[2343] - The Therapeutic Antibody Tregalizumab (BT-061) Induces Activation of Regulatory T Cells by Engaging a Unique CD4 Mediated Signaling that Strongly Differs from Signaling Events Induced by Standard Anti-CD4 Antibodies

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Abstract

Background: The humanized CD4 specific monoclonal antibody (mAb) tregalizumab is currently being tested in a phase II clinical trial in Rheumatoid Arthritis. In contrast to other anti-CD4 antibodies, tregalizumab is able to activate the suppressive properties of regulatory T cells (Tregs). Since tregalizumab was the first and only humanized anti-CD4 antibody described to be able to activate Tregs we asked for the underlying reason for this specific and unique functionality. To elucidate on the mode of action of this mAb, we focused on the signaling pathways engaged by tregalizumab.

Methods: Signaling events after cross-linking of tregalizumab, other anti-CD4 antibodies (RPA-T4, MT310, QS4120 or B-A1) or anti-CD3 (OKT-3) treatment were analyzed using intracellular staining of phosphorylated proteins (Lck, ZAP-70, LAT, SLP-76, PLC-γ, MEK, Itk, ERK, PKC, MAPK and NF-κB). Furthermore, the ability of tregalizumab to induce suppressive properties in Tregs was evaluated using a mixed lymphocyte reaction system. Other CD4 antibodies were included as controls. Additionally, the binding mode of tregalizumab to soluble CD4 was resolved by co-crystallization and subsequent x-ray crystallography with a resolution of 2.9 Å.

Results: Upon binding of tregalizumab to the CD4 molecule on T cells and subsequent cross-linking, an intracellular signal was induced. As described for anti-CD3 or other anti-CD4 antibodies, phosphorylation of the CD4 associated kinase Lck was observed. Nonetheless, significant signaling strength differences were observed between the different antibodies. Although tregalizumab induced the lowest phosphorylation signal on Lck, further downstream molecules were also activated. Tregalizumab mediated signaling additionally led to phosphorylation of ZAP-70, LAT, SLP-76, PLC-γ and MEK, thus engaging several components of the T cell receptor pathway. However, tregalizumab induced no phosphorylation of Itk, ERK, PKC, MAPK and NF-κB as observed for anti-CD3 treatment or other anti-CD4 antibodies tested. Although inducing the weakest phosphorylation signal of all anti-CD4 antibodies, only tregalizumab was able to induce full functional activation of Tregs via CD4.

The new mode of action of tregalizumab may be explained by the special binding epitope. While all other tested anti-CD4 antibodies bound to domain 1 of CD4, the crystal structure of tregalizumab in complex with CD4 revealed binding to domain 2.

Conclusion: In summary, we hypothesize that binding to domain 2 of CD4 may be the underlying reason for inducing weak but unique signaling in CD4 T cells that is sufficient to activate the function of Tregs without activation of T effector cells. Thus, tregalizumab represents a unique and novel mode of action for treatment of autoimmune diseases with insufficient Treg activity. A phase IIb clinical trial is currently ongoing in Rheumatoid Arthritis in european countries to further evaluate clinical use of tregalizumab (Biotest Study 979).

Background

Regulatory T cells (Tregs) are a specialized subset of CD4 positive T cells expressing high levels of the IL-2 receptor (CD25) and the forkhead box transcription factor FoxP3. Tregs are crucial for maintaining immune homeostasis and preventing fatal autoimmunity. Functional or numerical defects in Tregs is associated with disease activity in multiple sclerosis, psoriasis, rheumatoid arthritis (RA) and other diseases[1-3]. Circulating Tregs have to be considered non-activated and must be activated by presentation of auto-antigens. The therapeutic antigen-independent activation of Tregs thus represents an attractive opportunity to treat autoimmune diseases.

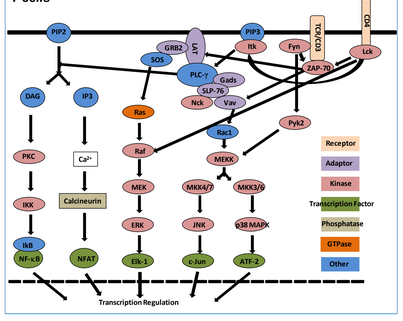
Tregalizumab (BT-061):

- Humanized agonistic monoclonal IgG1 antibody
- Binds to the human CD4 molecule on T helper cells and Tregs
- Selectively activates Tregs but not effector T cells
- Only therapeutic anti-CD4 antibody described to activate Tregs
- No evidence of ADCC or CDC and non-depleting
- Therapeutic antibody that is currently being developed clinically in rheumatoid arthritis and psoriasis
- The ability to activate Tregs offers great potential for any autoimmune indication where Tregs may play a role
- Therefore, BT-061 represents a unique and new mode of action not observed with other therapeutics
- However, the exact mechanism is still under intensive research and not completely understood

➡ We aimed at further deciphering the mode of action of BT-061 to explain why the antibody is able to activate Tregs without activation of effector T cells

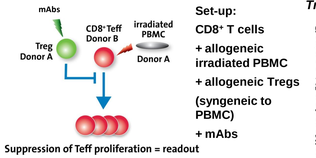
➡ To elucidate on the mode of action of BT-061, we focused on the signaling pathway (Figure 1), the Treg activation and the domain specificity of BT-061 in comparison to that of other anti-CD4 mAbs.

Figure 1: Simplified overview of the signaling pathway in T cells



Activation of suppressive properties of Tregs

Figure 2: Assay principle to determine Treg activation

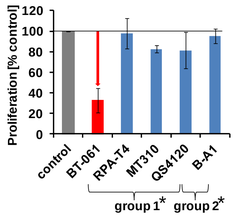


Set-up:
CD8⁺ T cells
+ allogeneic irradiated PBMC
+ allogeneic Tregs (syngeneic to PBMC)
+ mAbs

Suppression of Teff proliferation = readout

Tregs were transferred to a mixed lymphocyte reaction in which CD8⁺ T cells were stimulated with irradiated allogeneic PBMCs while the Tregs and PBMCs were syngeneic. Following this, the indicated mAb was added to the culture.

Figure 3: Only BT-061 is able to induce full functional activation of Tregs via CD4

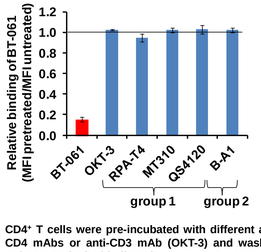


*** mAbs were grouped according to their phosphorylation signal strength induction: group 1 induces a higher phosphorylation signal intensity than OKT-3 while group 2 induces the same signal intenstiy as OKT-3**

➡ **In contrast to BT-061 the other tested anti-CD4 antibodies do not or only minimally activate Tregs**

Epitope recognized by anti-CD4 mAbs

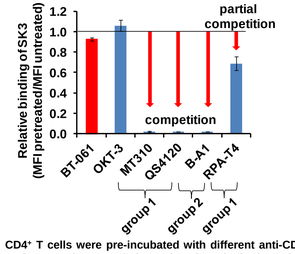
Figure 4: None of the standard anti-CD4 mAbs competes for binding of BT-061



CD4⁺ T cells were pre-incubated with different anti-CD4 mAbs or anti-CD3 mAb (OKT-3) and washed. Subsequently the binding ob BT-061 was measured.

➡ **BT-061 recognizes a different epitope than the standard anti-CD4 mAbs**

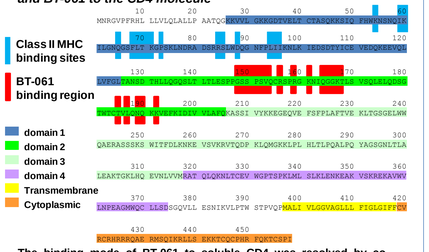
Figure 5: Standard Anti-CD4 mAbs compete for SK3 binding site on domain 1



CD4⁺ T cells were pre-incubated with different anti-CD4 mAbs or anti-CD3 mAb (OKT-3) and washed. Thereafter the binding of SK3, which is known to bind to the domain 1, was assessed.

➡ **All tested standard anti-CD4 antibodies bind to domain 1 of the CD4 molecule**

Figure 6: Crystal structure analysis: Binding of MHC class II and BT-061 to the CD4 molecule

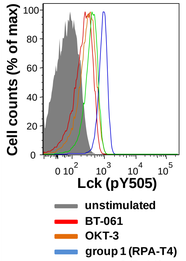


The binding mode of BT-061 to soluble CD4 was resolved by co-crystallization and subsequent x-ray crystallography with a resolution of 2.9 Å. The binding sites of BT-061 are displayed on the primary structure of the CD4 molecule.

➡ **BT-061 binds to a conformational epitope on domain 2 of the CD4 molecule**

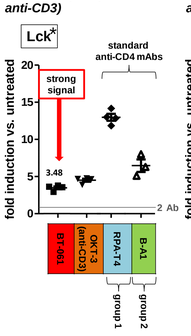
Signaling induced by BT-061

Figure 7: BT-061 induces phosphorylation of Lck



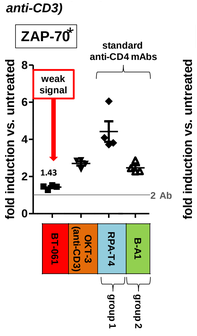
CD4⁺ T cells were pre-incubated with BT-061, anti-CD3 (OKT-3) or standard anti-CD4 mAbs. The antibodies were cross-linked by a secondary antibody. After stimulation for 10 min phosphorylation of different signaling molecules was measured using flow cytometry.

a) Strong signal induced by BT-061 (compared to anti-CD3)



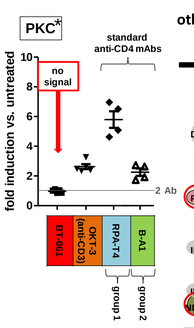
BT-061 agonism induces strong phosphorylation of Lck, PLC-γ and SLP-76

b) Weak signal induced by BT-061 (compared to anti-CD3)



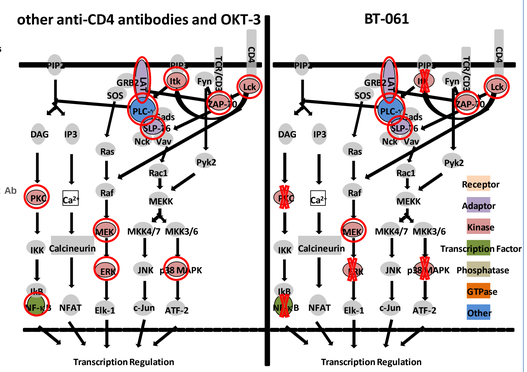
BT-061 signaling induces weak phosphorylation of ZAP-70, LAT and MEK

c) No signal induced by BT-061



Itk, ERK, MAPK and NF-κB are phosphorylated by signals of standard anti-CD4 mAbs but not by BT-061 signaling

Figure 8: Effects of BT-061, OKT-3 and standard anti-CD4 mAbs on the signaling pathway in T cells



Although the signaling cascade used by BT-061 involves many molecules that are phosphorylated by standard anti-CD4 or anti-CD3 (OKT-3) mAbs, the molecules Itk, ERK, MAPK, PKC and NF-κB are not engaged by the BT-061 pathway

*** For simplicity reasons only RPA-T4 from group 1 was presented. MT310 and QS4120 show similar results for all molecules analysed. Representative signaling molecules are shown.**

Conclusions

- BT-061 is the only anti-CD4 antibody that is able to induce full functional activation of Tregs via CD4.
- BT-061 recognizes a unique, conformational epitope on domain 2 on the CD4 molecule that is not recognized by the other tested anti-CD4 mAbs.
- BT-061 is the only anti-CD4 antibody that uses a different, weaker signaling pathway than the other anti-CD4 mAbs.
- The special epitope recognized by BT-061 may be the reason for inducing a weak but unique signaling in CD4 T cells that is sufficient to activate the function of Tregs without activation of effector T cells.
- Obviously not every signaling via CD4 is the same, which explains the uniqueness of BT-061.

Literature:

1) Haas, J., et al. Reduced suppressive effect of CD4⁺CD25^{high} regulatory T cells on the T cell immune response against myelin oligodendrocyte glycoprotein in patients with multiple sclerosis. Eur J Immunol 35, 3343-3352 (2005).
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3) Lindley, S., et al. Defective suppressor function in CD4⁺CD25⁺ T-cells from patients with type 1 diabetes. Diabetes 54, 92-99 (2005).

Conflict of interest declaration:

B. Helling, B. Daelken, S. Aigner, C. Zuber, M. König, A. Engling, F. Osterroth, N. Czeloth, C. Uherek: Biotest AG: Employment (full or part-time), Holger Wallmeier: Biotest AG: Consulting fees or other remuneration. Biotest sponsored the study. Abbott provided financial support to Biotest for scientific and clinical evaluation of BT-061.