From TGN1412 to TAB08: the return of CD28 superagonist therapy to clinical development for the treatment of rheumatoid arthritis

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ABSTRACT

CD28 superagonists (CD28SA) are CD28-specific monoclonal antibodies which are able to activate T-cells without overt TCR engagement. In rodents, CD28SA efficiently activate regulatory T-cells and are therapeutically effective in multiple models of autoimmunity, inflammation and transplantation. However, a phase I study of the human CD28SA TGN1412 in 2006 resulted in a life-threatening cytokine storm. This brief review summarises preclinical work before and since the failed phase I trial with an emphasis on understanding the reasons why there had been no warning of toxicity, and how a novel assay paved the way for a new phase I, phase Ib (both completed), and an ongoing phase II study.

Introduction

Numerous autoimmune and inflammatory diseases, including rheumatoid arthritis (RA), are associated with defects in the number or function of regulatory T-cells (Treg cells) (1). Furthermore, animal studies have shown that even in cases of non-immune system related tissue damage, Treg cells can counteract the manifestation of such diseases, and even promote tissue repair (2, 3). Accordingly, it is highly attractive to boost the regulatory T-cell compartment in diseases where Treg cells are known to have a protective or therapeutic effect. Ideally, such a Treg boost would lead to a transient wave of Treg activation, which results in numerical expansion, functional differentiation, and migration to sites of inflammation. Activated Treg cells could then interfere with the pathologic process on site, while the rest of the immune system returns to base line. Two different types of approaches have been developed towards this goal: First, treatment with low doses of IL-2 (4, 5), which makes use of the unique sensitivity of Treg cells to the stimulatory effects of this cytokine (6) or, in another variant of this approach which has not yet reached the clinic, the use of IL-2/anti-IL-2 complexes which selectively address the high affinity IL-2 receptor constitutively expressed by regulatory T-cells (7). In a second group of approaches, monoclonal antibodies to T-cell surface molecules have been developed which preferentially activate regulatory T-cells. One such reagent is the CD4-specific mAb Tregalizumab which, in spite of a setback in a recent phase II trial in RA, is being further developed for its potential usefulness in other disease entities (8). The second type of Treg promoting mAb are so-called CD28 superagonists (CD28SA), which are highly efficaceaous in rodents (9). The human CD28SA prototype, TGN1412, failed dramatically during a first-in-human FIH trial in 2006 due an unexpected cytokine release syndrome (10) caused by the activation of CD4 effector memory (CD4EM) cells (11, 12). The present review will briefly recount the research leading up to this trial, and describe the return of TGN1412, now called TAB08, to clinical development almost a decade later.

Preclinical rodent studies

CD28SA activity was originally discovered in rats, where a subset of CD28-specific mAb seemingly activated T-cells without the requirement for TCR engagement (13). We now know, however, that at least weak or "tonic" TCR signals are required for CD28SA activity to generate substrates for signal amplification by CD28 (14, 15). When injected into rats, CD28SA

expanded all CD4 T-cells at high doses, accompanied by a strong increase in the frequency and activity of Treg cells (16); at low doses, however, the response was restricted to the Treg cells themselves (17). We now know that besides the high sensitivity of Treg cells to IL-2 (6) and their ability to act as an IL-2 sink (18), it is the stronger TCR signalling input of this autoreactive cell type as compared to conventional T-cells (19) that reduces the amount of CD28SA required to reach an activation threshold (unpublished results).

The transient wave of polyclonal Treg activation observed in response to CD28SA treatment in rats prompted the study of its therapeutic potential in models for human diseases including multiple sclerosis, Guillain-Barré syndrome, type-1 diabetes, glomerulonephritis, and in several models of transplantation (Table I). When later on, also a mouse-specific CD28SA was developed (20), these studies were further extended to graft-*versus*-host disease, infection-associated inflammation and various autoimmune and inflammatory disorders caused by genetic manipulations (Table I). Recently, tissue repair after myocardial infarction and stroke has extended the scope of experimental CD28SA treatment (Table I). In all instances, strong therapeutic effects were observed without discernable adverse side effects. Furthermore, we could demonstrate that as postulated earlier (9), polyclonal Treg activation resulted in the sequential induction of proliferation, a switch in chemokine receptors directing migration to inflamed sites, and expression of effector function (21) (Fig. 1).

In 2002, TeGenero, a spin-off company from the University of Würzburg, initiated the development of a human CD28-specific superagonist. TGN1412, a fully humanised mouse antibody of the IgG4 subclass was extensively tested in cynomolgus macaques, where it binds to CD28 with the same affinity as in humans (22). Since up to 50 mg/kg was well tolerated in that species, a FIH dose of 0.1 mg/kg was expected to be safe. Shockingly, however, all 6 healthy volunteers (HV) receiving the drug respondedwith a massive cytokine release syndrome requiring intensive care treatment (10). Fortunately, they all eventually recovered, but as a consequence of the incident, TGN1412 development was interrupted and TeGenero went into insolvency.

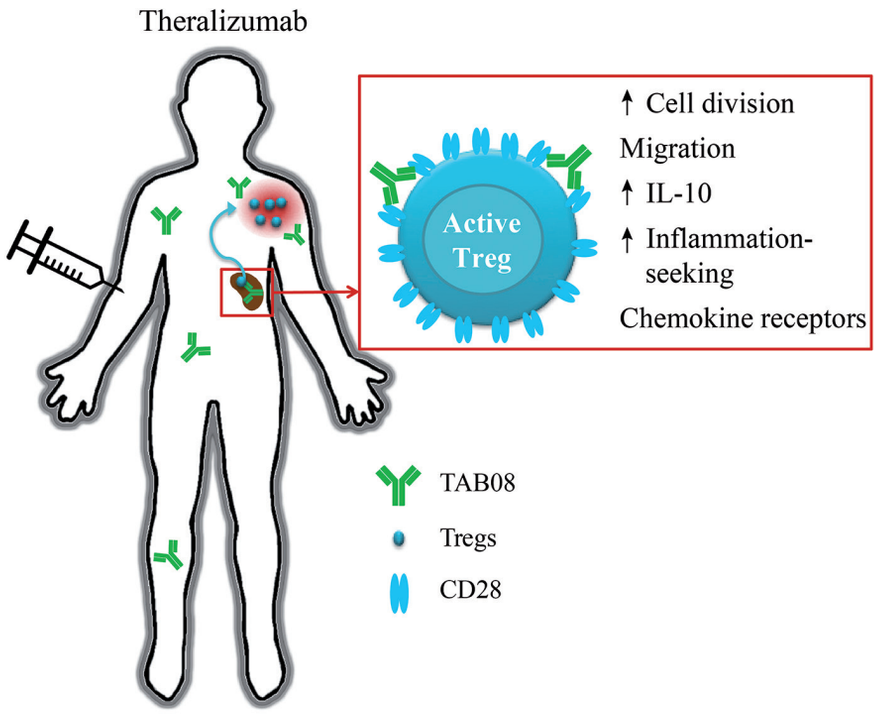

Fig. 1. Proposed mode of action of Theralizumab (TAB08). Treatment with a low dose of the antibody selectively activates Treg cells in lymphoid organs, which divide, enter the circulation, express homing receptors guiding them to sites of inflammation, and use effector functions such as IL-10 to fight inflammation on-site. This model is based on observations in mice, and on *in vitro* studies with human PBMC.

Table I. Rodent models of autoimmunity, inflammation, transplantation and tissue repair in which CD28SA-mediated Treg activation have been found therapeutically effective.

Disease / indication	Rodent model	References
Guillain-Barré-Syndrome	EAE	(27)
Multiple Sclerosis	EAE	(17) (28) (29)
Acute arthritis	AA	(30)
Chronic arthritis	G6PI-arthritis	(31)
Inflammatory bone destruction	TNF-tg mouse	(32)
Type I diabetes	BB rat	(33)
Glomerulonephritis	rat glomerulonephritis	(34)
IBD	DSS colitis	(35)
Trypanosome inflammation	T.cruzii infected mice	(36)
Pemphigus vulgaris	desmoglein 3 immunized mice	(27)
GvHD	rat, mouse GvHD	(37) (38)
Solid organ graft rejection	kidney, heart, liver, trachea	(39) (40) (41) (42)
Stroke	mouse central artery occlusion	(43)
Myocardial infarction	mouse transient coronary occlusion	(44)

Resumption of preclinical development

The most important insight into why the cytokine storm experienced in the 2006 HV trial had not been announced by preclinical work is the finding by the group of Richard Stebbings that CD4 effector memory cells, which are the source of the multiple pro-inflammatory cytokines released by CD28SA stimulation in humans, selectively down-regulate the target molecule CD28 in cynomolgus macaques (11). Accordingly, these monkeys can tolerate extremely high amounts of this potent antibody without discomfort, making the no-observed-adverse-events method used

to determine the FIH dose misleading.
Furthermore, since in contrast to polyclonal T-cell activators addressing the TCR such as the CD3-specific mAb OKT3, PBMC cultures do not respond to TGN1412 with cytokine release, modified PBMC based *in vitro* assays were developed to reveal its stimulatory capacity and make it amenable to mechanistic analysis. Strong responses were indeed observed to plastic-immobilised TGN1412 (23). While this approach does reveal the stimulatory potential of the antibody, it precludes the establishment of dose-response relationships with soluble antibody as is applied in vivo. We therefore developed an alternative assay which allows to analyse the responses of PBMC T-cells to soluble CD28SA.
This "Restore" assay (for resetting T-cells to original reactivity) resets the signalling-defective circulating T-cells to tissue-like reactivity by culturing PBMC for 2 days at high density (24). During the high density pre-culturing step, T-cells regain the cell contact dependent tonic signalling of the TCR which they exhibit in the tissues but lose during recirculation. Such basal TCR signals are an absolute requirement for signal transduction in response to CD28SA (14, 15). The Restore assay not only allows to study the cytokine and proliferative responses of PBMC to soluble CD28SA, it also repairs the poor the sensitivity of circulating memory CD4 (24) and CD8 (25) T-cells to microbial and tumour-associated antigens, making it a useful diagnostic tool.
With the help of the Restore assay, TheraMAB, the new owner of TGN1412, resumed development of the antibody, which was renamed TAB08. TAB08 titrations in Restore cultures from a large number of healthy donors, but also from patients suffering from rheumatoid arthritis (26), multiple sclerosis, systemic lupus erythematosus, and psoriasis (unpublished) were analysed for the dose requirements for both, the desired activation of regulatory T-cells, and the unwanted pro-inflammatory cytokine release from CD4 effector memory cells. Exactly as had been noted in rats and mice, Treg cells turned out to require much lower CD28SA doses for activation and proliferation than conventional T-cells. Furthermore, inclusion of corticosteroids completely eliminated the toxic response seen at high TAB08 doses, while a substantial amount of Treg activation was corticosteroid-resistant (26), providing an additional safety net for clinical application.

Resumption of clinical development
These encouraging findings led to the design of a new healthy volunteer trial, which started at 0.1 mg/kg, *i.e.* a 1000-fold lower dose than the one applied in the ill-fated trial of 2006 (Clinical trials identifier: NCT01885624). After careful monitoring of each patient, the dose was gradually increased to a maximum of 7 mg/kg, still well below what had been applied in the first HV trial. To our satisfaction, pro-inflammatory cytokine release remained completely absent, whereas at the highest doses employed, all volunteers responded with transient release of IL-10 into the circulation (26). Since IL-10 is a Treg signature cytokine, this strongly suggested that the desired selective activation of regulatory T-cells is possible if TAB08 is appropriately dosed.

A Phase Ib trial in RA patients
Subsequently, a total of 18 patients with RA diagnosed at least 6 months earlier was performed (Clinical trials identifier: NCT01990157). TAB08 was applied in four weekly intervals, and laboratory parameters, adverse effects and clinical response were recorded. While the details of this trial are currently being prepared for publication, it can be summarised that adverse events (AEs) remained at an acceptable level (brief episodes of fever associated with circulating IL-6), and the majority of patients responded with ACR20 or higher scores. Obviously, this result awaits confirmation by a double blinded phase II study, which is currently under way.

Outlook
If the encouraging proof-of-concept study of TAB08 in RA patients will be confirmed in phase II, transient polyclonal Treg stimulation by CD28SA may provide a novel approach to the treatment of other immunopathologies and inflammatory disorders as well. Whilst the individual effector mechanisms mediating pathology vary between such diseases and hence are currently addressed by an array of different mAb or soluble receptor fusion proteins, the power of transient controlled polyclonal Treg activation lies in the versatility of this cell type in interfering with most of these pathomechanisms through a set of countermeasures including interruption of co-stimulation, provision of anti-inflammatory cytokines, cytokine elimination, induction of apoptosis in pathogenic immune cells and immunomodulatory interactions between cell surface receptors on Treg and target cells. It is an interesting parallel that both, IL-2 and CD28SA therapy, were initially wrought with strong toxicity due to activation of effector cells (which, in case of IL-2, was intended to fight tumours) and vascular leakage. Now, at much reduced doses, both agents appear to be useful to activate the control of immunopathology by regulatory T-cells. Further clinical studies are eagerly awaited with the hope that this strategy will help those who do not respond to the current standard treatments.

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