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REVIEW ARTICLE

TEGENERO CLINICAL TRIALS DISASTER

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ABSTRACT

CD28 and Tumor necrosis factor (TNF) co-stimulatory signals are required for recruitment and expansion of naïve T-cell, CD28 regulates both the development of T-regulatory cells in the Thymus and their peripheral homeostasis. These T-regulatory cells (CD25+ and CD4+) constitute 5-15% of the peripheral T-cells these cells are involved in the autoimmune disease as they act as immunosuppressant, studies shown that targeting of CD28 with monoclonal antibodies (mAbs) produces as significant increase in CD4 cell count in peripheral blood, spleen and the lymph nodes. In case of CD28, stimulatory mAbs act as artificial ligand that mimic the effect of natural ligands and cause co-stimulation of the T-cells and some of these mAbs have super agonistic activity. TeGenero, a German company, developed a recombinantly expressed humanized super agonist anti- CD28 mAbs (TGN1412). TGN1412 produced T-cell expansion in the absence of stimulation by T-cell receptor in the ex-vivo studies without provoking proinflammatory response; hence, TGN1412 shown to have the ability to activate regulatory T-cells therefore TGN1412 thought to possess a therapeutic effect in autoimmune disorders, and after extensive preclinical studies, TGN1412 approved for clinical trials. Unfortunately, the clinical trial does not went well as six of the volunteers suffered from severe adverse effects of the drug as discussed in this review.

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INTRODUCTION

CD28 and Tumor necrosis factor (TNF) co-stimulatory signals are required for recruitment and expansion of naïve T-cell (Boesteanuand Katsikis 2009). CD28 has been shown to regulate both the development of T-regulatory cells in the Thymus and their peripheral homeostasis (Tang *et al.* 2003). These T-regulatory cells (CD25+ and CD4+) constitute 5-15% of the peripheral T-cells these cells are involved in the autoimmune disease as they act as immunosuppressant (Tang *et al.* 2003, Masteller *et al*. 2005). It has been shown that targeting of CD28 with monoclonal antibodies (mAbs) produces as significant increase in CD4 cell count in peripheral blood, spleen and the lymph nodes (Shi *et al*. 2012), thesem Abs could be used either to block or stimulate specific cell receptor by mimicking the action of endogenous ligand (Hünig and Dennehy2005), in case of CD28 stimulatory mAbs act as artificial ligand that mimic the effect of natural ligands and cause co-stimulation of the T-cells and some of these mAbs have superagonistic activity (Hünig and Dennehy2005). The superagonistis defined as a ligand that binds to specific receptor and produces a response greater than that of endogenous ligand with an efficacy exceeding 100 % (Carlier *et al*. 2002).

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The difference between conventional and superagonist CD28 mAbs is that the conventional CD28 antibodies bound to the same binding site of the natural ligand i.e. top of the molecule, while CD28 superagonists in lateral manner to IgV domain at the bottom of the CD loop (Hünig and Dennehy2005)figure-1.TeGenero, a German company, developed a recombinantly expressed humanized superagonist anti- CD28 mAbs (TGN1412), TGN1412 shown to produce T-cell expansion in the absence of stimulation by T-cell receptor in the ex-vivo studies, moreover preclinical data shown that TGN1412 produced a well tolerated T-cell expansion without provoking proinflammatory response, and since TGN1412 shown to had the ability to activate regulatory T-cells therefore TGN1412 could produce a therapeutic effect in autoimmune disorders, and after extensive preclinical studies, TGN1412 was approved for clinical trials (Attarwala 2010). In March 2006 TGN1412 used in a randomized, double blinded, placebo controlled phase I clinical trials, unexpectedly within 90 minutes after TGN1412 intravenous dose 6 of the volunteers developed systemic proinflammatory response and other symptoms like headache, nausea, myalgias, hypotension andas the time was progressing the severity of symptoms continued to increase; consequently all of the 6 volunteers were hospitalized and placed under close monitoring and received a several medications in order to reverse the condition (Suntharalingam *et al.* 2006). Below is a review of steps of TGN1412 development, trials and lessons learnt from it.

CD28 Super agonists: Naïve T-cells require two signals to be fully activated; the first signal is the interaction T-cell antigen receptor (TCR) with the complimentary major histocompatibility complex/peptide complex and the second is the co-stimulatory signal mediated by interaction of CD28 with the ligands CD80 (B7-1) and CD86 (B7-2) (Luhder *et al.* 2003,Margulies2003) figure-2. In vitro studies showed that absence of CD28 signaling did not affect number and differentiation of the T-cells, but will lead to the loss of T-regulatory (T-reg) cells CD4+ CD25+ which function as inhibitors of the auto reactive T-cells in the periphery; thus upon loss of T-reg cells function and/or number may lead to autoimmune disorders (Beyersdorf *et al.* 2005).

Therefore targeting CD28 with stimulatory mAbs that mimic the effect of endogenous ligand could be of valuable therapeutic potential (Hünig and Dennehy2005). There are two types of anti-CD28 mAbs: conventional co-stimulatory mAbs which depends on TCR signaling and superagonistic mAbs which have ability to activate naïve T-cells independently from TCR ligation in both in vivo and in vitro models (Luhder*et al.* 2003). Conventional mAbs bindto a site located near the binding site of B7 (MYPPY) which is located in the membrane distal part of the CD28 extracellular region, while superagonists binding site is located near C"D loop which is close to the membrane (Rileyand June2005) figure-2.More recently Integration between TCR and CD28 signals was shown to be mediated by protein kinase C isoform (PKC θ), and this will lead to activation of the transcription factor (NF-κB) which is necessary for proliferation of T-cell and interleukin-2 (IL-2) production (Dennehy *et al*. 2003). Interestingly CD28 superagonists shown to activate PKCθ-NF-κB pathway and stimulate all T-cells proliferation in the periphery without TCR ligation signals (Dennehy *et al*. 2003).

Role of CD28 in disease: CD28 is a 44 KDa glycoprotein expressed as a homodimer on the cell surface; almost all CD4 and majority of CD8 cells express CD28 on their cell surfaces (Daikh, Wofsy and Imboden1997). Homodimeric receptor family includes CD28, inducible co-stimulator (ICOS) and cytotoxic T-lymphocyte antigen-4 (CTLA-4, CD152) (Hünig and Dennehy2005). During infection CD4+ and CD8+ T-cell response is mediated by the co-stimulator molecule (CD28) which will interact with CD80/86 expressed by the antigen presenting cells together with TCR activation will lead to increase expression of BcL-X_L(an anti-apoptotic protein), increase in production of IL-2, and increase in stability and transcription of IL-2 mRNA (Topp *et al*. 2003). Moreover it has been shown that during chronic illness and elderly CD28 expression in T-cell is reduced significantly suggesting the relationship between CD28 loss and senescence (Riley and June 2005). Surprisingly Topp *et al*. (2003) restored IL-2 expression in HIV-specific $CD8^+$ and cytomegalovirus-specific $CD28^-$ $CD8^+$ T-cells by retroviral-mediated CD28 gene transfer. Moreover CD28 regulates TCR signals required for the activation of CD4+CD25+ T-reg cells which in turn, once activated, suppress and modify the autoimmune response (Rileyand June 2005), and it has been shown that CD28 inhibition will exacerbate autoimmune response while CD28 activation will prevent or delay diabetes in non-obese diabetic mice (Salomon *et al*. 2000).

TeGenero Preclinical studies: After the discovery of CD28 superagonists and how they were different from conventional antibodies in terms of binding to different epitope binding site and their capability of activate naïve T-cells independently from TCR signals; TeGenero Company started screening of several mouse mAbs that possess super agonistic activity and upon this screening TeGenero produced a genetically engineered humanized monoclonal antibody by adding the complimentary determining regions from the light and heavy chains variable regions of mouse anti-human CD28 (5.11A1) to human light and heavy chain variable regions (Attarwala 2010, TeGenero 2010).

In Vitro studies: Epitope mapping analysis using mutated CD28 molecule was used to determine topological requirement of TGN1412 and it showed that TGN1412 bind specifically to the Ig-like extracellular region of CD28 molecule(C"D loop) (TeGenero 2010). Flowcytometry and Biacore analysis were used to evaluate TGN1412 specificity to CD28; flowcytometric analysis showed that peripheral blood mononuclear cells (PBMC) were stained by TGN1414 in a CD28-specific pattern, while Biacore analysis revealed that TGN1412 has an affinity of $Kd=1.88*10^{-9}$ to CD28, furthermore TGN1412 bound only to cell lines transfected with human CD28 gene, but not to cell lines transfected with genes of closely related receptors such as ICOS and CTLA-4 with (TeGenero 2010).In vitro analysis shown that there was no cross reactivity with rodents and monkeys CD28 as TGN1412 bound with low affinity to CD28 expressed on T-cells derived from rodents, in contrast TGN1412 exhibited a high binding affinity towards CD28 expressed on T-cells from Rhesus and Cynomolgus monkeys; later on analysis of C"D loop sequence homology between Rhesus monkeys and human revealed only one amino acid difference while marmoset monkeys the difference was two amino acids, and low sequence homology when compared to rodents (Attarwala 2010). Finally TGN1412 was capable of inducing rapid proliferation of T-cells independent from TCR signals when incubated with T-cells obtained from healthy individuals, while the conventional CD28 antibody couldn't provoke such stimuli (Attarwala 2010) figure-3.

In vivo studies: After TGN1412 exhibited efficacy in vitro studies, further In vivo studies were performed to evaluate its efficacy and safety; thus rhesus and cynomolgus monkeys were used due to the fact that the extracellular region of their CD28 receptor is 100% homologous to human CD28 and hence have the same binding affinity toward TGN1412 also these species have a highly conserved Fc receptors and motifs like human thus similar response and affinity could be obtained (Attarwala 2010).

Since degradation pathways of proteins are well known therefore classical absorption, metabolism, distribution, and excretion studies for TGN1412 were not performed as it was thought that TGN1412 is degraded into amino acids by lysosomal enzymes in liver and/or kidney which will subsequently reabsorbed (TeGenero 2010). Rat, rhesus and cynomolgus monkeys were used to determine plasma/serum concentration and kinetics of T-cell activation/expansion (TeGenero 2010). Moreover repeated doses of TGN1412 were used to evaluate its toxicokinetics in cynomolgus monkeys, with a dose range of 5-50mg/Kgfor 28 days and it was shown that TGN1412 plasma half life was 8 days after the first dose and this was expected because all relatively large biological molecules like antibodies have a slower elimination rate (TeGenero 2010).

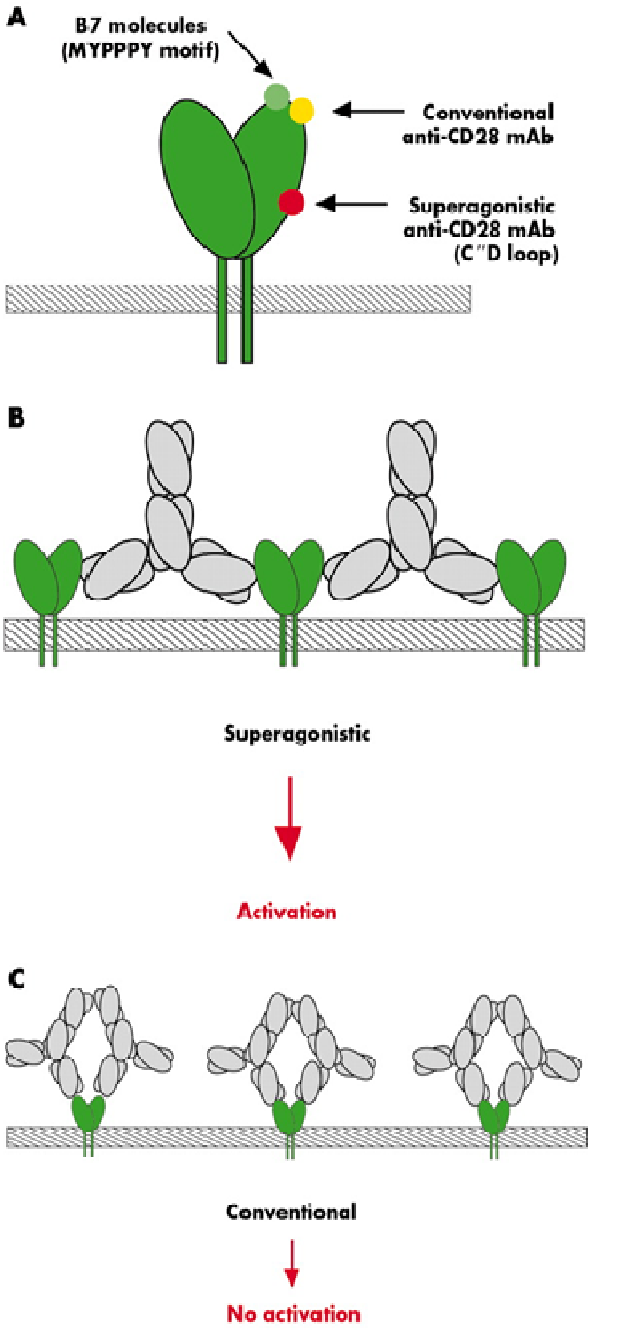

Figure-1: (A and B) binding sites of conventional and superagonist anti-CD28 mAbs. Superagonists (red) bind laterally to motif of CD28, while B7 molecule (green) and conventional bind to the normal binding site of the natural ligand, therefore Cd28 superagonist will form liner complexes which will consequently activate signaling components, probably as aggregates, leading to strong activating signals (Beyersdorf *et al.* 2005) Figure-1(C):binding of the conventional mAbs to CD28 in bivalent manner, unlike superagonist they doesn't form linear complex but rather considered to be tangled (Beyersdorf *et al.* 2005)

Phrmacodynamic studies shown that TGN1412 produced efficient expansion of CD4+ and CD8+ T-cells at a dose of 5mg/kg within 13 days from the initial dose (Attarwala 2010). Although TGN1412 achieved 4 peaks plasma concentration upon administration of 4 increasing repeated increasing doses, however T-cell number in blood achieved only one peak because TGN1412-induced T-cell expansion is greatly dependent on availability of T-cells and co-stimulator receptor saturation kinetics (Attarwala 2010). Furthermore the pilot toxicological studies in rhesus and cynomolgus monkeys shown that even higher doses of TGN1412 (50mg/kg) did not produce adverse effect and was well tolerated (TeGenero 2010).

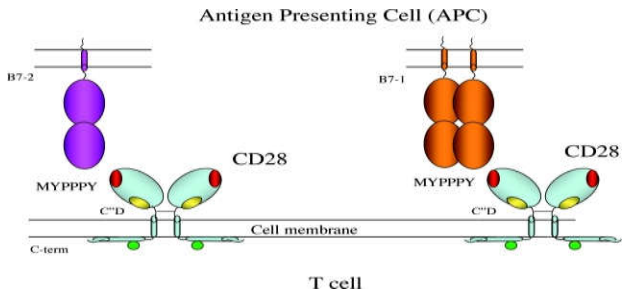

Figure 2. illustrate the binding site of the ligands CD86 (B7-2) and CD80 (B7-1) which is the MYPPPY domain colored with red, the binding sites of conventional mAbs is located near this region. While superagonists ant-CD28 mAbs bind to C"D loop colored with yellow (Margulies2003)

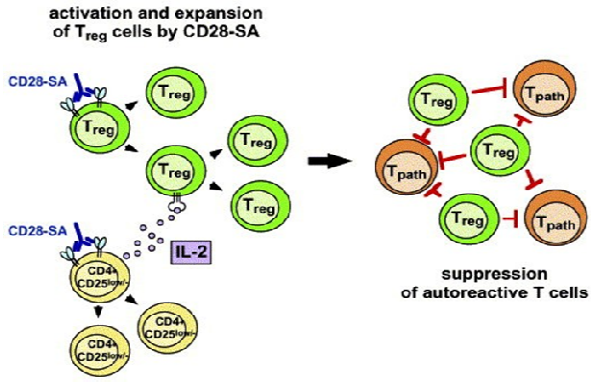

Figure 3. Activation of CD28 by super agonist will lead to expansion of regulatory T-cell proliferation and also enhance IL-2 production by CD4+CD25low- which in turn lead to further activation and proliferation of T-regulatory cells (Beyersdorf *et al.*2006).

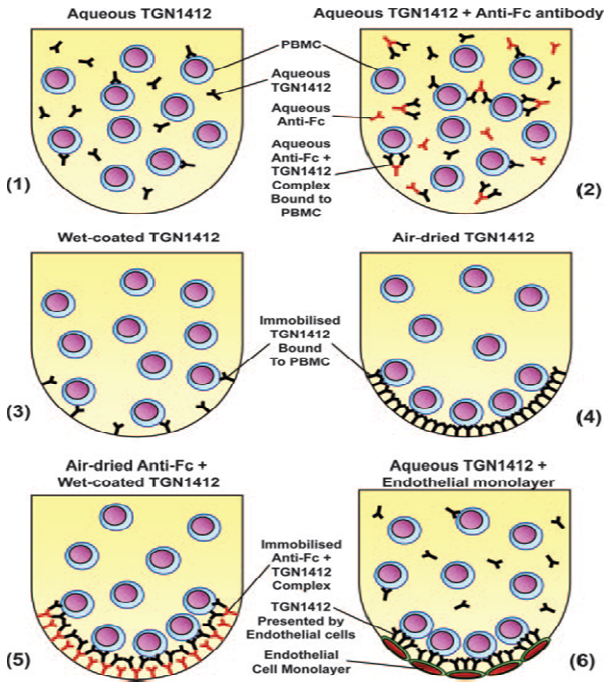

Figure-4. Different protocols of TGN1412 presentation to PBMC, (1)TGN1412 aqueous solution, (2) TGN1412 aqueous solution with anti-human Fc aqueous solution, (3) TGN1412 direct wet coat onto the walls of the wells, (4) TGN1412 air-dried coat onto the walls of the wells, (5) TGN1412 wet coat on an air dried anti-human FC antibody coat, (6) TGN1412 wet coat on human endothelial cell layer to mimic the effect of interaction between endothelial cells and TGN1412 with. Only Protocols 1 and 2 were used in the original in vitro studies and did not produce T-cell proliferation and cytokine release, Protocols 3-6 tried to see the effect of Ab immobilization on T-cells response, protocol 3 did not stimulate T-cells or induce cytokine release, while protocols 4-6 evoked cytokine release TNFα, IL-8, and IL-6 in human PBMC but not cynomolgus monkeys" blood (Stebbings *et al.* 2007).

Table 1. Summary of safety pharmacological and toxicological studies of TGN1412 (TeGenero 2010)

	System	TGN1412 effect
1	Respiratory system	• TGN1412 bound to the CD28 of pulmonary lymphocytes of Cynomolgus monkeys • normal histological finding • no treatment related adverse effects were seen.
2	Cardiovascular system	• no cross reaction with human or cynomolgus heart tissues • no ECG changes were observed throughout treatment period in Cynomoglus monkeys(28 days using repetitive toxic dose) • no histological changes in cardiovascular tissues like aorta and heart
3	Central nervous system	• TGN1412 distributed in CNS tissues including brain spinal cord and pituitary gland • no clinical manifestation recorded during treatment period including 28 days of repeated TGN21412 doses in cynomolgus monkeys • no altered histological findings were observed in tissues like eye, sciatic nerve or optic nerve.
4	Genital system	• no effect of TGN1412
5	Mutagenicity and carcinogenicity	• no effect of TGN1412
6	In vitro cytotoxicity	• TGN1412 did not produce complement dependent cytotoxicity(CDC) in peripheral blood mononuclear cell line • TGN1412 did not produce antibody dependent cellular cytotoxicity in modified human Jukart cell line.

Table 2. Summary of adverse events observed in the 6 volunteers and time after which these events were developed; patients 5 and 6 were mostly affected and events were more severe.

Patients	Adverse events	time followed the first dose
Patients 1-6	Headache, lumbar myalgia, rigors	50-90
Patients 1-6	*Hypotension and tachycardia	210-280 minutes
Patients 1-6	Hyperthermia(39.5-40C^0)	240-390 minutes
Patient 1	Respiratory failure and tachypnea	300 minutes
Patient 6	Hypotension after initial recovery, metabolic acidosis, hypoxemia and respiratory distress, abnormal coagulation and hemodynamic	12 hours
Patients 1-6	Tachypnea, bilateral pulmonary infiltrates, patients were unable to finish spoken sentences.	16-12 hours
Patients 1-6	Intravascular coagulation and renal impairment, decreased platelet count, prolonged prothrombine time, monocytopenia and lymphopenia	16-12 hours
Patients 1-6	metabolic acidosis, oliguria and elevated creatinine levels	8-16 hours
Patients 5 and 6	Recurrent fever, diffused erythematous flushing, increase capillary permeability lasted for several days	After 48 hours
Patients 6	Peripheral ischemia, necrotic patches on all toe and fingers	after 48 hours to day 30
Patients 1-6	Generalized desquamation(severe in patients 5 and 6), muscles weakness	After 30 days

*Hypotension defined as more than 20 mmHg drop in systolic blood pressure, Tachycardia: heart rate was 110-145 beat per minute (Suntharalingam*et al.* 2006).

Table 3. Phases of the clinical status progression and the time after TGN1412 infusion (Suntharalingam*et al.* 2006)

Phase	Time after TGN1412 infusion	Characteristics
Phase I	1 hour to day 2 or 3 for patients 5 and 6	Cytokine storm, sever monocytopenia and lymphopenia, rapid activation of cytokines type 1 and 2
Phase II	1 day through day 3 or 8 for patients 5 and 6	Renal and respiratory failure, disseminated intravascular coagulation
Phase III	Day 3 to day 15, or day 5 to 20 for patients 5 and 6	Recovery of pulmonary and renal function, increase platelet count, increase in monocytes and lymphocytes
Phase IV	Day 15 or day 20 for patients 5 and 6	Recovery of the normal reading for the measured markers

Table 4. Factors that could affect immunogenicity of biological therapies (Strand*et al*. 2007)

	Factors	Comments
1-	Patient related	1-Genetic variation 2-immunocompetence, 3-polymorphism of the Fcγ recptor which affect cytokine release and cytotoxicity 4- underlying immunosuppressive disease like rheumatoid arthritis 5-haplotype of the major histocompatibility (MHC) which affect antigen presentation to the immune system
2-	Product related	1- presence of non-human sequence resulted from the recombination process 2-conjucation with polyethylene glycols 3-binding of mAbs idiotypic regions to cell surface antigens are often immunogenic 4-immpurities that contaminate product during packaging 5-vaiability of the constant C and variable V regions of the antibody
3	Treatment related	1-intravenous route is least immunogenic while subcutaneous route is most immunogenic 2-intermetant administration of mAb to avoid resistance commonly associated with immunogenicity 3-high doses of biological agents are associated with less frequent immunogenicity
4-	Concomitant drug administration	Co-administration of immunosuppressive agents like methotrexate and azathioprim which reduce immune response in patients with rheumatoid arthritis treated with infliximab

Safety pharmacology and toxicology: Usually novel drugs are subjected to a series of studies to confirm its safety by testing its effect on organs other than that intended for therapeutic use (TeGenero 2010) thus TGN1412 underwent assessment for it unwanted effects on respiratory system, central nervous system and cardiovascular system (TeGenero 2010) Table1.

Surprisingly no clinical manifestations related to cytokine release observed, no signs and symptoms of anaphylactic reaction and no suppression of immune system or induction of autoimmune disorders (Attarwala 2010).

Effect on cytokine secretion: TGN1412 dose range (5-50mg/kg) in cynomolgus monkeys caused a transient increase

Table-5. a summary of the Expert Scientific Group ESG recommendations for first-in-human trails (St Clair 2008)

	Issue	Recommendations
1-	Preclinical and early clinical development	Sufficient safety evaluation studies, regular review of the first of human studies regulatory guidelines, data of unpublished studies should be accessed, safety data of phase I trials safety should be shred
2-	Preparation and review of clinical trial applications	Sponsors and regulators communication at early developmental stages, outside expert consultation to evaluate the risk associated with the trails, plenty of time required to review complex cases.
3-	Phase I trials designing	Dealing with New agents with novel mechanism that cannot be evaluated by animal studies require special considerations, starting dose calculations should rely on several factors not only on 'no-observable-effect level in animals' , selection of a safe starting dose, rate of drug administration should be considered in trails, trails design and dosing interval should be appropriate for new agents, careful consideration for volunteers selection for the trials.
4-	Clinical environment	Principal investigator qualification, risk management and suitable staff, equipment and facilities are required.
5-	Developing expertise	Higher number of principal investigators required, specialized center required for conduction of first in-human trails.

in the plasma levels of IL-2, IL-6(inflammatory cytokine) and IL-5(anti-inflammatory cytokine) but not affected IL-4 levels even at higher dose (50mg/kg), this transient increase was shown to be dose-dependent, moreover the proinflammatory cytokines TNFγ and TNFα levels were not significantly increased after TGN1412 first dose (TeGenero 2010). From the data of efficacy and pre-clinical safety in vivo studies it was shown that TGN1412 is effective, not associated with potential adverse effects, and based on no-observed adverse effects level of 50mg/kg it was estimated that 0.1mk/kg will be the starting dose in clinical trials which represents a 500-folds margin of safety. Consequently TeGenero approved for phase I clinical trials (TeGenero 2010).

TeGenero Clinical Trials: TGN1412, the novel recombinantly expressed humanized monoclonal antibody (IgGκ4 subclass), was shown to be safe and effectively expanded CD4+CD25+ T-reg cells in the in vivo models (Suntharalingam *et al.* 2006) thus TGN1412 underwent phase I clinical trial which was performed by Parexel international, a contract research organization, in St. Mark's and Northwick park hospitals on 13th March 2006 (Suntharalingam *et al.* 2006). The phase I trial was double blinded placebo controlled and involved 8 volunteers, 2 of them received placebo while the other 6 volunteers receivedTGN1412 all volunteers were provided with consent form. Treatment group receivedTGN1412 dose of 0.1mg/Kg by intravenous infusion, infusion rate was 2 mg per minute for 3-6 minutes period at a 10 minutes intervals, while control group received saline with similar volume (Suntharalingam *et al.* 2006).

After 60-90 minutes from the first dose all six individuals developed several adverse effects ranged from headache, diarrhea and vasodilatation to multi-organ failure and disseminated intravascular coagulation, table-2 summarizes adverse events, time at which they were developed (Suntharalingam *et al.* 2006). The progression of the volunteers' clinical conditions divided into 4 phases, Table-3. After development of these signs and symptoms all patients received an initial empirical treatment (Suntharalingam *et al.* 2006). 1-intravenous fluids, hydrocortisone 200mg (i.v), chlorphenramine 10mg (i.v), acetaminophen 1 gram(i.v), ondasetron 4-8mg (i.v), metaraminol 0.3-0.5 mg(i.v). After that all patients moved to critical care unit and received a second dose of hydrocortisone, followed by 3 intravenous doses of 1gm methylprednisolone administered at 16, 40 and 64 hours from TGN1412 infusion, furthermore to reverse the effect of TGN1412 on T-cells all patients received 1mg/kg daclizumab, an IL-2 receptor antagonist, after this treatment low dose of steroid therapy initiated for 21-33 days (Duff report 2006). All these adverse effect were due to TGN1412-induced rapid and sudden release of proinflammatory cytokines, which was not expected according to pre-clinical data. Cytokine release and systemic inflammatory response syndrome were similar to that when exposed to bacterial endotoxin, however regulatory authorities confirmed that TGN1412 was free from pyrogens and other contaminants (Suntharalingam *et al.* 2006).

Later on a study performed by Stebbings *et al.* 2007 tried to explain these events and interestingly it was shown that TGN1412 pre-clinical data failed to predict TGN1412 toxicity in vitro and in vivo models,in which:

- TGN1412 presentation protocols to white blood cells (WBC) in vitro did not mimic its presentation in vivo models as pre-clinical in vitro studies involved cross linking of TGN1412 via Fc receptors in aqueous solution or simply adding TGN1412 to WBC both presentations protocol did not provoked cytokine release figure-4.
- TGN1412 can produce T-cell proliferation andcytokine release when presented in the right way to PBMC and TGN1412 specific and/or nonspecific binding to cell surface is required for cytokine release and T-cell activation.
- 3-Clinical trial dose appeared (0.1mg/kg) to be near to the maximum stimulatory dose required for T-cell proliferationas the superagonist anti-CD28 mAb 5.11A1, from which TGN1412 was produced, can evoke maximal human T-cell activation at a dose of 0.1mg/kg in vitro.
- Cynomolgus white blood cells respond to TGN1412 in a different manner from human cells in vivo and in vitro, as in vitro cynomolgus cells stimulation by immobilized TGN1412 did not induced cynomolgus lymphocytes proliferation unless IL-2 added to cultures.

Thus TGN1412 in cynomolgus monkeys' models did not act as a superagonist (Stebbings *et al.* 2007).Although CD28 extracellular region is 100% identical between human and cynomolgus, however there were functional differences, also CD28 in cynomolgus is different from human in the 3 transmembrane domains which may affect the binding properties, Moreover it has been suggested that the lack of Siglec expression (an immunoihibitory) in human T-cell was responsible for the difference in response to TGN1412 (Stebbings *et al*. 2007).

Lessons from TGN1412: Several lessons learnt from TGN1412 catastrophe (Strand *et al*. 2007). The first lesson is that although theory behind biological therapy is simple, however it is hard to predict phrmacodynamic properties, efficacy and toxicity of these therapies, second lesson is that Fc region in mAbs and immunoglobulin mediate different cellular functions; Fc increase mAbs serum levels via interaction with neonatal Fcγ receptors which in turndecreasem Abs catabolism and enhance mAbs recycling, beside this function the binding to classical Fcγ receptors is mediate via Fc region, in turn this binding can activate other cellular functions like apoptosis (Strand *et al*. 2007). The third lesson is all biological therapies can induce immunogenicity and several factors can affect immunogenicity of biological therapies, table-4 (Strand *et al*. 2007). The forth lesson was the adverse effects, efficacy and safety of biotherapies might not completely predicted in pre-clinical studies due to differences in target molecules and physiology of immune system in animal models; for example it has been shown that blockade of the interaction of CD40 with its ligand (CD40L), in which together act as co-stimulatory molecule, with anti-CD40mAbs produced a beneficial effect in animal models of Systemic Lupus Erythematosus, however in phase I clinical trials it produced episodes of thromboembolism suggesting the presence of CD40 ligand on human platelets, therefore safety parameters and adverse effect should be studied carefully (Strand *et al*. 2007).Consequently an Expert Scientific Group (ESG) put 22 recommendations for development process of these novel agents before being used or tested in man; one of these recommendations emphasized on safety profile of these agents and full explanation of their mechanism of action and information available should be revised by independent experts and the TeGenero trial regulators should have asked for more data about TGN1412safety and untested mechanisms of action in human before the trials (St Clair 2008). On the other hand 9 of these recommendations focused on the design of the study and accurate calculation of the first in-man study dose, as mentioned before dose of TGN1412 was not estimated correctly, also the dosing interval as the ESG stated that 10 minutes interval between infusion was too short to monitor adverse effect related to the TGN1412 infusion(St Clair 2008).Finally the ESG stated that first in-human trails should be conducted by highly qualified and trained investigators and they suggested introducing national accreditation system for principal investigators involved in clinical trials, summary of these recommendations listed in table-5(St Clair 2008).

After TeGenero: After the TeGenero catastrophe the regulatory authorities raised the level of regulations, for example in the United Kingdome, the Medicines and Health care Regulatory Authority (MHRA) should ask for Expert Advisory Group(EAG)/Commission on Human Medicines (CHM) advise before giving approval for phase I clinical rails of high risk investigational drugs, Moreover the Sponsor must provide sufficient data so that expert advice could be given and also Sponsors should communicate with Agency before application for Clinical trials authorization (CTA) for such trails, and then followed by normal CTA application process (Dayanand Wraith2008).New therapies have been defined as medicinal products with potential high-risk when the serious adverse effects are not expected to occur in first-time-in-human trails (Dayanand Wraith2008). In conclusion to void problems associated with new treatments in the future; firstly a greater effort should be given to improve pre-clinical evaluation techniques, for example *in silico* assessment techniques or artificial immune system, secondly greater involvement of independent immunological assessment of biopharmaceuticals; thirdly agents that target immune system should be examined as much as possible in the ex-vivo and preclinical studies to successfully estimate the possible risks and finally the regulatory process should be reviewed by qualified experts in this field (Dayanand Wraith2008).

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