

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

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Abstract

Tregs down-regulate excessive immune responses. In patients with autoimmune diseases, reduced numbers, or functional impairment, of Tregs is observed. The humanized agonistic monoclonal antibody (mAb) BT-061 binds to a unique epitope of CD4, thereby selectively activating Tregs but not helper T cells. In the first placebo-controlled, double-blind clinical trial of BT-061, 55 patients with moderate to severe chronic plaque psoriasis received a single dose of BT-061 or placebo with doses ranging between 0.5 mg and 20 mg i.v. or 12.5-25 mg s.c. BT-061 was well tolerated and the majority of adverse events were mild or moderate without increasing in frequency and intensity with increasing doses. There was no evidence of an increased risk of infections, no significant increase in cytokines and no depletion of CD4 T cells. Efficacy parameters showed a long-lasting effect in some of the patients with an improvement in PASI-score persisting for up to 90 days after single dose administration. 19 out of 55 patients exceeded a PASI 50 response including two patients with more than 75% improvement. Responses were seen in four patients receiving placebo, but none reached a PASI 75 response. The strongest improvements in PASI were observed in 2.5 mg i.v. and 25 mg s.c. dose groups. Based on these promising results, phase II clinical trials of BT-061 in patients with rheumatoid arthritis and chronic plaque psoriasis are ongoing.

Background

Tregs modulate and balance the immune system by negative regulation of immune responses, thereby limiting excess inflammation and by maintenance of immunological tolerance, prevention of development of autoimmunity. In patients with autoimmune diseases, reduced numbers or functional impairment of Tregs results in loss of this finely-tuned mechanism. Like normal T cells, Tregs require receptor activation to efficiently suppress proliferation and cytokine secretion from normal T cells. Once activated, Tregs suppress T cells in an antigen-independent manner (Figure 1 A).

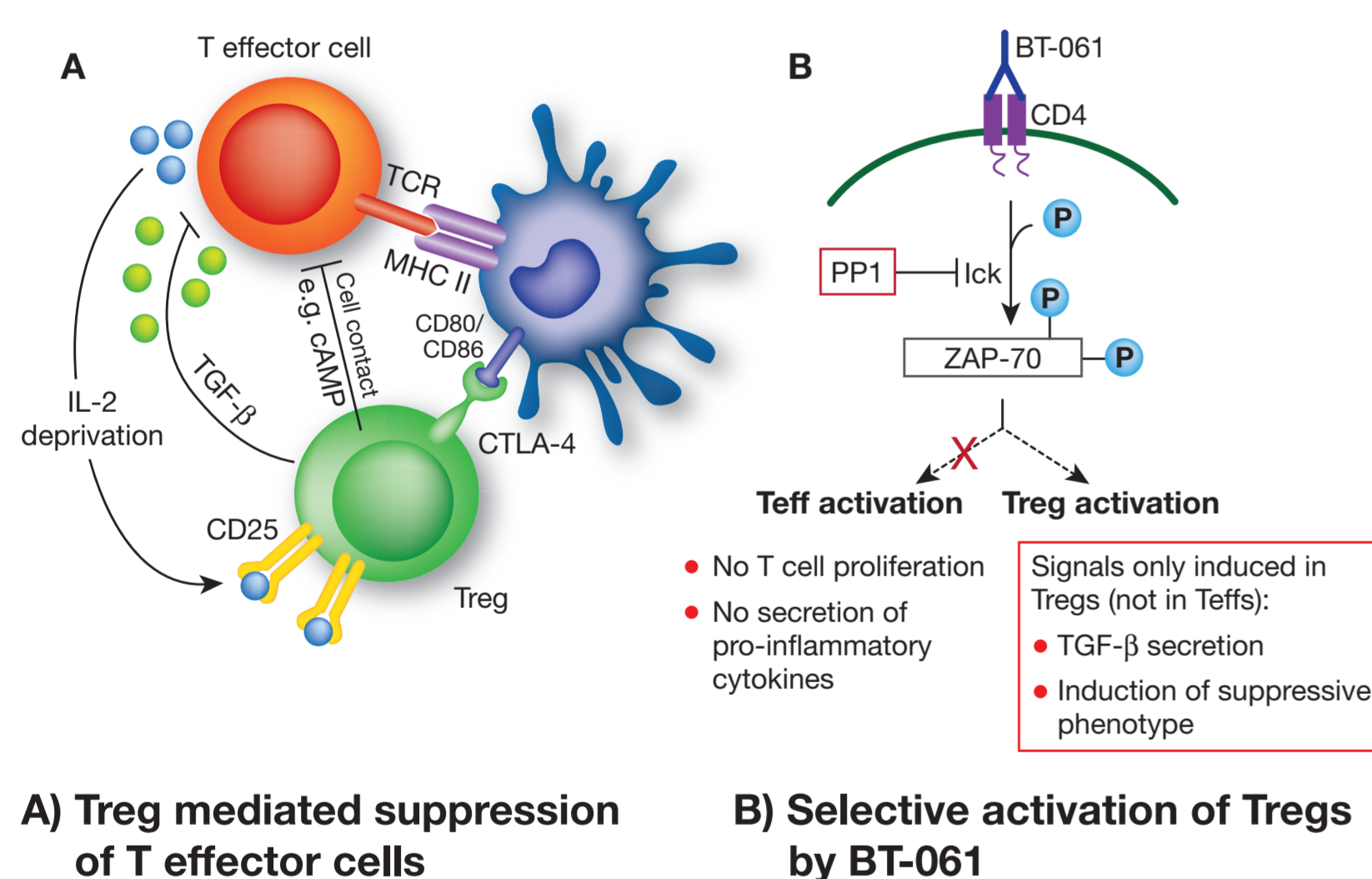
Here we report on the first clinical efficacy data of the humanized agonistic monoclonal antibody (mAb) BT-061, a selective activator of Tregs, in chronic plaque psoriasis.

BT-061 compound specifics:

- humanised monoclonal antibody (IgG1)
- binds to a unique epitope of CD4 (low nanomolar affinity)
- non-depleting (no antibody dependent cell-mediated cytotoxicity (ADCC) or complement dependent cytotoxicity (CDC))
- binds to both T helper and Treg cells
- provides an activation signal to naturally occurring Tregs, but not to conventional T cells, binding to a unique epitope (not reported for other therapeutic CD4 antibodies)
- specifically activates Tregs

Surface plasmon resonance studies and X-ray structure analysis of a CD4/Fab(BT-061) complex demonstrated that BT-061 binds with low nanomolar affinity to a conformational epitope of the CD4 molecule. Binding of BT-061 to this epitope induces activation of the CD4 associated kinase Ick that leads to phosphorylation of the T cell receptor associated kinase ZAP70 (Figure 1 B). This phosphorylation induces Treg-specific signalling events which result in activation of their suppressive functions.

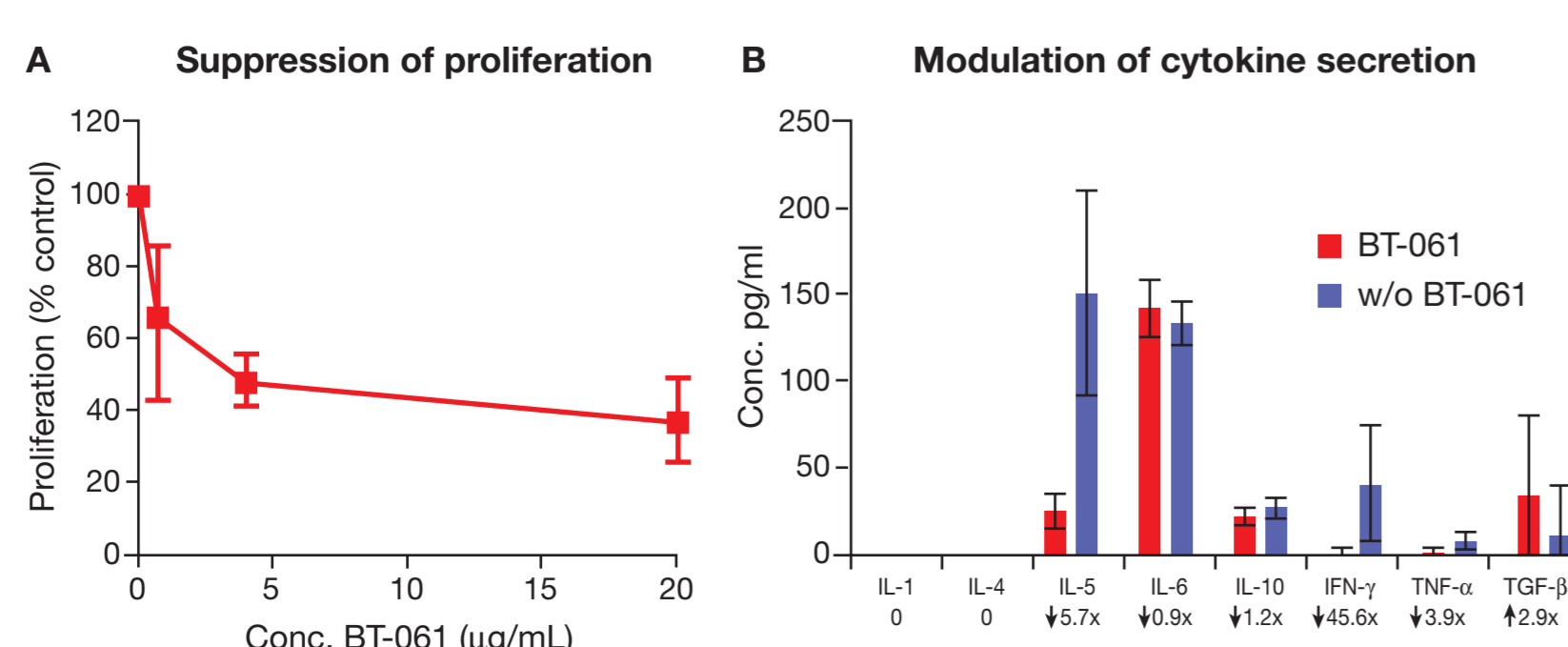
Figure 1. Effect of Tregs and mechanism of action of BT-061



In vitro we have demonstrated that while freshly isolated resting Tregs do not effectively inhibit proliferation of conventional T cells, pre-treatment of Tregs with BT-061 led to induction of their suppressive activity (Czeloth *et al.*). We have shown that BT-061 treated Tregs are able to strongly suppress proliferation and cytokine secretion of CD4 and CD8 effector T cells following allogeneic or antigen-specific activation. In contrast to reports of Treg activation with anti-CD3 mAbs, we reported that although BT-061 appears to similarly bind to T helper cells, BT-061 does not induce secretion of inflammatory cytokines or proliferation of conventional T cells.

The influence of BT-061 on proliferation and cytokine secretion was assessed using isolated PBMC stimulated with the recall antigen tetanus toxoid in an antigen-specific manner. PBMC proliferation was assessed by ³H thymidin incorporation and cytokine levels were determined in cell culture supernatants after 2 days. Increasing concentrations of BT-061 led to a reduction of PBMC proliferation in a dose dependent manner (Figure 2 A). Analysis of cytokine levels show that BT-061 reduces inflammatory cytokines such as IFN- γ and moderately induces the regulatory cytokine TGF- β (Figure 2 B).

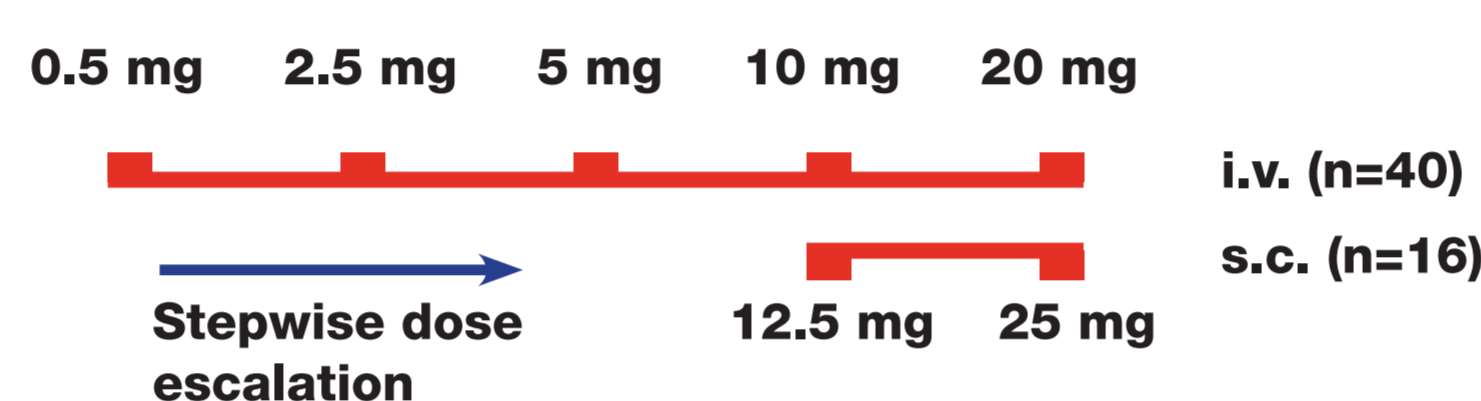
Figure 2. BT-061 reduces proliferation and pro-inflammatory cytokines



Biotest Study 967-The first placebo-controlled, double-blind clinical trial of BT-061 in chronic plaque psoriasis

Clinical methodology: Written informed consent was obtained from 55 patients with chronic plaque psoriasis, with a PASI score ≥ 10 and with $\geq 10\%$ body surface area involvement of at least 6 months' duration. Before baseline, systemic psoriasis treatments, phototherapy, and high potency topical treatments had to be discontinued. Only emollients and low potency topical steroids were permitted as rescue medications. Patients were randomised to receive a single dose of either BT-061 or placebo in a double-blind setting. They were then followed-up for 75 days (extended to 90 days in some dose groups) to assess safety, pharmacokinetics and development of their PASI score. Dose escalation started at a dose of 0.5 mg BT-061 i.v. and 12.5 mg BT-061 s.c. At each dose level, six patients received the active drug and two patients received placebo. The maximum doses tested were 20 mg i.v. and 25 mg s.c. (Figure 3). Pharmacokinetic parameters were assessed by measuring BT-061 antibody in plasma samples taken from treated patients at different timepoints.

Figure 3. Trial graph of Study 967 – each square dot represents a group of 8 (6 active, 2 placebo)



An extensive safety screening was applied at inclusion (e.g. excluding latent infections) which led to delayed recruitment. Recruitment was therefore halted after 55 of 56 planned patients in order to start a follow-up trial with multiple dosing.

Results

Study population: 34 male and 21 female patients were recruited, with a mean age of 49.6 years (median 53 years). Baseline PASI scores ranged from 10 to 28.2, with a mean of 14.5 and a median of 13.5. Three patients discontinued treatment prematurely (lack of study compliance, loss to follow-up and withdrawal of consent). At the end of the study, seven patients were rated as major protocol violators, after undergoing high potency antipsoriatic treatment (steroids, infliximab, UV-therapy).

Pharmacokinetic and pharmacologic results

BT-061 could be detected in plasma after i.v. administration of doses ≥ 10 mg, but not after s.c. administration. Peak concentrations were seen immediately at the end of a 2 hour i.v. infusion. Preliminary analysis showed that C_{max}, systemic exposure and apparent half-life of BT-061 are dose dependent (Table 1).

Based on *in vitro* studies, clearance is dominated by binding of BT-061 to CD4, with subsequent internalization of the BT-061/CD4 complex. Further mechanistic studies revealed that internalisation is a result of the agonistic mode of action of BT-061 which involves activation of the CD4-bound kinase Ick and subsequent cellular signalling events leading to active internalisation of the BT-061/CD4 complex (data not shown).

Table 1. Pharmacokinetics

Dose (mg)	t _{1/2} * (h)	C _{max} (μ g/ml)	AUC _{0-1h} * (h* μ g/ml)
10 i.v.	3.4	0.75	4.94
20 i.v.	9.9	1.09	15.74

*estimated

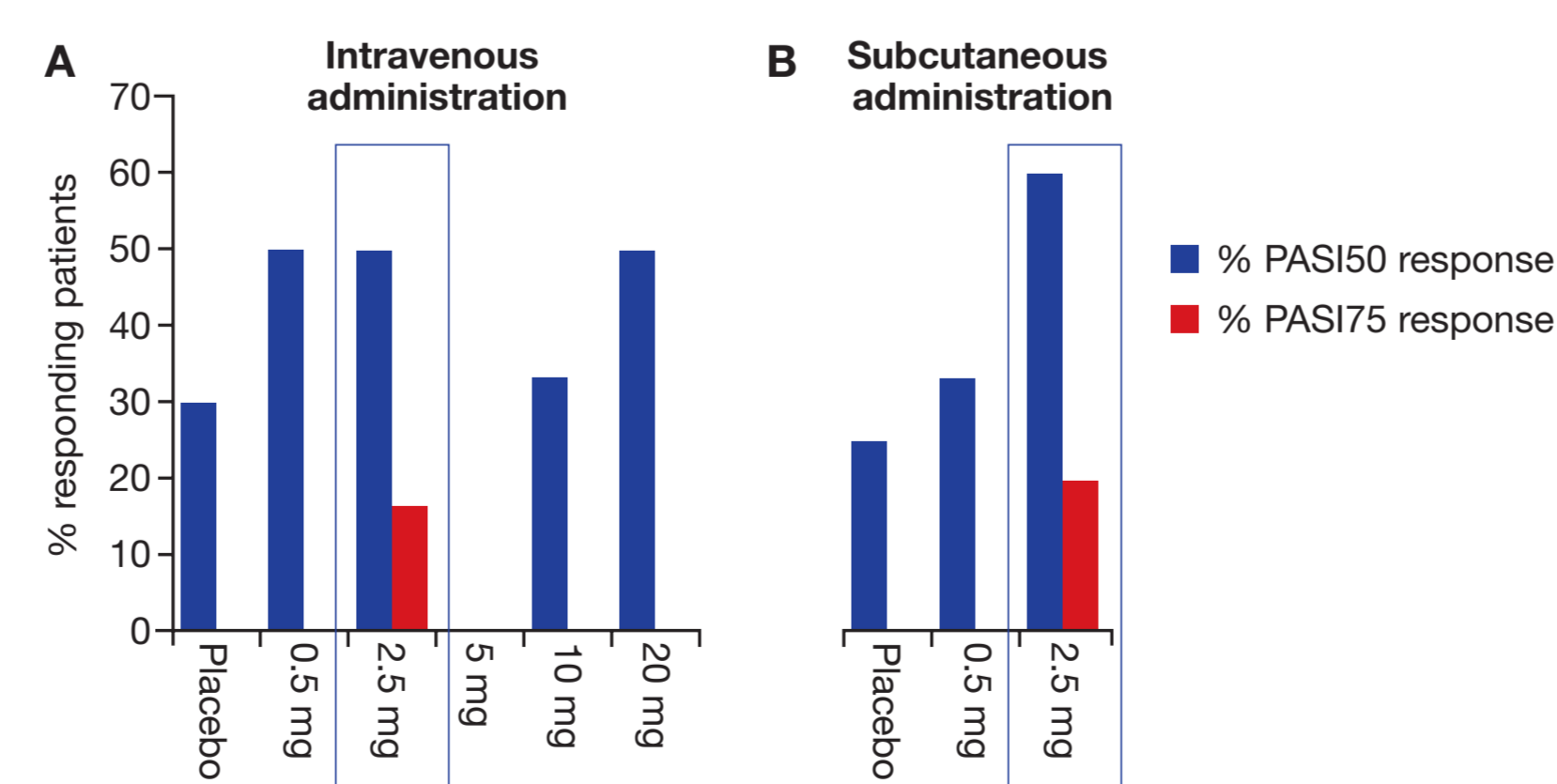
Pharmacologic effects

- Immunogenicity – no immunogenicity of BT-061 was detected
- Cell counts – no depletion of CD3/CD4-positive cells was found
- Cytokines – no increase in the proinflammatory cytokines IL-1beta, IL-2, IL-4, IL-5, IL-8, IL-10, IFN- γ . Limited and transient increase (max. 10x upper limit of normal [ULN]) of IL-6 was seen after i.v. doses ≥ 2.5 mg. A similar effect was observed for TNF- α (max. 2x ULN) after i.v. dosing but not after s.c. dosing. ULN values for IL-6 and TNF- α were determined to be 5.1 pg/ml and 4.1 pg/ml, respectively.

Clinical efficacy (per protocol analysis)

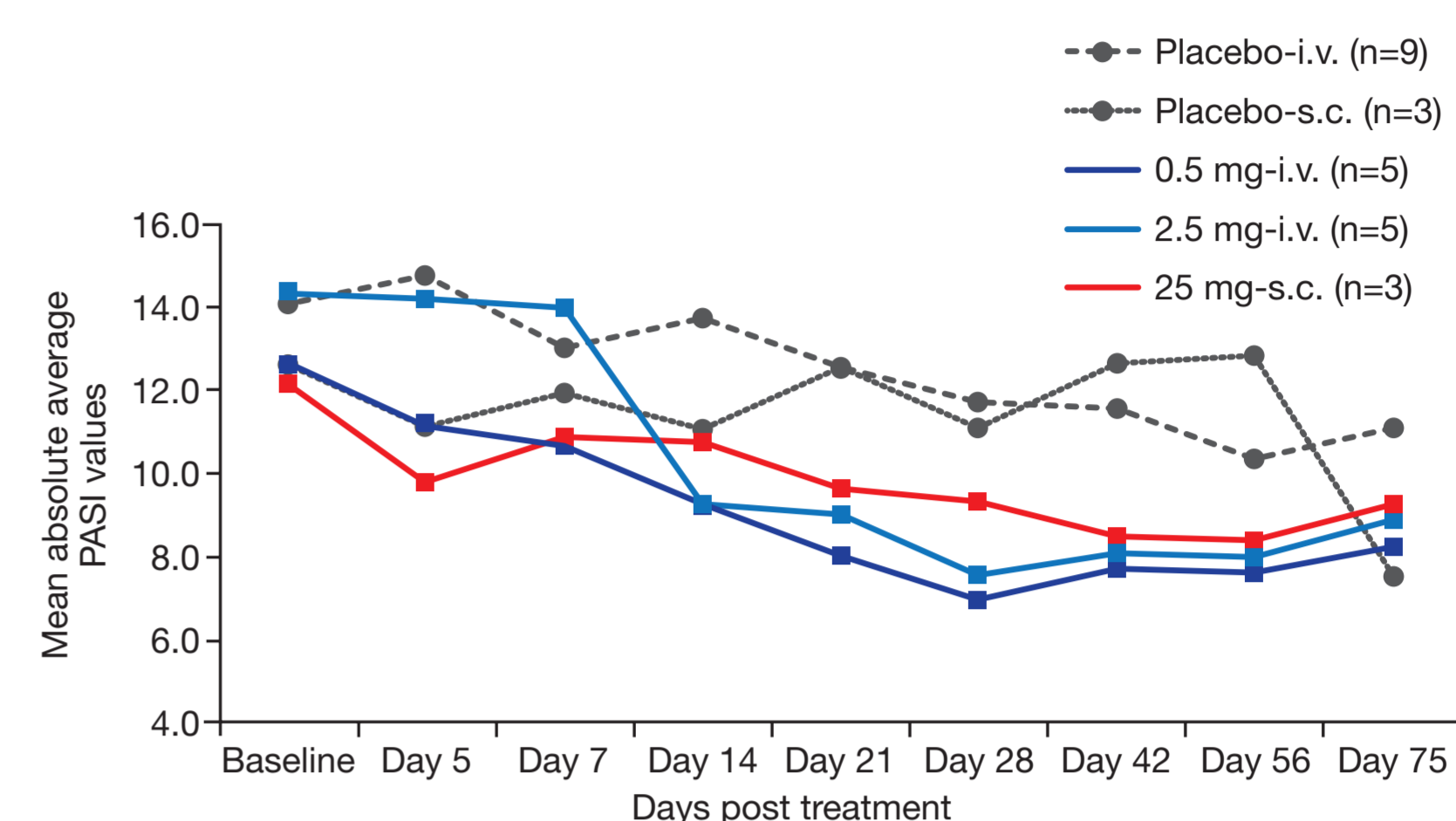
15 of 41 patients (37%) receiving BT-061 exceeded a PASI 50 response including two patients with more than 75% improvement (Figure 4). Responses were also seen with placebo (29%), but no patients reached a PASI 75 response. The strongest improvements in PASI were observed in the 2.5 mg i.v. and 25 mg s.c. dose groups (boxed).

Figure 4. PASI 50 and PASI 75 responses after single dose BT-061



PASI response over time: Some of the patients experienced a long-lasting improvement in PASI and PGA scores, with an improvement in PASI-score persisting for up to 90 days after single dose administration. At the more efficacious doses, the benefit from BT-061 persisted over several weeks (Figure 5). The arithmetic means of absolute PASI scores over time post-treatment is shown for the dose groups with the strongest responses and also for the placebo arms. The average benefit of one single dose of BT-061 vs. placebo was most apparent 3-7 weeks post dosing, but persisted until day 90 in the subjects with the strongest individual response.

Figure 5. Kinetics of PASI response after single dose*



*Excluding protocol violators using forbidden high potency antipsoriatic treatments.

Patient photograph showing clear improvement (>50%) of psoriatic plaques after a single injection of BT-061 12.5 mg s.c.



Patient 65: Predose PASI = 15.3

Day 28 PASI = 5.3

Safety and adverse events (ITT population)

Overall, BT-061 was well-tolerated and no serious safety signals were identified. Adverse events (AEs) were reported in all patients, but the mean number of adverse events in all patients was similar in the placebo (14.5/16.3) and in the active groups (14.2/24.8). There was no increase in the number of AEs with increasing dose (Table 2 and Table 3). The most frequently reported AEs were headache and erythema. The majority of AEs were mild to moderate in intensity. Six patients experienced serious adverse events. Similar numbers of adverse events in the System Organ Class *Infections and infestations*, were reported in the active groups and the placebo controls. No adverse event required delay or interruption of the infusion in i.v. dosing. Injection site reaction was not reported for the s.c. treated cohort.

Table 2. Number of adverse events reported in Study 967

	Placebo		BT-061						
	i.v.	s.c.	0.5mg i.v.	2.5mg i.v.	5mg i.v.	10mg i.v.	20mg i.v.	12.5mg s.c.	25mg s.c.
N	10	4	6	6	6	6	6	6	5
Number of AEs reported	163	58	97	104	149	120	116	111	71
Mean Number of AEs*	16.3	14.5	16.2	17.3	24.8	20.0	19.3	18.5	14.2

* Per patient, over all patients in the respective dose group.

Table 3. Patients with adverse events in Study 967

	Placebo		BT-061						
	i.v.	s.c.	0.5mg i.v.	2.5mg i.v.	5mg i.v.	10mg i.v.	20mg i.v.	12.5mg s.c.	25mg s.c.
N	10	4	6	6	6	6	6	6	5
Patients with (100) AE (%)	10 (100)	4 (100)	6 (100)	6 (100)	6 (100)	6 (100)	6 (100)	6 (100)	5 (100)
Patients with (10) serious AEs (%)	1 (10)	0	3 (50)	1 (17)	1 (17)	0	0	0	0
Patients withdrawing due to AEs	0	0	0	0	0	0	0	0	0
Deaths	0	0	0	0	0	0	0	0	0

Conclusion

BT-061 possesses a unique mode of action and shows long-lasting clinical efficacy after a single dose. BT-061 was adequately safe with the noteworthy absence of injection site reactions and safety issues related to cytokine release or cell depletion. Currently, BT-061 is being tested in phase II clinical trials in patients with rheumatoid arthritis and chronic plaque psoriasis, to investigate the suitability of Treg activation with repeated doses of BT-061. The unique and specific mode of action of BT-061 has great potential for the management of several different autoimmune diseases, where other biologic treatments are failing to reach or maintain adequate response.

Conflict of Interest Declaration: A. Abufarag, S. Aigner, N. Czeloth, B. Dälken, H. Koch, G. Niemann, C. Uherek, F. Osterroth, A. Wartenberg-Demand: Employment by Biotest AG; A. Enk, W.E. Haefeli, R. Schopf received research funding from Biotest AG.

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.