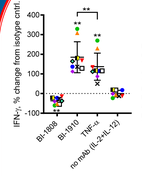


Figure shows individual values +/- SD of 9 donors, represented by one specific symbol. **= p<0,01 as calculated using one-way ANOVA. BI-1808 induce significantly lower IFN-γ production compared to either isotype cntrl (set to 0) or no antibody (+IL-2 & IL-12), while BI-1910 induces significantly more. BI-1910 also induces more IFN-γ compared to the positive cntrl. which is addition of 100 ng/ml TNF-α

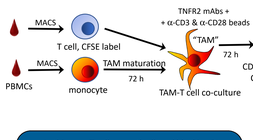
- In this assay, the ligand blocking BI-1808 is antagonistic and the non-ligand blocker BI-1910 is agonistic.
- The NK cells express TNFR2
- Addition of exogenous soluble TNF-α increases IFN-γ release ca 3-fold
- In the cultures, low concentrations of TNF-α (ca 100 pg/ml) is produced
- *Due to species differences in FcγR expression and TNF-α production, the NK cell assay is not transferrable to mouse*

Agonism/antagonism assay #2

T cells and CD14⁺ cells were isolated by MACS from human PBMCs. CD14⁺ cells were matured to TAM like cells for 3 days (using material derived from cancer patients) and thereafter co-cultured at different ratios (1:4 or 1:8 TAM:T cell) with CFSE labeled T cells for 48-72 h in presence of a-CD3 and a-CD28 beads. Proliferation, and CD25 upregulation were measured as a sign of activation.

Similar set-up for murine with splenic T cells from naive mice and CD11b⁺ cells from MC38 tumors

**= p<0,01 *=p<0,05 as calculated using one-way ANOVA. BI-1808 significantly inhibits, while BI-1910 significantly induces T cell proliferation, compared to isotype cntrl (set to 0). Equivalent data is seen with the respective murine surrogates (3F10 and 5A05)

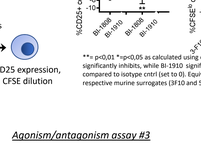


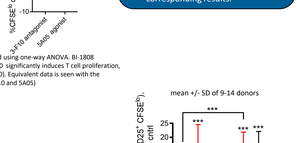
- In this pure T cell assay, the ligand blocking BI-1808 does not significantly alter proliferation or T cell activation. BI-1910 is still agonistic.
- When BI-1808 is strongly cross-linked, it behaves as an agonistic antibody indistinguishable from BI-1910. BI-1910 is not affected by cross-linking.

Agonism/antagonism assay #3

T cells were isolated by MACS from human PBMCs Cells were CFSE labeled and stimulated with anti CD3 and anti CD28. Cross-linking is mediated by hyFab', Fragments directed against the human Fc part (XL). After 72 h, proliferation, and CD25 upregulation were measured by FACS as a sign of activation.

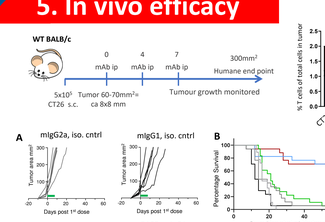
Similar set-up for murine with splenic T cells from naive mice ongoing.



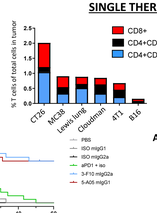


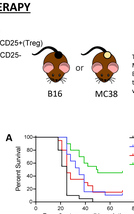
***= p<0,001 as calculated using one-way ANOVA. BI-1808 does not affect T cell proliferation compared to isotype cntrl. (set to 0), while BI-1910 induces significantly more.

5. In vivo efficacy

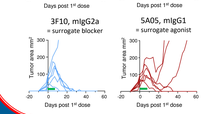


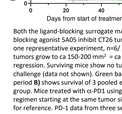
SINGLE THERAPY



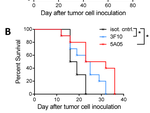


Treatment started at ca 6x6 mm for MC38 and at day 4 after injection for B16. Mice treated with 10 mg/kg 3 times as for CT26. Mice culled when volume >2000 mm³



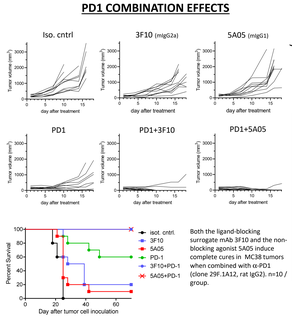


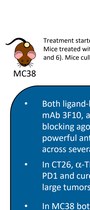
Both the ligand-blocking surrogate mAb 3F10 and the non-blocking agonist 5A05 inhibit CT26 tumor growth. **A)** shows one representative experiment, n=6/ group. Note that some tumors grow to ca 150-200 mm³ = ca 13x13 mm before regression. Surviving mice show no tumor growth upon re-challenge (data not shown). Green bars indicate treatment period **B)** shows survival of 3 pooled experiments, n=17 per group. Mice treated with α-PD1 using the same treatment regimen starting at the same tumor size have been included for reference. PD-1 data from three separate exp, n= 18.



Both the ligand-blocking surrogate mAb 3F10 and the non-blocking agonist 5A05 induce significant tumor-specific T cell responses, both in the tumor and in the periphery, here spleen.

PD1 COMBINATION EFFECTS



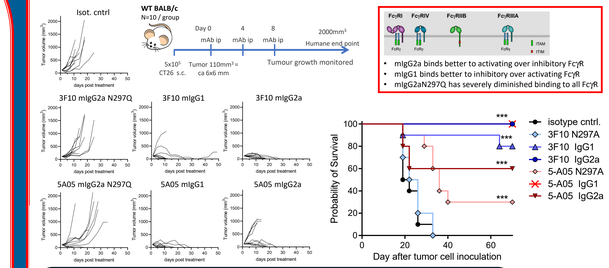


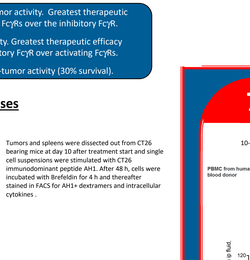
Treatment started at ca 150 mm³ or 6x7 mm Mice treated with 10 mg/kg 3 times (day 1,4 and 6). Mice culled when volume >2000 mm³

- Both ligand-blocking surrogate mAb 3F10, and the non-blocking agonist 5A05, induce powerful anti-tumor responses across several models.
- In CT26, α-TNFR2 outperforms PD1 and cures 75% of very large tumors
- In MC38 both antibodies synergize with α-PD1 to induce cures.
- In treatment resistant and poorly infiltrated B16, α-TNFR2 slows tumor growth. Combination effects under investigation

6. Mode-of-action

FcγReceptor dependency

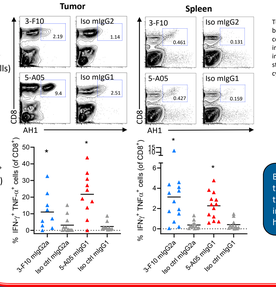




- mIgG2a binds better to activating over inhibitory FcγR
- mIgG1 binds better to inhibitory over activating FcγR
- mIgG2aN297Q has severely diminished binding to all FcγR

- "Blocker / deleter" 3F10 α-TNFR2 mAb has Fc:FcγR-dependent in vivo anti-tumor activity. Greatest therapeutic efficacy observed with isotype (mIgG2a) that preferentially engages activating FcγRs over the inhibitory FcγR.
- "Agonist" 5A05 α-TNFR2 mAb has Fc:FcγR-dependent in vivo anti-tumor activity. Greatest therapeutic efficacy observed with antibody isotype (mIgG1) that preferentially engages the inhibitory FcγR over activating FcγRs.
- "Agonist" 5A05 α-TNFR2 mAb also has some Fc:FcγR-independent in vivo anti-tumor activity (30% survival).

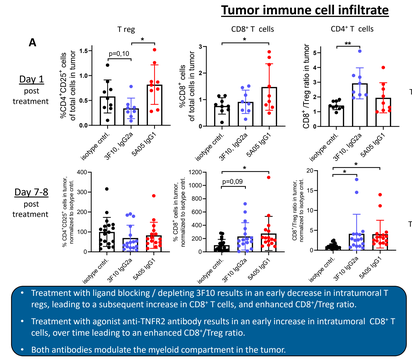
Tumor specific CD8⁺ T cell responses

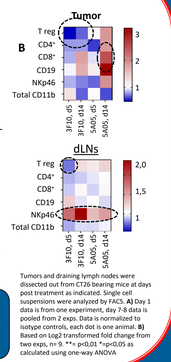


Tumors and spleens were dissected out from CT26 bearing mice at day 10 after treatment start and single cell suspensions were stimulated with CT26 immunodominant peptide AH1. After 48 h, cells were incubated with Brefeldin for 4 h and thereafter stained in FACS for AH1+ dextramers and intracellular cytokines .

Both the blocker /deleter 3F10 and the agonist 5A05 induce significant tumor-specific T cell responses, both in the tumor and in the periphery, here spleen.

Tumor immune cell infiltrate

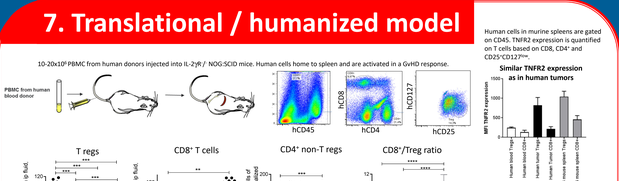


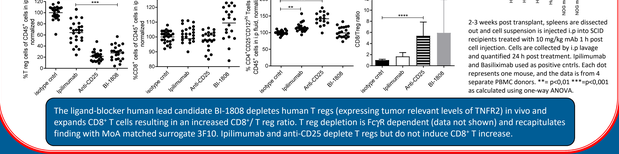


Tumors and draining lymph nodes were dissected out from CT26-bearing mice at days post treatment as indicated. Single cell suspensions were analyzed by FACS. **A)** Day 1 data is from one experiment, day 7-8 data is pooled from 2 exps. Data is normalized to isotype controls, each dot is one animal. **B)** Based on Log2 transformed fold change from two exps, n= 9. **= p<0,01 *=p<0,05 as calculated using one-way ANOVA

- Treatment with ligand blocking / depleting 3F10 results in an early decrease in intratumoral T regs, leading to a subsequent increase in CD8⁺ T cells, and enhanced CD8⁺/Treg ratio.
- Treatment with agonist anti-TNFR2 antibody results in an early increase in intratumoral CD8⁺ T cells, over time leading to an enhanced CD8⁺/Treg ratio.
- Both antibodies modulate the myeloid compartment in the tumor.

7. Translational / humanized model



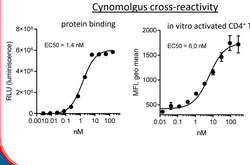


The ligand-blocker human lead candidate BI-1808 depletes human T regs (expressing tumor relevant levels of TNFR2) in vivo and expands CD8⁺ T cells resulting in an increased CD8⁺/ T reg ratio. T reg depletion is FcγR dependent (data not shown) and recapitulates finding with MoA matched surrogate 3F10. Ipilimumab and anti-CD25 deplete T regs but do not induce CD8⁺ T increase.

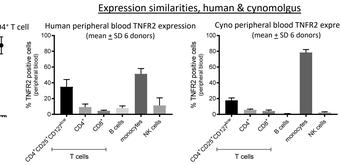
2-3 weeks post transplant, spleens are dissected out and cell suspension is injected i.p into SCID recipients treated with 10 mg/kg mAb 1 h post cell injection. Cells are collected by i.p lavage and quantified 24 h post treatment. Ipilimumab and Basiliximab used as positive cntrls. Each dot represents one mouse, and the data is from 4 separate PBMC donors. **= p<0,01 ***=p<0,001 as calculated using one-way ANOVA.

8. Toxicology & and safety

Cynomolgus cross-reactivity



Expression similarities, human & cynomolgus



- BI-1808 and BI-1910 (data not shown) are cross-reactive with cynomolgus TNFR2. TNFR2 is similarly expressed in cyno and human blood cells
- DRF tox study of BI-1808 showed no adverse events up to 200 mg/kg
- GLP tox study of BI-1808 completed (2, 20 and 200 mg/kg). NOAEL set to 200 mg/kg. Findings:
  - Reversible peripheral T cell reduction in all doses (ca 40-50% reduction). Similar findings in blood in mice using BI-1808 surrogate (data not shown)
  - Increase in neutrophils in a dose dependent manner. Similar findings in mice using surrogates (data not shown)
  - Transient increase in monocytes in a dose –dependent manner
  - Good PK.
- Tissue cross-reactivity GLP study of BI-1808 shows expected binding profile to immune cells (particularly T cells and some tissue-resident macrophages), no observed off-target effects.
- In vitro cytokine release assays (three different systems) performed with both BI-1808 and BI-1910. No significant increases in cytokine release observed
- In vivo cytokine release experiments (NOG mice engrafted with human PBMC until > 20% of the blood cells are human then i.v. challenged with antibody) performed with both BI-1808 and BI-1910. No signs of cytokine storms observed.

9. Summary & Future

Ligand blocking lead candidate antibody BI-1808 (and surrogate antibody 3F10):
- Depletes intratumoral T reg's in vivo, works best in depleting format and is dependent on activating FcγR's
- Induces/expands intratumoral CD8⁺ T cells
- Increases tumor-specific effector CD8⁺ T cells intratumorally and systemically
- Synergizes with α-PD1
- Is well tolerated in multiple systemic doses up to 200 mg/kg in cynomolgus monkeys. NOAEL in GLP tox study = 200 mg/kg

Agonistic lead candidate antibody BI-1910 (and surrogate antibody 5A05):
- Agonistic in all in vitro assays tested
- Agonises intratumoral CD8⁺T cells in vivo, works best in mIgG1 format, partly FcγR dependent and partly FcγR independent effects
- Increases tumor-specific CD8⁺ T cells intratumorally and systemically
- Synergizes with α-PD1

BI-1808 and BI-1910 have different characteristics which translate into different Mode of Action and different FcγR dependency. This could affect both the types of patients that benefit from either of the mAbs as well as safety profile.

BI-1808 will enter a PhI study in patients with solid cancer in Q4 2020

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