

Disclaimer

While all reasonable care has been taken to ensure the information contained in this presentation is accurate and up-to-date, Biotest makes no warranties, express or implied, that the information and forward looking statements contained in this presentation are free from error or omission. Scientific statements - in particular statements related to ongoing clinical trials - are by nature subject to elements of uncertainty that could result in deviation of actual developments from expected developments.

Conflict of interest declaration

Anatoliy Rudnev: Biotest AG: Employment (full or part-time). Sukanya Ragavan: Biotest AG: Research grants. Christina Trollmo: Biotest AG: Research grants. Vivianne Malmstroem: Biotest AG: Research grants. Christian Becker: Biotest AG: Research grants. Helmut Jonuleit: Biotest AG: Research grants. Vibeke Strand: Biotest AG: Consulting fees. Silke Aigner: Biotest AG: Employment (full or part-time). Niklas Czeloth: Biotest AG: Employment (full or part-time). Benjamin Daelken: Biotest AG: Employment (full or part-time). Andre Engling: Biotest AG: Employment (full or part-time). Helga Koch: Biotest AG: Employment (full or part-time). Gabriele Niemann: Biotest AG: Employment (full or part-time). Frank Osterroth: Biotest AG: Employment (full or part-time). Christoph Uherek: Biotest AG: Employment (full or part-time). Andrea Wartenberg-Demand: Biotest AG: Employment (full or part-time). Olga Ershova: Biotest AG: Research grants. Tatiana Sotnikova: Biotest AG: Research grants. Alexander Orlov-Morozov: Biotest AG: Research grants.

Contact

Benjamin_Daelken@biotest.de

[1125] - Selective Activation of Naturally Occurring Regulatory T Cells (Tregs) by the Monoclonal Antibody (mAb) BT-061. Markers of Clinical Activity and Early Phase II Results in Patients with Rheumatoid Arthritis (RA)

Anatoliy Rudnev¹, Sukanya Ragavan², Christina Trollmo², Vivianne Malmstroem², Christian Becker³, Helmut Jonuleit³, Vibeke Strand⁴, Silke Aigner¹, Niklas Czeloth¹, Benjamin Daelken¹, Andre Engling¹, Helga Koch¹, Gabriele Niemann¹, Frank Osterroth¹, Christoph Uherek¹, Andrea Wartenberg-Demand¹, Olga Ershova⁵, Tatiana Sotnikova⁶, Alexander Orlov-Morozov⁷

¹Biotest AG, Dreieich, ²Karolinska Institute, Stockholm, ³Johannes-Gutenberg University, Mainz, ⁴Division of Immunology, Stanford University, Portola Valley, CA, ⁵Clinical Hospital for Emergency Medical Care, Yaroslavl, ⁶Botkin Clinical Hospital, Moscow, ⁷City Clinical Hospital Nr. 23 n.a. Medsantrud, Moscow

Abstract

Naturally occurring Tregs are essential for maintaining normal immune homeostasis in healthy individuals. In patients with autoimmune diseases reduced numbers or functional impairment of Tregs has been observed. A humanized agonistic mAb, BT-061 selectively activates Tregs. It binds to a unique epitope of the CD4 molecule, leading to induction of Treg specific signaling events. While freshly isolated and resting Tregs do not inhibit T cell proliferation, pre-treatment of Tregs with BT-061 leads to suppression of CD4 and CD8 T effector cell proliferation, reduction of pro-inflammatory cytokines and a moderate increase in the anti-inflammatory cytokine TGF-beta.

To further assess the potential of BT-061 to modulate immune responses, *in vitro* studies with synovial fluid derived mononuclear cells from patients with active RA were performed. Addition of BT-061 at concentrations between 0.01 and 50 micro g/mL to isolated CD4-positive cells derived from the highly inflammatory milieu of RA synovial fluid suppressed proliferation as well as IFN-gamma production following antigen-specific stimulation.

In a Phase I/IIa trial in patients with psoriasis, a single dose of BT-061 resulted in PASI 50/75 responses for up to 90 days at doses of 0.5, 2.5, 10, 20 mg i.v. and 12.5, 25 mg s.c. A Phase IIa, multicenter, randomized placebo-controlled trial with BT-061 monotherapy was performed in 96 patients with active RA and inadequate responses to one or more traditional DMARD despite 3 months or more of treatment. Patients were randomized to 12 treatment groups: from 1.25–100 mg s.c. or 0.5–25 mg i.v. once weekly for 6 weeks: 6 patients received BT-061 and 2 received placebo in each group.

Initial data analysis confirmed the clinical activity of BT-061 by ACR20/50/70 responses in a meaningful proportion of patients despite the short duration of therapy. No major safety signals were identified. Final analyses of safety and efficacy data are ongoing and will be presented.

Phase II multiple dose trials with BT-061 are underway to further evaluate the clinical benefit of BT-061 in patients with RA and psoriasis.

American College of Rheumatology 2010 Annual Scientific Meeting. Abstract 1125
Presented Tuesday, 9th November, 2010

[1125] - Selective Activation of Naturally Occurring Regulatory T Cells (Tregs) by the Monoclonal Antibody (mAb) BT-061. Markers of Clinical Activity and Early Phase II Results in Patients with Rheumatoid Arthritis (RA)

Anatoliy Rudnev¹, Sukanya Ragavan², Christina Trollmo², Vivianne Malmstroem², Christian Becker³, Helmut Jonuleit³, Vibeke Strand⁴, Silke Aigner¹, Niklas Czeloth¹, Benjamin Daelken¹, Andre Engling¹, Helga Koch¹, Gabriele Niemann¹, Frank Osterroth¹, Christoph Uherek¹, Andrea Wartenberg-Demand¹, Olga Ershova⁵, Tatiana Sotnikova⁶, Alexander Orlov-Morozov⁷

¹Biotest AG, Dreieich, ²Karolinska Institute, Stockholm, ³Johannes-Gutenberg University, Mainz, ⁴Division of Immunology, Stanford University, Portola Valley, CA, ⁵Clinical Hospital for Emergency Medical Care, Yaroslavl, ⁶Botkin Clinical Hospital, Moscow, ⁷City Clinical Hospital Nr. 23 n.a. Medsantrud, Moscow

Abstract

Naturally occurring Tregs are essential for maintaining normal immune homeostasis in healthy individuals. In patients with autoimmune diseases reduced numbers or functional impairment of Tregs has been observed. A humanized agonistic mAb, BT-061 selectively activates Tregs. It binds to a unique epitope of the CD4 molecule, leading to induction of Treg specific signaling events. While freshly isolated and resting Tregs do not inhibit T cell proliferation, pre-treatment of Tregs with BT-061 leads to suppression of CD4 and CD8 T effector cell proliferation, reduction of pro-inflammatory cytokines and a moderate increase in the anti-inflammatory cytokine TGF- β .

To further assess the potential of BT-061 to modulate immune responses, *in vitro* studies with synovial fluid derived mononuclear cells from patients with active RA were performed. Addition of BT-061 at concentrations between 0.01 and 50 micro g/mL to isolated CD4-positive cells derived from the highly inflammatory milieu of RA synovial fluid suppressed proliferation as well as IFN- γ production following antigen-specific stimulation.

In a Phase I/IIa trial in patients with psoriasis, a single dose of BT-061 resulted in PASI 50/75 responses for up to 90 days at doses of 0.5, 2.5, 10, 20 mg i.v. and 12.5, 25 mg s.c. A Phase IIa, multicenter, randomized placebo-controlled trial with BT-061 monotherapy was performed in 96 patients with active RA and inadequate responses to one or more traditional DMARD despite 3 months or more of treatment. Patients were randomized to 12 treatment groups: from 1.25–100 mg s.c., or 0.5–25 mg i.v., once weekly for 6 weeks: 6 patients received BT-061 and 2 received placebo in each group.

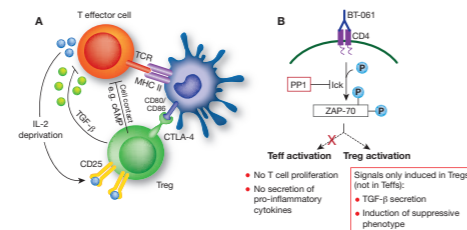
Initial data analysis confirmed the clinical activity of BT-061 by ACR20/50/70 responses in a meaningful proportion of patients despite the short duration of therapy. No major safety signals were identified. Final analyses of safety and efficacy data are ongoing and will be presented.

Phase II multiple dose trials with BT-061 are underway to further evaluate the clinical benefit of BT-061 in patients with RA and psoriasis.

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.

Figure 1: Effect of Tregs and mechanism of action of BT-061



BT-061:

- Humanized monoclonal IgG1 antibody
- Binds to a unique epitope of CD4 on T helper and regulatory T cells
- Provides an activation signal to naturally occurring regulatory T cells (Figure 1B); not known from other therapeutic CD4 antibodies (Czeloth *et al.*)
- Selectively activates Tregs but not normal T cells
- No evidence of ADCC or CDC and non-depleting.

Study Design (Biotest Study 962)

- Randomized, placebo-controlled, Phase IIa trial
- 96 patients with RA who had a history of DMARD failure
- BT-061 s.c. (1.25–100 mg) or i.v. (0.5–25 mg) (n=6 per group) or matching placebo (n=2 per group) weekly for 6 weeks
- Primary endpoint: ACR 20 response after 6 weeks' of treatment
- Secondary endpoints included ACR 50/70, DAS28 and EULAR criteria, safety, cytokine assessment, lymphocyte phenotyping.

Results

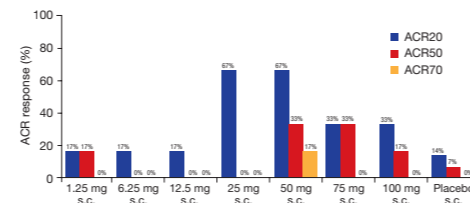
The most efficacious route was identified to be s.c. Patient demographics for this patient population are shown in Table 1.

Table 1: Patient Demographics

	BT-061 s.c.							Placebo s.c. (n=14)
	1.25 mg (n=6)	6.25 mg (n=6)	12.5 mg (n=6)	25 mg (n=6)	50 mg (n=6)	75 mg (n=6)	100 mg (n=6)	
Age (mean)	46.5	57.8	60.7	54.2	53.8	56.7	48.7	59.4
Tender joint count (mean)	15.8	17.0	19.5	14.3	20.2	27.2	22.2	19.9
Swollen joint count (mean)	10.0	10.3	9.8	9.0	7.7	15.8	13.5	11.9
HAQ (mean)	2.00	1.83	2.02	2.02	1.40	1.79	1.92	1.88
DAS28 (mean)	6.5	6.4	6.8	6.1	6.0	6.7	6.7	6.5

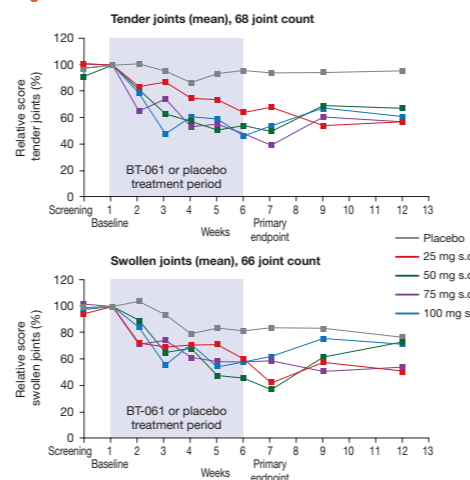
ACR responses (Figure 2) indicate that the effective dose range is between 25 mg and 100 mg s.c. Additional assessment of secondary endpoints was undertaken on these dose groups.

Figure 2: ACR 20/50/70 Responses (Day 43 ± 1)



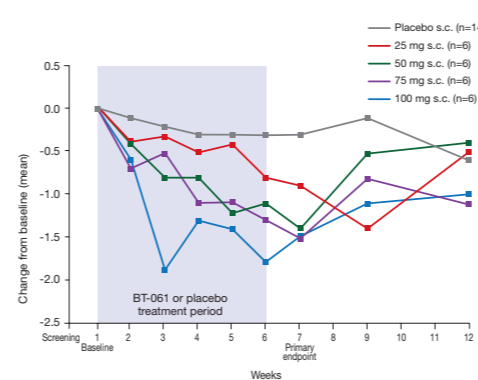
Tender and swollen joint counts showed rapid improvement, which in some cases was sustained beyond the 6 week dosing period (Figure 3).

Figure 3: Tender / Swollen Joint Scores



DAS28 responses also showed a similar pattern, with improvement over the dosing period being sustained in some patients (Figure 4). Overall, DAS28 responses were observed at study endpoint in 67% of patients treated with BT-061 50 mg and 33% of those treated with 25 mg, 75 mg, or 100 mg.

Figure 4: DAS28 score - change from baseline



Safety

No depletion of CD4 cells was observed at any dose level. BT-061 was generally well tolerated, serious AEs were reported in 2 patients of 72 treated with BT-061 (both received 6.25 mg s.c.); and in 1 of 24 patients who received placebo. There were no deaths reported in the study. The number of patients with any AE was similar in all dose groups, with no evidence of a dose-response relationship, and at a similar level to placebo-treated patients.

More patients withdrew due to adverse events in the placebo groups (Table 2), compared to those in the BT-061 treatment groups. Infections were reported in 13 patients (18%) who received BT-061 compared with 1 patient (7%) who received placebo. There were no serious infections; all infections reported were mild or moderate in severity.

Table 2: Adverse Events

	Placebo s.c. (n=14)	Placebo i.v. (n=10)	BT-061 s.c.						BT-061 i.v.					
			1.25 mg (n=6)	6.25 mg (n=6)	12.5 mg (n=6)	25 mg (n=6)	50 mg (n=6)	75 mg (n=6)	100 mg (n=6)	0.5 mg (n=6)	2.0 mg (n=6)	6.25 mg (n=6)	12.5 mg (n=6)	25 mg (n=6)
Patients with any AEs (%)	8 (57%)	6 (60%)	4 (67%)	6 (100%)	2 (33%)	3 (50%)	3 (50%)	4 (67%)	4 (67%)	3 (50%)	3 (50%)	6 (100%)	4 (67%)	1 (17%)
Patients with serious AEs	0	1 (10%)	0	2 (33%)	0	0	0	0	0	0	0	0	0	0
Patients withdrawing due to AE	2 (14%)	3 (30%)	0	0	0	0	0	0	1 (17%)	1 (17%)	0	0	0	0

Exploratory Analysis of Cytokine Levels

Analysis of cytokines was undertaken at each study visit before treatment with BT-061 or placebo by multiplex array and ELISA (TGF- β). Analysis revealed that there was no change in TNF- α , TGF- β , interferon gamma, IL-5, IL-6, IL-8, or IL-10. This is consistent with *in vitro* findings that BT-061 does not induce secretion of inflammatory cytokines or proliferation of conventional T cells.

Data from CD4 cells isolated from the synovial fluid of patients with RA indicated that BT-061 reduced but did not abrogate proliferation of such cells in response to the recall antigens PPD (purified peptide from *M. tuberculosis*) or influenza vaccine. IFN- γ secretion was also reduced and there was no increase in pro-inflammatory cytokine secretion.

Conclusions

- This is the first study of a CD4 antibody given via the s.c. route for the treatment of RA.
- In this trial, in patients with a history of DMARD failure, clinical effects of BT-061 given as monotherapy were identified across the dose ranges tested. ACR responses could be demonstrated after just 6 weeks of therapy. Based on these responses, doses between 25 mg and 100 mg s.c. were identified as offering the greatest efficacy.
- BT-061 at the active dose range (25–100 mg s.c.) demonstrated rapid improvements in tender and swollen joint counts, which in some cases was sustained beyond the 6 week dosing period. Therapy was generally well tolerated.
- Further trials are being performed with BT-061 including BT-061 in combination with methotrexate.
- Activation of Tregs by the monoclonal agonistic antibody BT-061 is a promising approach for management of RA. In addition, BT-061 has shown activity in psoriasis showing long-lasting clinical effects after a single dose (Abufarag *et al.*).

References

- Czeloth N, Dälken B, Engling A *et al.* Selective activation of naturally occurring regulatory T cells (Tregs) by the monoclonal antibody BT-061 as a novel therapeutic opportunity: pre-clinical and early clinical results. Annual European Congress of Rheumatology, EULAR 2010. Abstract OP0138. Presented 18th June 2010.
- Abufarag A, Aigner S, Czeloth A *et al.* Selective activation of naturally occurring regulatory T cells (Tregs) by the monoclonal antibody BT-061 as a novel therapeutic opportunity in psoriasis: Early clinical results after single doses. European Society for Dermatological Research 2010. Abstract 379. Presented 9th September 2010.

Disclosure:

Anatoliy Rudnev: Biotest AG: Employment (full or part-time). Sukanya Ragavan: Biotest AG: Research grants. Christina Trollmo: Biotest AG: Research grants. Vivianne Malmstroem: Biotest AG: Research grants. Christian Becker: Biotest AG: Research grants. Helmut Jonuleit: Biotest AG: Research grants. Vibeke Strand: Biotest AG: Consulting fees. Silke Aigner: Biotest AG: Employment (full or part-time). Niklas Czeloth: Biotest AG: Employment (full or part-time). Benjamin Daelken: Biotest AG: Employment (full or part-time). Andre Engling: Biotest AG: Employment (full or part-time). Helga Koch: Biotest AG: Employment (full or part-time). Gabriele Niemann: Biotest AG: Employment (full or part-time). Frank Osterroth: Biotest AG: Employment (full or part-time). Christoph Uherek: Biotest AG: Employment (full or part-time). Andrea Wartenberg-Demand: Biotest AG: Employment (full or part-time). Olga Ershova: Biotest AG: Research grants. Tatiana Sotnikova: Biotest AG: Research grants. Alexander Orlov-Morozov: Biotest AG: Research grants.