
The Novel Regulatory T Cell (Treg) Agonistic Monoclonal Antibody (mAb) Tregalizumab (BT-061): Further Characterization of Mechanism of Action, Epitope Binding, and Clinical Effects in Patients with Rheumatoid Arthritis

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Disclosure

This study was designed and sponsored by Biotest AG.

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Abstract

We have previously demonstrated that the humanized, agonistic mAb, tregalizumab, selectively activates Tregs by binding to CD4, leading to phosphorylation of the ZAP70 protein kinase associated with the T cell receptor complex. This induces Treg specific signaling events which result in activation of Treg suppressive functions. To further characterize the unique mode of action of tregalizumab, the crystal structure of tregalizumab in complex with CD4 was analyzed, and the epitope was compared to that of other CD4 antibodies.

Analysis of the crystal structure showed that tregalizumab binds to a conformational epitope of the IgG-like C2 type 1 domain of CD4. In contrast to most other CD4 mAbs analyzed, tregalizumab did not interfere sterically with MHC class II binding to CD4. In competition experiments it was demonstrated that this epitope is distinct from the epitopes recognized by the other CD4 antibodies analyzed, which did not show activation of Tregs. It was identified that binding to the IgG-like C2 type 1 domain of CD4 is not sufficient to activate Tregs, but binding to the exact epitope is critical and may be involved in the unique mode of action of tregalizumab.

Clinical effects of tregalizumab have been demonstrated in Phase II trials in RA and psoriasis.

In addition, activation of the suppressive activity of Tregs has been linked to the induction of cyclic AMP (cAMP) and cellular transfer to effector cells (Bopp, et al). Tregalizumab, but not other anti-CD4 mAbs tested, induced cAMP in Tregs but not in T effector cells (Figure 3).

Figure 3: Induction of intracellular cAMP in conventional and regulatory T cells by anti-CD4 mAbs

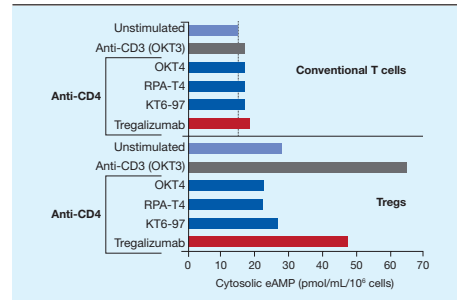


Figure 6: Superimposition of CD4-tregalizumab complex with CD4-MHC II complex

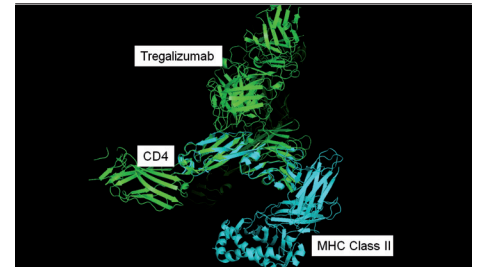
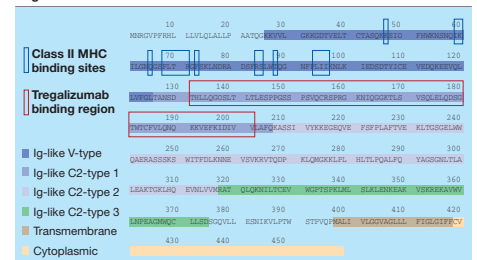


Figure 7: Crystal structure analysis: Binding of MHC class II and tregalizumab to CD4



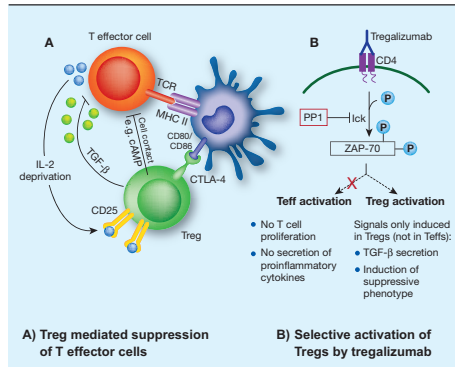
Background

Tregs modulate and balance the immune system. Once activated, they suppress T cells in an antigen-independent manner (Figure 1A). In patients with autoimmune diseases, reduced numbers or functional impairment of Tregs results in loss of this finely-tuned mechanism.

Tregalizumab (BT-061):

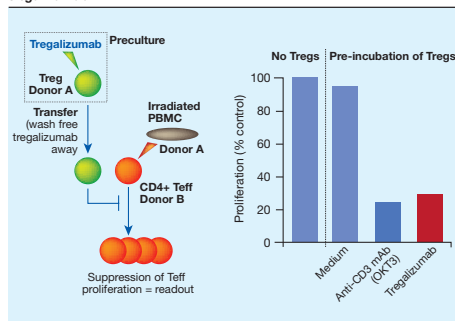
- Humanized monoclonal IgG1 antibody
- Binds to the IgG like C2 type 1 domain of CD4 on T helper cells and Tregs
- Receptor activation induces phosphorylation of the ZAP70 protein kinase
- Provides an activation signal to naturally occurring Tregs (Figure 1B); not known with other therapeutic CD4 antibodies (Czeloth, et al)
- Selectively activates Tregs but not normal T cells
- No evidence of ADCC or CDC and non-depleting.

Figure 1: Effect of Tregs and the mechanism of action of tregalizumab



Suppression of CD4+ T cells via Tregs by tregalizumab was previously demonstrated in mixed lymphocyte reactions; pre-incubation of Tregs with tregalizumab led to reduced proliferation of CD4 T effector cells, while freshly isolated Tregs had no effect (Figure 2) (Czeloth, et al).

Figure 2: Inhibition of T cell proliferation by Tregs incubated with tregalizumab

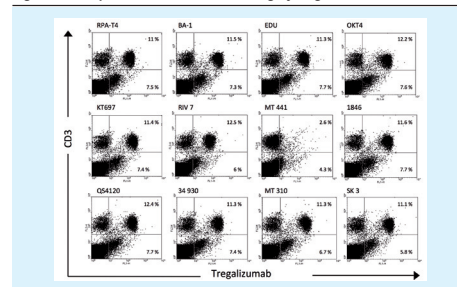


Methods / Results

In order to better characterize the novel mechanism of action of tregalizumab, the crystal structure of tregalizumab in complex with CD4 was analyzed and the epitope was compared to that of other CD4 antibodies.

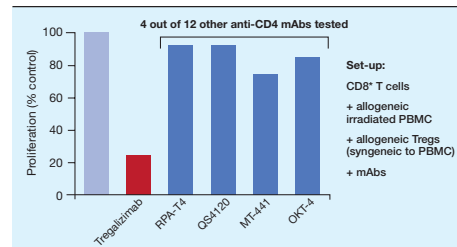
CD4+ PBMC were pre-incubated with different commercially available anti-CD4 mAbs, and subsequently with tregalizumab and anti-CD3. Only the mAb MT-441 was able to compete with tregalizumab (Figure 4); this was likely due to steric hindrance (Song, et al).

Figure 4: Competition of CD4 mAb binding by tregalizumab



In addition, only Tregalizumab was able to induce strong suppression of CD8 T cell proliferation (via Tregs) while the other antibodies tested (including MT-441) had no such effect (Figure 5).

Figure 5: Treg mediated suppression of CD8 T cell proliferation by anti-CD4 mAbs



Analysis of the 2.9 Angstrom-resolution crystal structure was undertaken which demonstrated that tregalizumab binds to a conformational epitope of the IgG-like C2 type 1 domain of CD4. Moreover, the epitope was identified to be outside of the MHCII site on the CD4 molecule (Figures 6, 7).

In summary, it was shown that binding of an antibody to the IgG-like C2 type 1 domain of CD4 is not sufficient to activate Tregs, but binding to the exact epitope is critical and may be involved in the unique mode of action of tregalizumab.

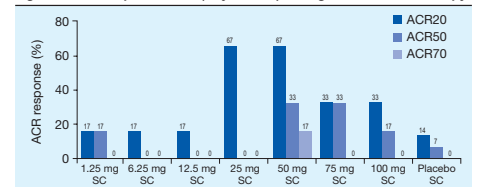
Clinical Results

The results from Phase I/IIa studies of tregalizumab in rheumatoid arthritis (RA) and psoriasis have demonstrated the clinical activity of tregalizumab (Czeloth, et al).

Phase IIa Study of Tregalizumab Monotherapy in Rheumatoid Arthritis

The most efficacious route of administration of tregalizumab was found to be SC and the optimal dose 50 mg, based on ACR responses (Figure 8). Tender/swollen joint scores also demonstrated that 50 mg SC was the most effective dose; with this regimen rapid and sustained improvements were reported. (Rudnev, et al).

Figure 8: ACR response rates (Day 43 +/- 1) to tregalizumab monotherapy



No depletion of CD4 cells was observed at any dose level. Tregalizumab was generally well tolerated and there were no serious infections and no deaths reported in the study.

Conclusions

- The binding of tregalizumab was identified to be in the IgG like C2 type 1 domain of CD4.
- Competition experiments showed that this epitope is not recognized by other anti-CD4 antibodies.
- This binding does not interfere sterically with the binding of MHCII to CD4.
- Binding of an antibody to the IgG-like C2 type 1 domain of CD4 is not sufficient to activate Tregs, but binding to the exact epitope is critical and may be involved in the unique mode of action of tregalizumab.
- Results of a clinical trial in active RA with tregalizumab as monotherapy indicate promising efficacy and good tolerability.
- Larger Phase II trials are underway to further evaluate the clinical potential of Treg activation by tregalizumab.

References

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