

# TEGENERO CLINICAL TRIALS DISASTER

LECTURER

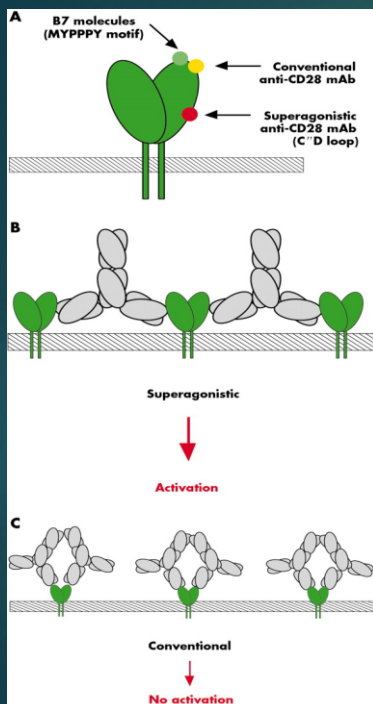
MAHMOOD K. SALIH

## Introduction

- ▶ CD28 and Tumor necrosis factor (TNF) co-stimulatory signals are required for recruitment and expansion of naïve T-cells.
- ▶ CD28 regulate 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.
- ▶ Targeting of CD28 with monoclonal antibodies (mAbs) produces a significant increase in CD4+ cell count in peripheral blood, spleen and the lymph nodes

## Introduction cont.

- ▶ MAbs could be used either to block or stimulate specific cell receptor by mimicking the action of endogenous ligand, 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.
- ▶ some of these mAbs have superagonistic activity .
- ▶ The superagonists defined as a ligand that binds to specific receptor and produces a response greater than that of endogenous ligand with an efficacy exceeding 100 %.
- ▶ 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



- ▶ **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 linear complexes which will consequently activate signaling components, probably as aggregates, leading to strong activating signals.

**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.

# Role of Cd28 in disease

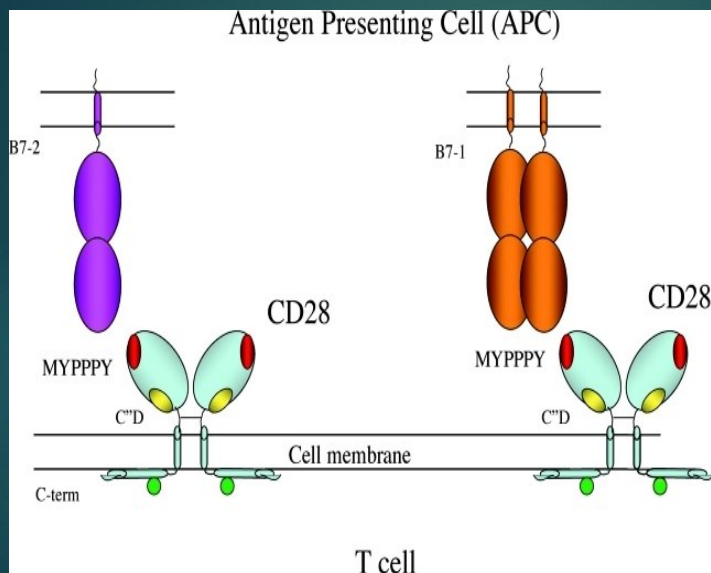
CD28 is a 44 kDa glycoprotein expressed as a homodimer on the cell surface.

almost all CD4 and majority of CD8 cells express CD28 on their cell surfaces.

Homodimeric receptor family includes CD28, inducible co-stimulator (ICOS) and cytotoxic T-lymphocyte antigen-4 (CTLA-4, CD152).

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<sub>L</sub> (an anti-apoptotic protein), increase in production of IL-2, and increase in stability and transcription of IL-2 mRNA

During chronic illness and elderly CD28 expression in T-cell is reduced significantly suggesting the relationship between CD28 loss and senescence



► **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 anti-CD28 mAbs bind to C'D loop colored with yellow

# CD28 Superagonists

- ▶ 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

the second is the co-stimulatory signal mediated by interaction of CD28 with the ligands CD80 (B7-1) and CD86 (B7-2).

- ▶ 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 autoreactive T-cells in the periphery; thus upon loss of T-reg cells function and/or number may lead to autoimmune disorders.

Therefore targeting CD28 with stimulatory mAbs that mimic the effect of endogenous ligand could be of valuable therapeutic potential.

# Types of Anti CD-28 MAbs

- ▶ There are two types of anti-CD28 mAbs

Conventional co-stimulatory mAbs which depends on TCR signaling

Superagonistic mAbs which have ability to activate naïve T-cells independently from TCR ligation in both in vivo and in vitro models

# TeGenero

- ▶ 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.
- ▶ Preclinical data shown that TGN1412 produced a well tolerated T-cell expansion without provoking proinflammatory response.
- ▶ Thus TGN1412 shown to have the ability to activate regulatory T-cells therefore TGN1412 could have a therapeutic effect in autoimmune disorders,
- ▶ After extensive preclinical studies, TGN1412 was approved for clinical trials

# TeGenero development

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 superagonistic activity

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.

## Preclinical studies

In vitro

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  $K_d=1.88 \times 10^{-9}$  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

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

## Preclinical studies contd.

In vivo

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

Rat, rhesus and cynomolgus monkeys were used to determine plasma/serum concentration and kinetics of T-cell activation/expansion (TeGenero 2010).

Pharmacodynamic 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.

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.

Moreover repeated doses of TGN1412 were used to evaluate its toxicokinetics in cynomolgus monkeys, with a dose range of 5-50mg/Kg for 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.

# Safety pharmacology and toxicology

- ▶ **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



	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.

## Clinical trials

- ▶ 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 and as 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

## Events during the trials

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°)	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



## Phases of adverse effects

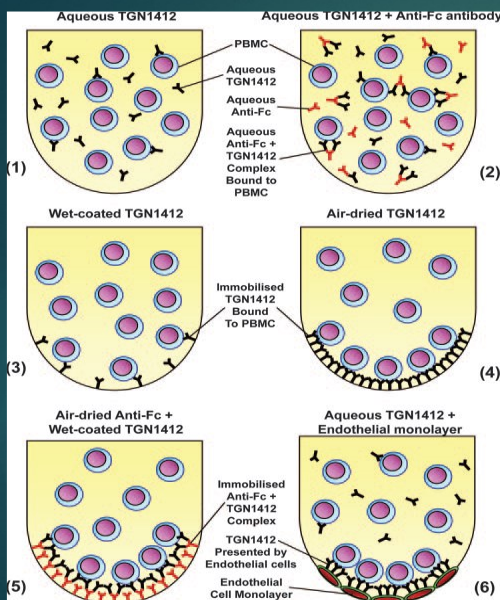
Phase	Time after TGN1412 infusion	Characteristics
Phase I	1 hour to day 2 or 3 for patients 5 and 6	Cytokine storm, severe monocytopenia and lymphopenia, rapid activation of cytokines type 1 and 2
Phase II	1 day through day 3 or 8 for patients 5 and 6	Renal and respiratory failure, disseminated intravascular 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

## Treatment of cytokine storm

- ▶ intravenous fluids, hydrocortisone 200mg(i.v), chlorpheniramine 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.

# What went wrong

- ▶ 1- 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-3.
- ▶ 2- TGN1412 can produce T-cell proliferation and cytokine 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 proliferation as 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.
- ▶ 4- 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. 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 immunoinhibitory) in human T-cell was responsible for the difference in response to TGN1412.



**Figure-3:** 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 TNF $\alpha$ , IL-8, and IL-6 in human PBMC but not cynomolgus monkeys' blood

# Lessons from the trail

- ▶ The first lesson is that although theory behind biological therapy is simple, however it is hard to predict pharmacodynamic 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 turn decrease mAbs 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
- ▶ The third lesson is all biological therapies can induce immunogenicity and several factors can affect immunogenicity of biological therapies, table-1&2
- ▶ The fourth 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

## factors that could affect immunogenicity of biological therapies

	Factors	Comments
1-	Patient related	1-Genetic variation 2-immunocompetence, 3-polymorphism of the Fcγ receptor 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-conjugation with polyethylene glycols 3-binding of mAbs idiotypic regions to cell surface antigens are often immunogenic 4-impurities that contaminate product during packaging 5-vaibility 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-intermittent administration of mAb to avoid resistance commonly associated with immunogenicity 3-high doses of biological agents are associated with less frequent immunogenicity
4-	Concomitant administration	drug Co-administration of immunosuppressive agents like methotrexate and azathioprim which reduce immune response in patients with rheumatoid arthritis treated with infliximab

## summary of the Expert Scientific Group ESG recommendations for first-in-human trails

	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 shared
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.

## After TeGenero

- ▶ 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 TGN1412 safety and untested mechanisms of action in human before the trials.

## After TeGenero contd.

- ▶ After the TeGenero catastrophe the regulatory authorities raised the level of regulations, for example in the United Kingdom, 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 trials 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 trials, and then followed by normal CTA application process.
- ▶ 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 trials.

**thanks for  
listening!**