“Immunotoxicity of Biologics”

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What Is Immunotoxicology?

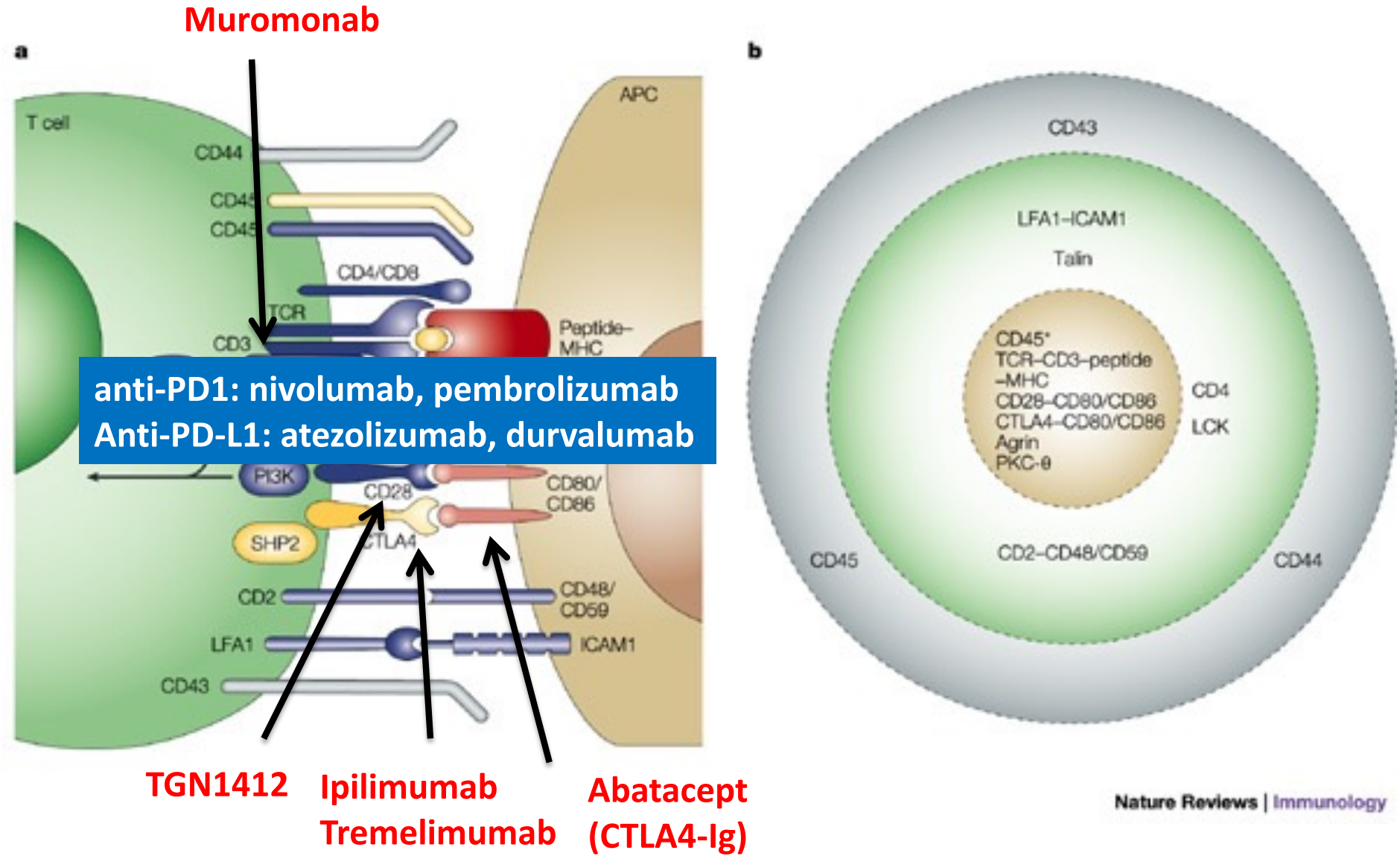
- **Immunosuppression**
  - Infections, viral-induced leucoencephalopathy, viral-induced cancer
- **Inappropriate stimulation of the immune system**
  - Cytokine release syndrome (CRS)
  - Adjuvant effects
- **Hypersensitivity, allergy**
  - Anaphylactic shock, asthma, contact dermatitis
  - Stevens-Johnson Syndrome (SJS), Drug Reaction with Eosinophilia and Systemic Symptoms (DRESS), Lyell syndrome
  - Immune-Related Drug-induced Liver Injury (DILI)
- **Autoimmunity**
  - Lupus, Bowel inflammation, Autoimmune hepatitis, Thyroiditis…

Biotherapeutics ?

- **Proteins**
  - Growth factors (EPO…)
  - Cytokines (IL-2…)
  - Antibodies: monoclonals, bi-specific…
- **Cells**
  - Cell therapy: Replacement of a damaged tissue…
  - Bone marrow transplantation
  - chimeric antigen receptors (CAR) T-cells: tumor cells killing
- **Gene therapy**
  - Vector (AAV, lentivirus…)-based gene expression
  - Cells with genetic modifications (CD34+…)

Very diverse

The immunological synapse = target for biologics



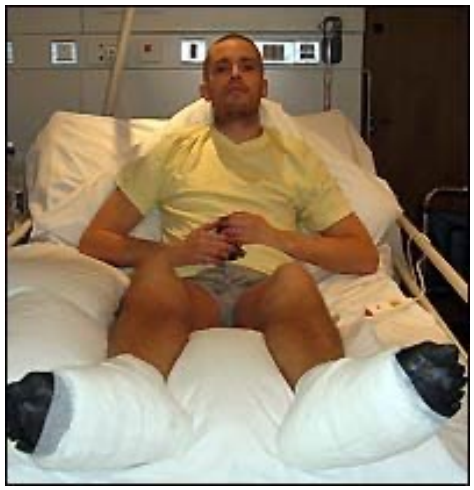
Side effects and “biologics”

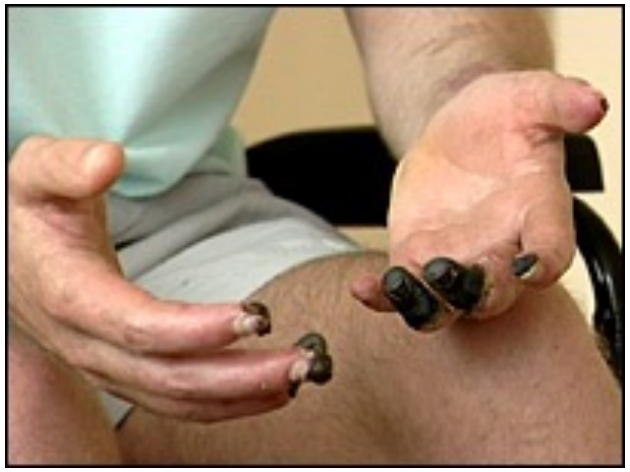
What did we learned or not from the clinic ?

- **From the clinic**
  - **1988** Anti-CD3 (Muromonab): Cytokine Release Syndrome (CRS)
  - Anti-CD52 (Alemtuzumab): CRS
  - TGN1412 (non-conventionnal anti-CD28) : severe CRS
  - IL-2: Vascular Leak Syndrome
  - Alzheimer vaccine (AN1792): meningoencephalitis in 6% of immunized patients
  - Ipilimumab (anti-CTLA-4) and severe colitis
  - Natalizumab (anti-integrin) and PML (progressive multifocal encephalopathy)
  - Daclizumab (anti-CD25) and liver effects

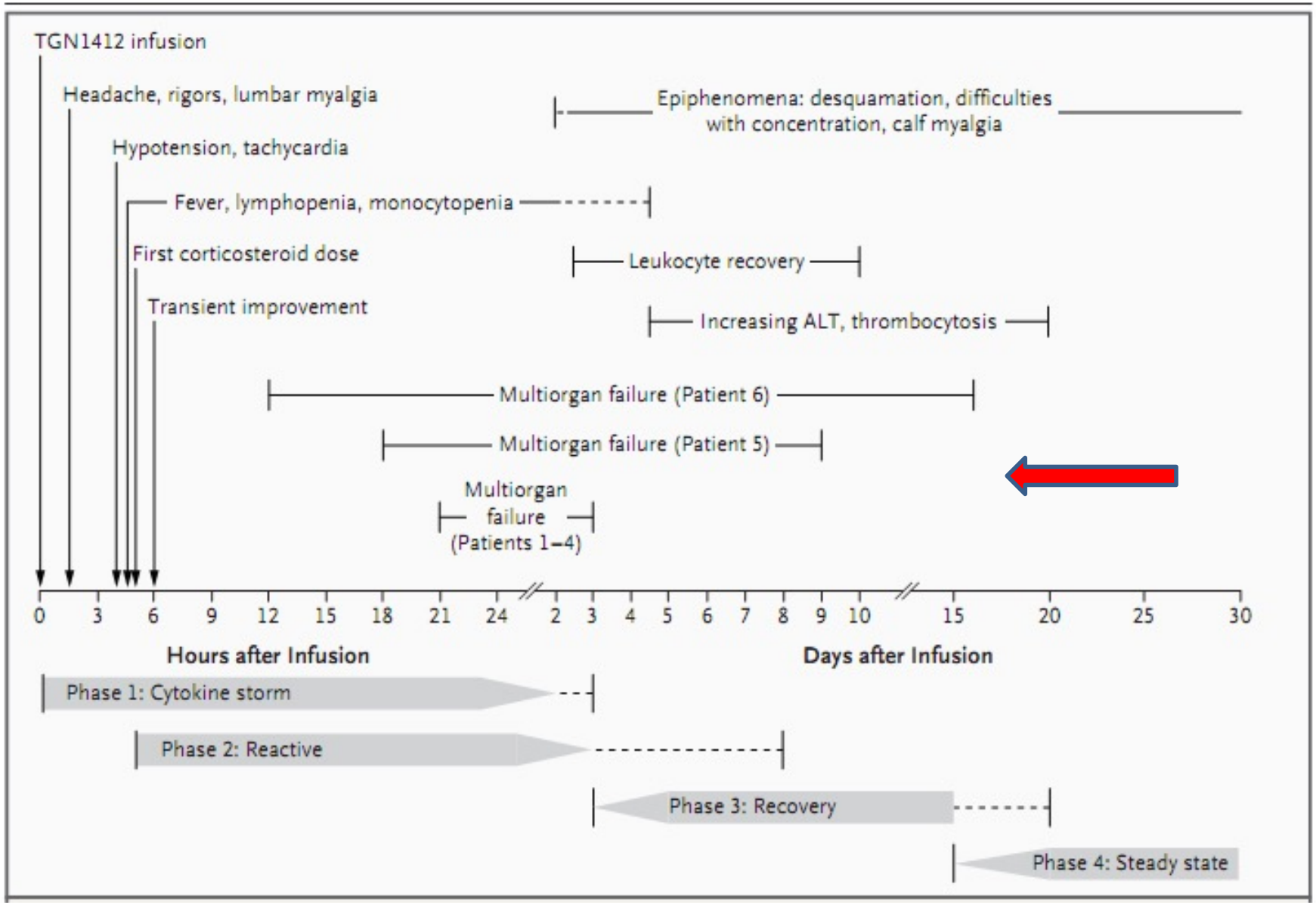
The TGN 1412 story

- March 2006
- Phase I clinical trials with a superagonist anti-CD28 monoclonal antibody
  - Stimulate Treg cells (CD4+CD25+CTLA-4+CD45RlowFoxP3+) ?
  - Superagonist (Binds CD28, activates T cells with no TCR engagement)
- **Potential therapeutic indication: auto-immune diseases**
- 6 human volunteers received the product
  - 0.1 mg/kg, 2 mg/min, as 3-6 min IV infusion, at 10 min intervals
- **Major Cytokine Release Syndrome** with lungs affected
- Hospitalisation in intensive care
- Not observed in toxicology studies using cynomolgus monkeys



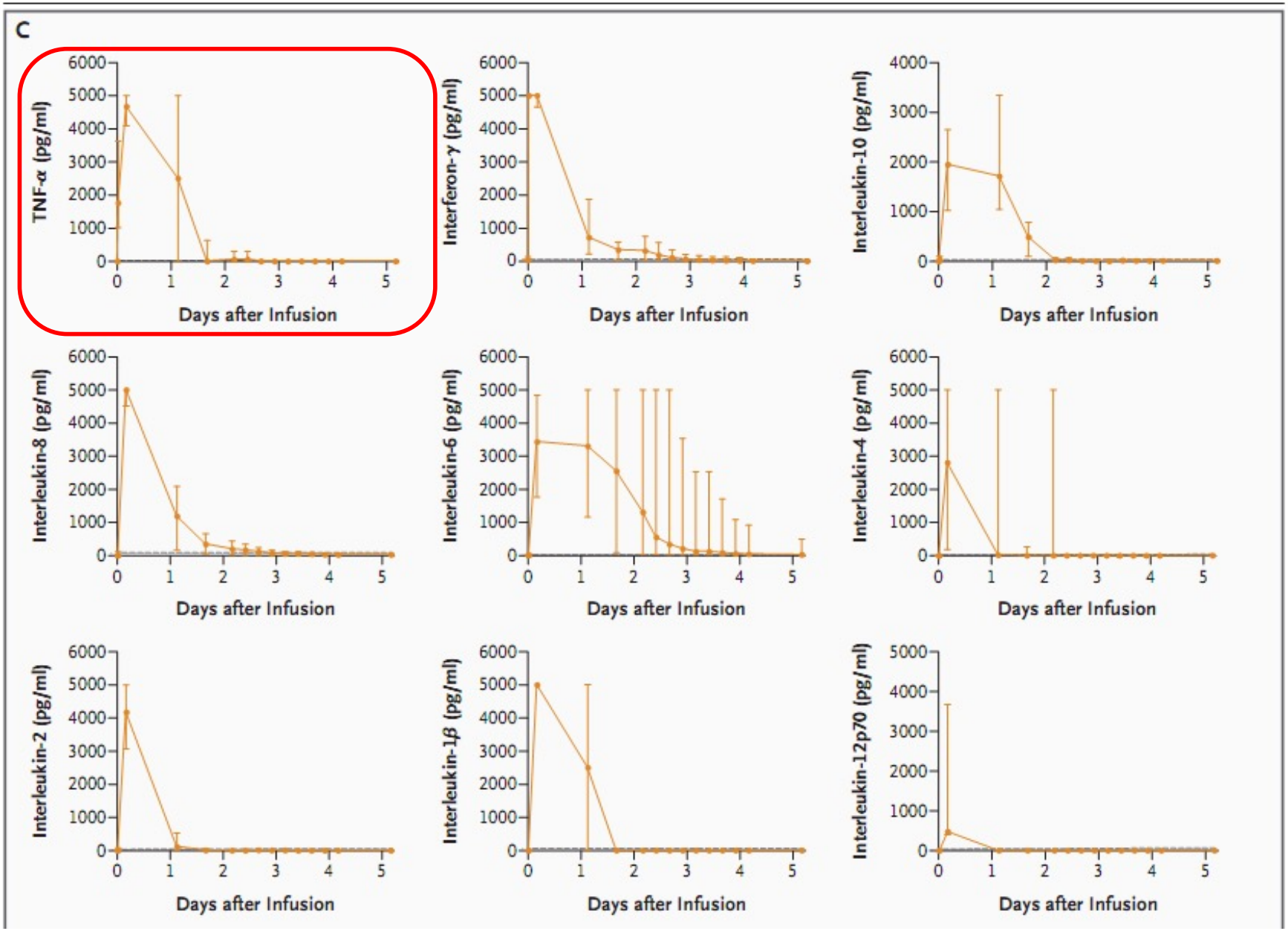


Summary Timeline of the Main Events after Infusion of TGN1412



G. Suntharalingam, N Eng J Med, 2006

Summary of Laboratory Results for the Six Patients during the First 30 Days (Panels A and B) and the First 5 Days (Panel C) after Infusion of TGN1412



G. Suntharalingam, N Eng J Med, 2006

Human PBMC

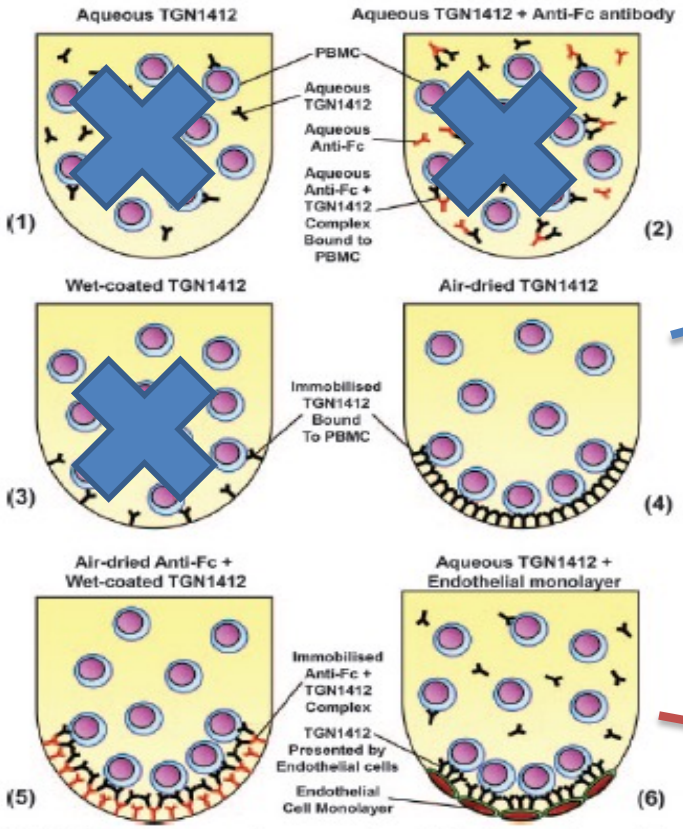
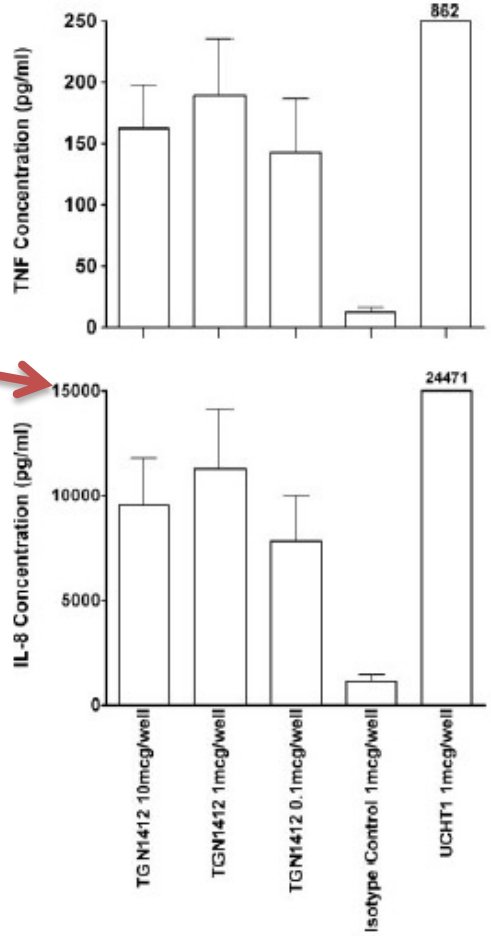
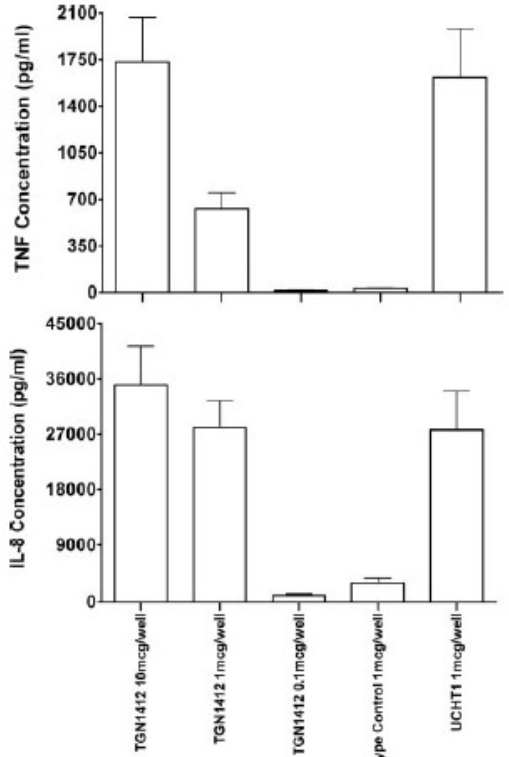


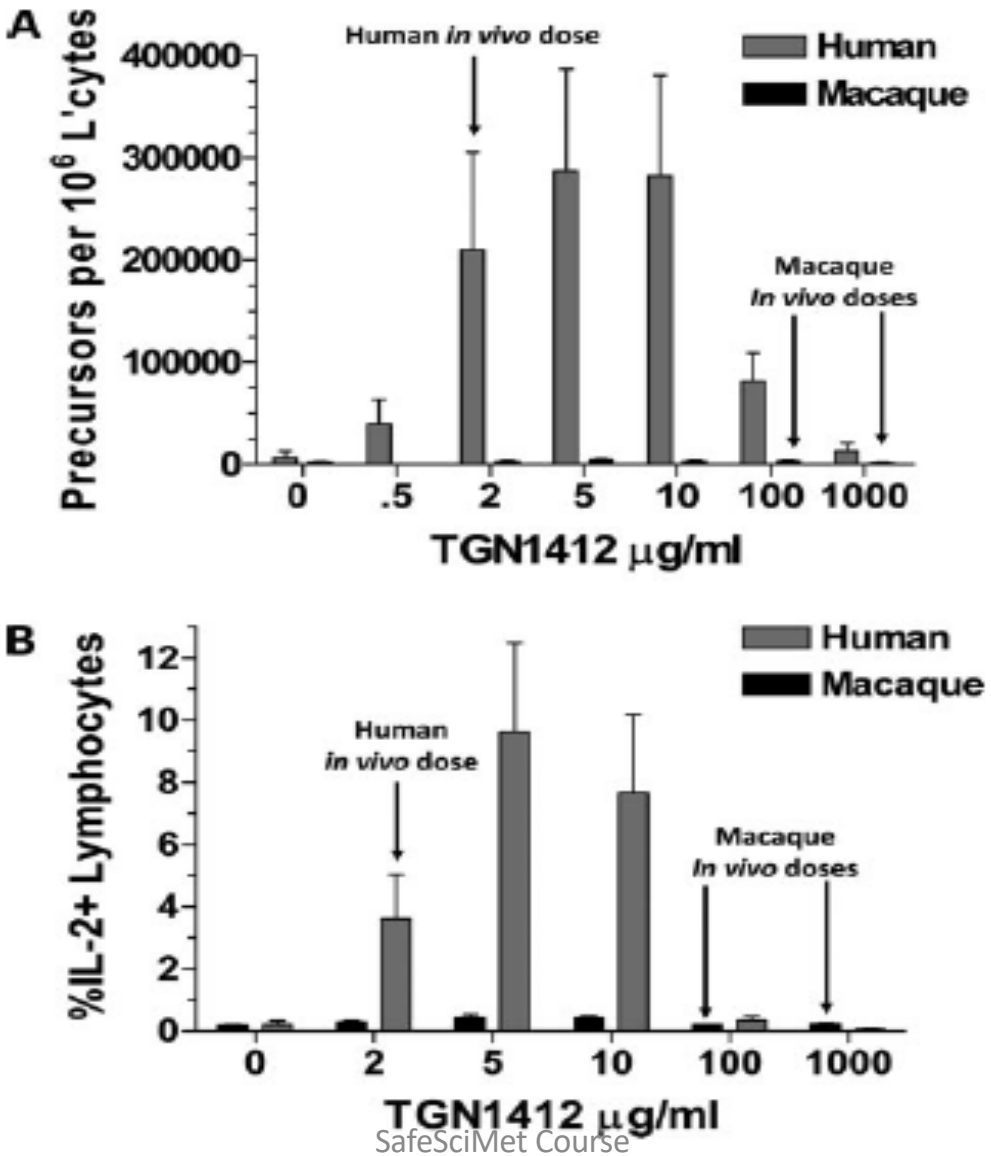
FIGURE 1. Diagrammatic representation of the six protocols used for presentation of TGN1412 to white blood cells.

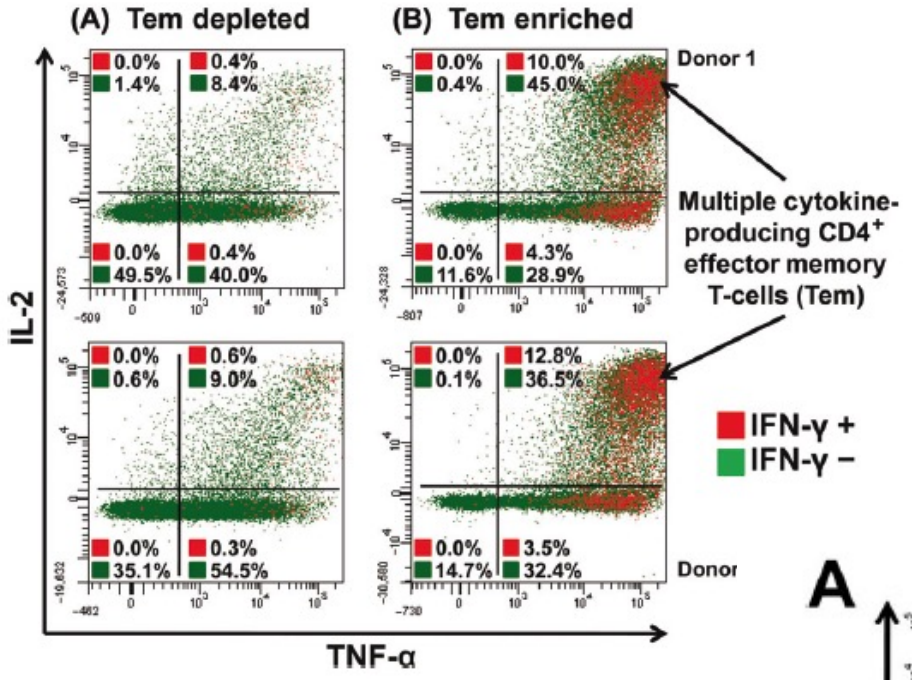




Stebbings R et al, J. Immunol (2007)

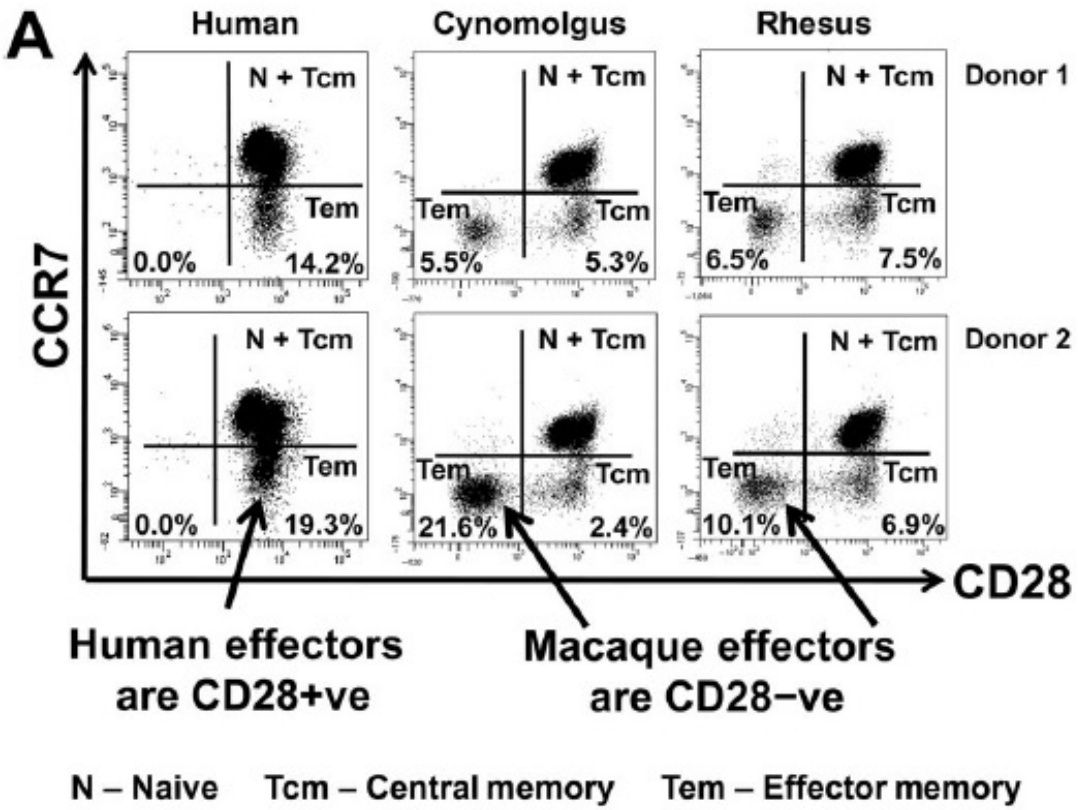
huPBMC vs monkey PBMC



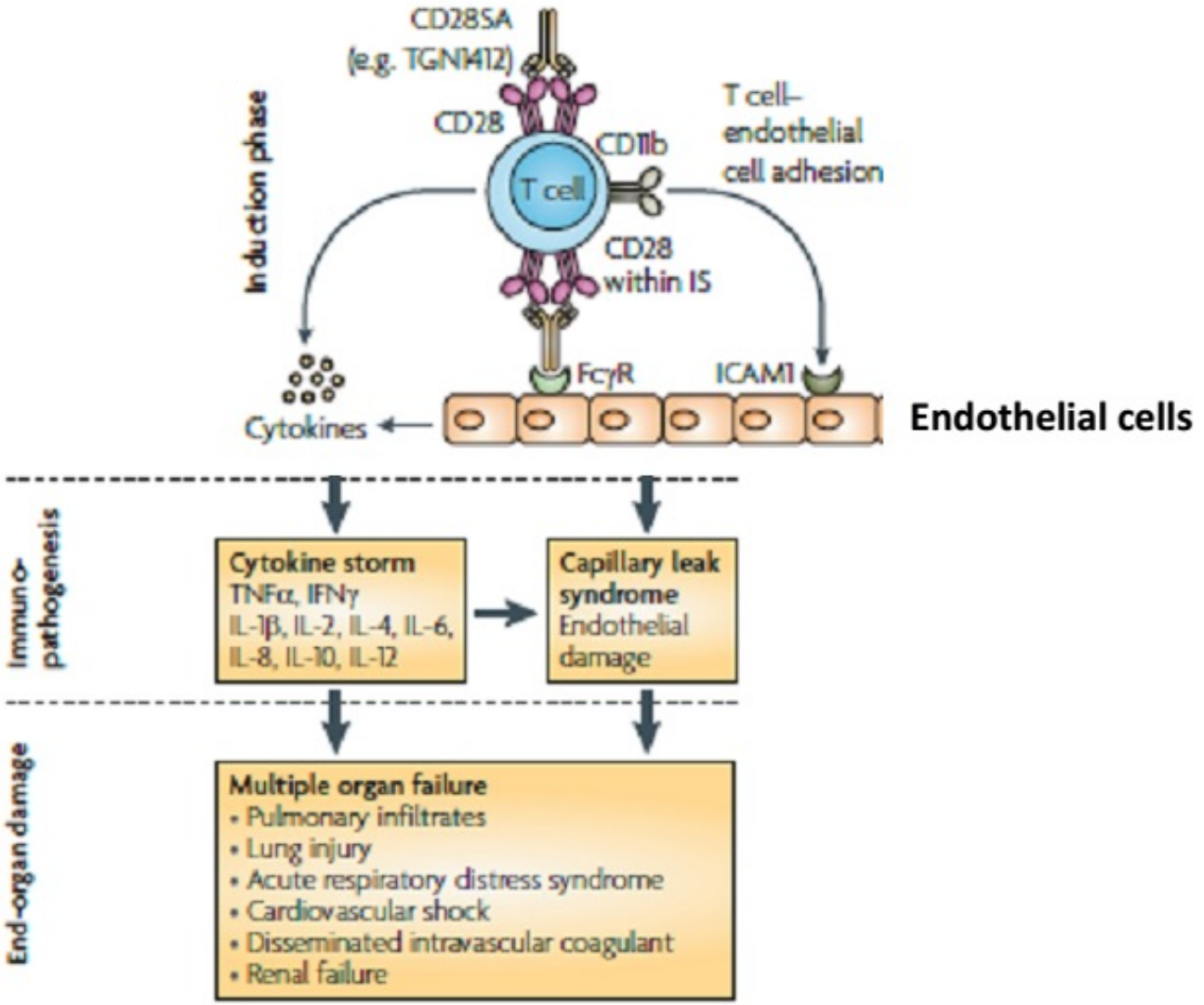


- **Effector memory cells are the key T-cell subpopulation responding to anti-CD28 in humans**

- **CD28 is not express on Tem memory cells in monkeys**



Possible Mechanism for TG1412 effects



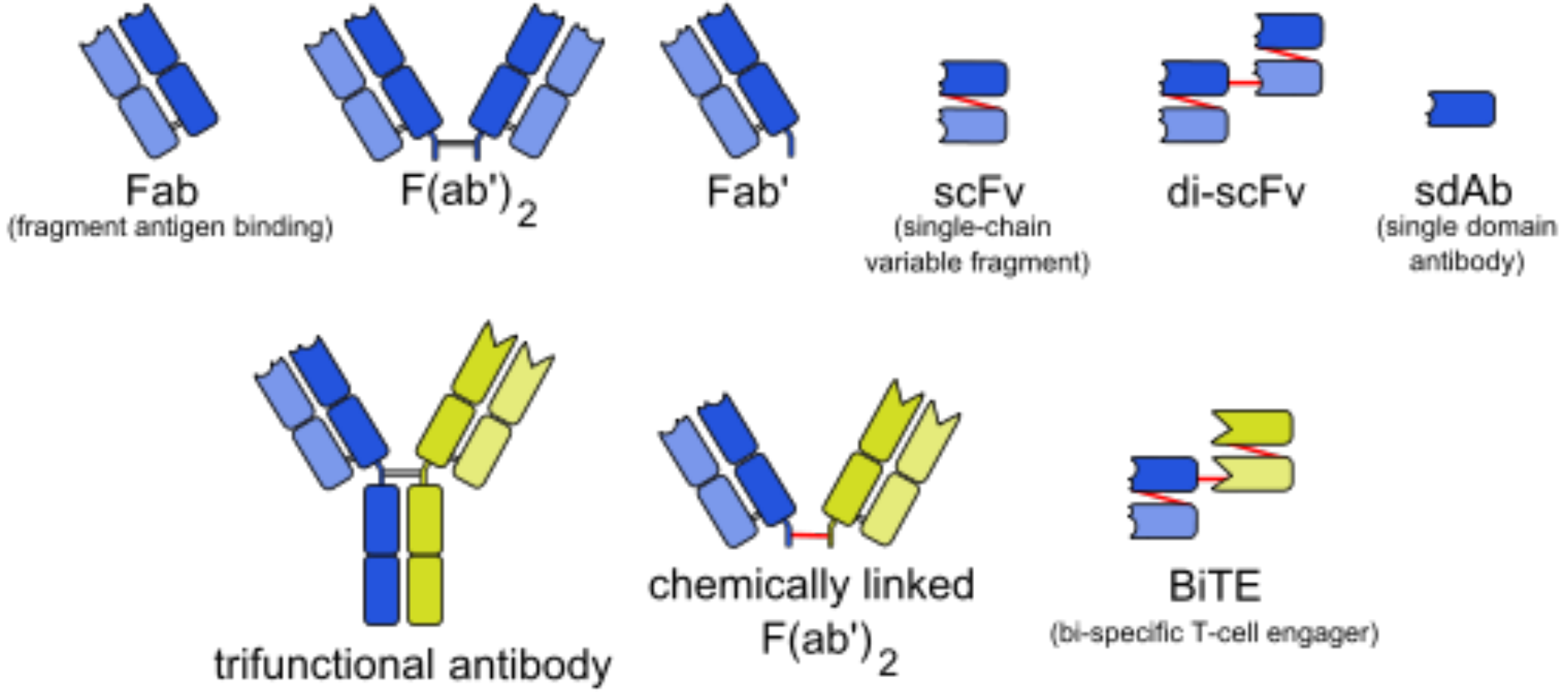
Hansel et al, Nat Rev Drug Discov, 2011

Issues and Consequences

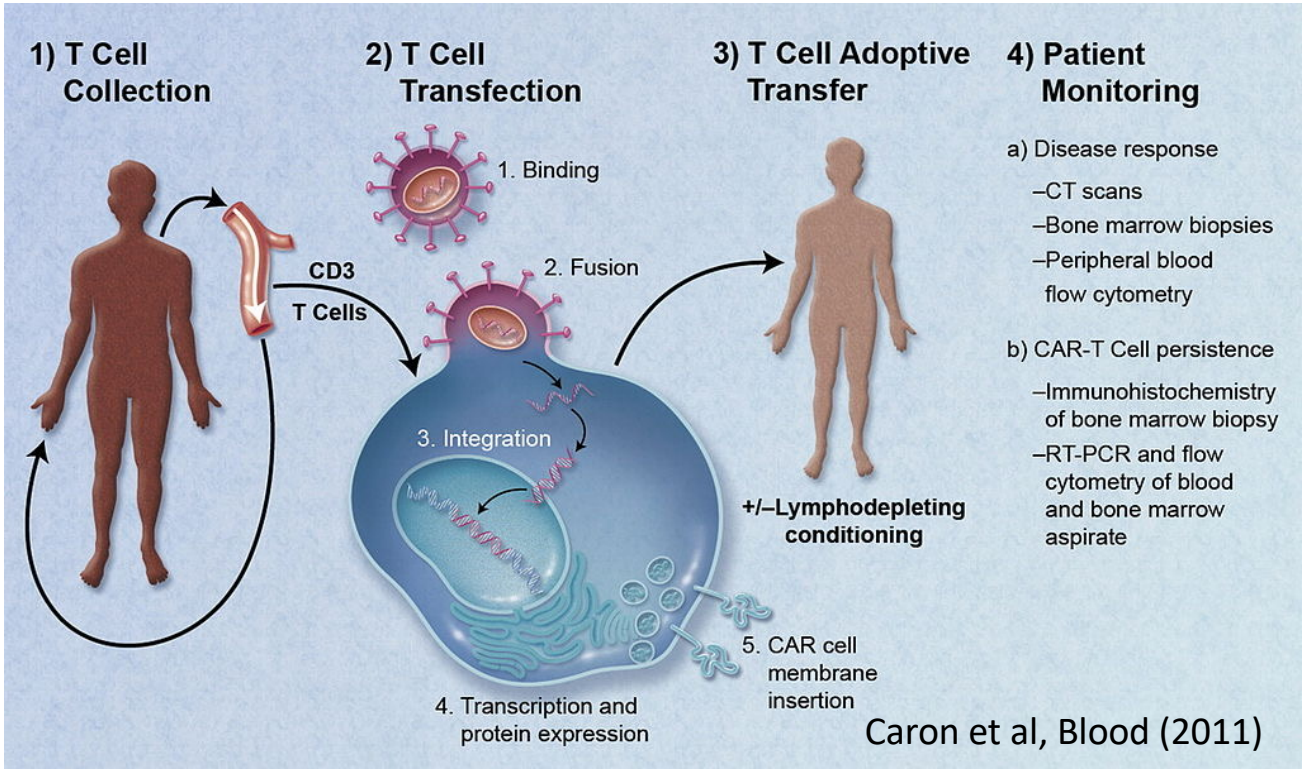
- Toxicology studies didn't predict this effect
  - Monkey CD28 was recognized by TGN 1412
  - But one month monkey study with 50 mg/kg: no effect
  - CD28 is not express on monkey effector memory T-cells
  - Species differences: alter extrapolation of animals to humans
- New EMA guideline (September 2007)
  - «Guideline on strategies to identify and mitigate risks for first-in human clinical trials with investigational medicinal products»
- Use of the MABEL (minimal anticipated biological effect level) vs NOAEL if needed to define first-in man starting dose
- Still very present in the mind of evaluators

Different types of monoclonal antibodies

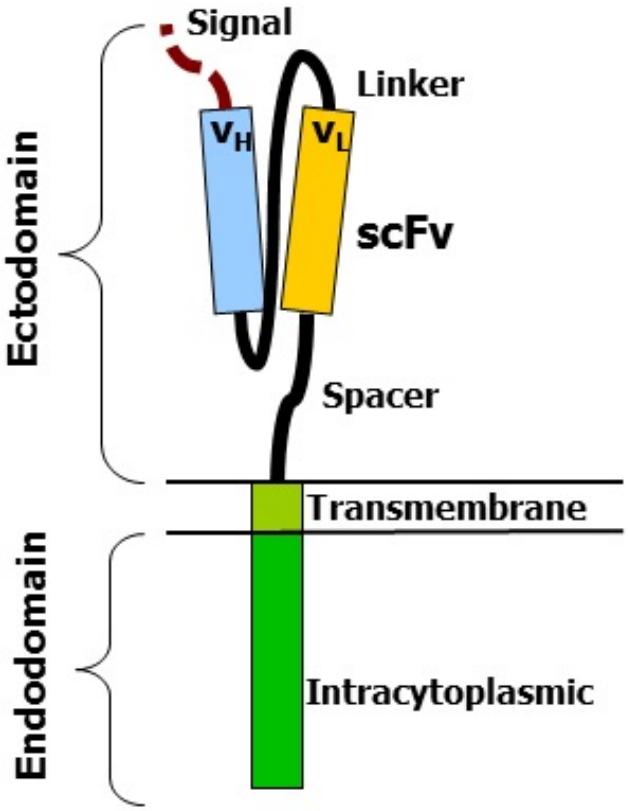
- Fab, F(ab')2 fragment, Fab' fragment, single-chain variable fragment, di-scFv, single domain antibody
- Bispecific antibodies (trifunctional antibody, Chemically linked F(ab')2, Bi-specific T-cell engager

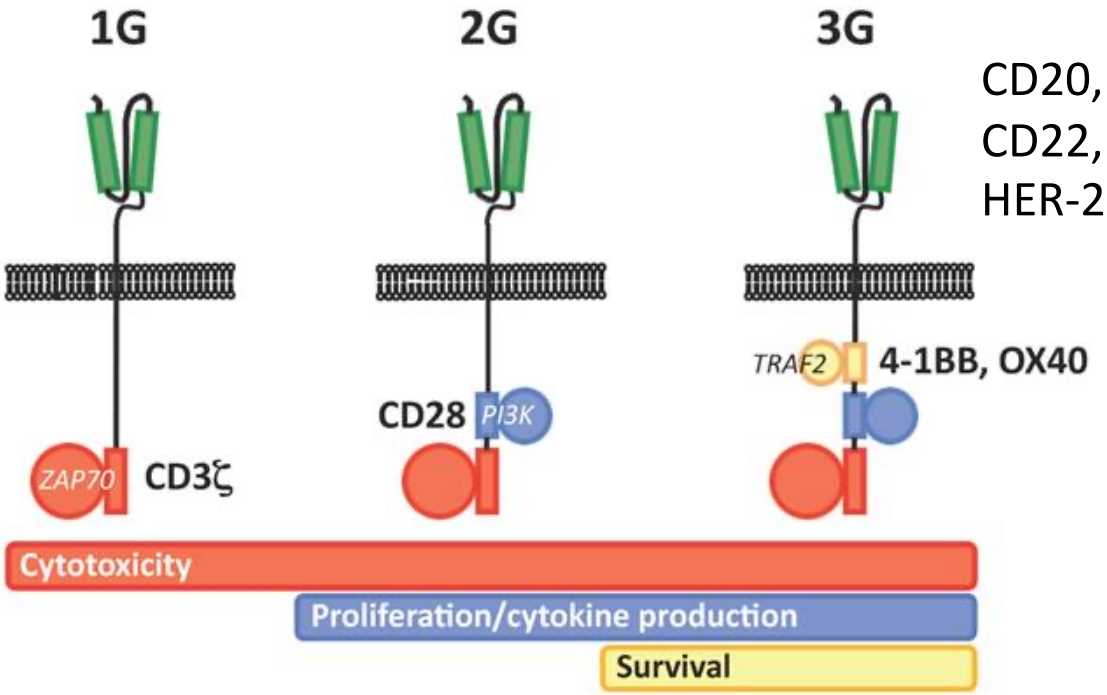


Anti-CD3/Anti-CD20; Anti-CD3/Anti-CD19…..



Caron et al, Blood (2011)



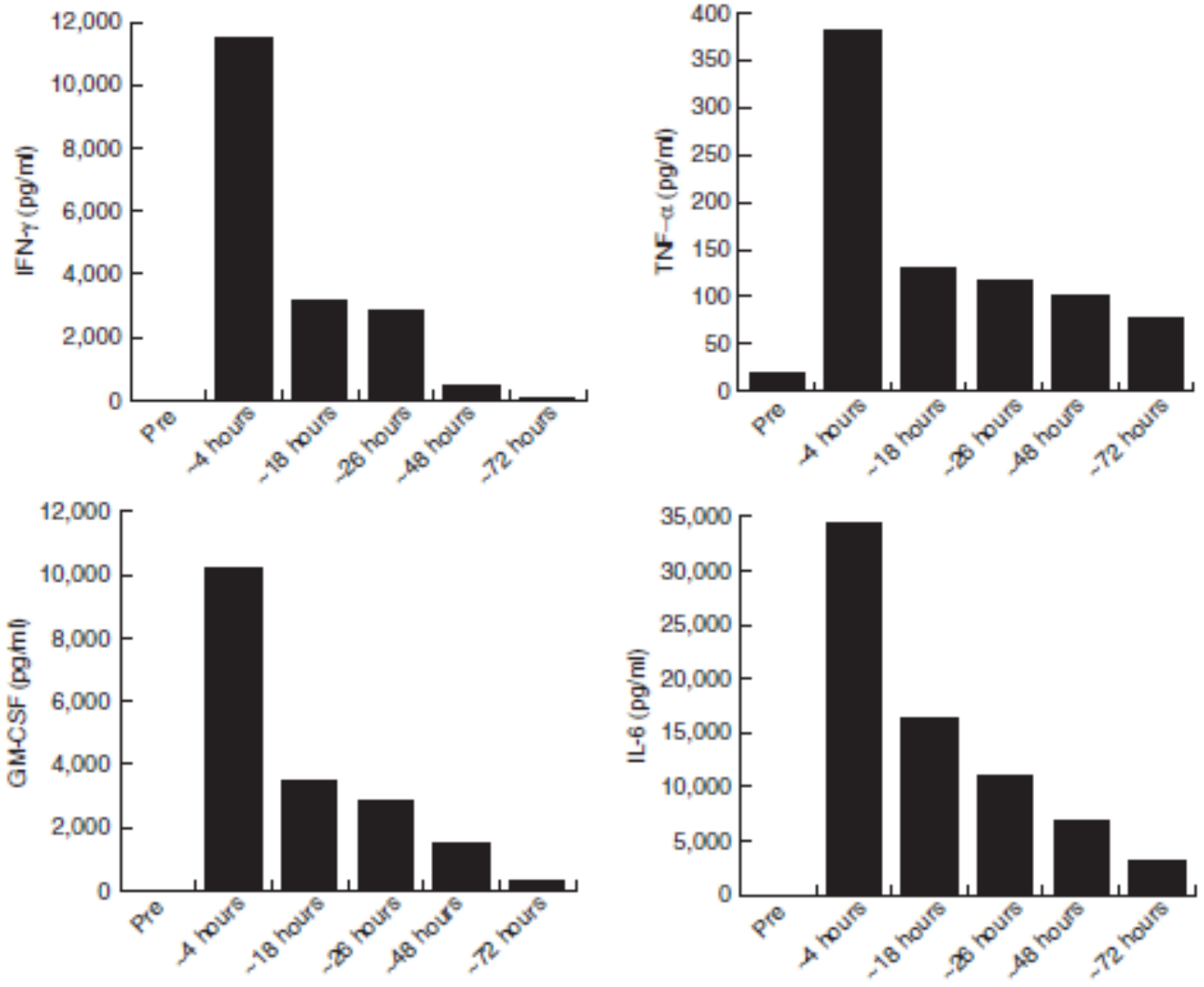


CD20, CD19,
CD22, CD30
HER-2…

- **Fever**
- **Cytokine release**
- **Tumor lysis syndrome**
- **Long persistence (months)**

New issues with new antibody formats (Bi-specific, BITE…) or cellular products ?

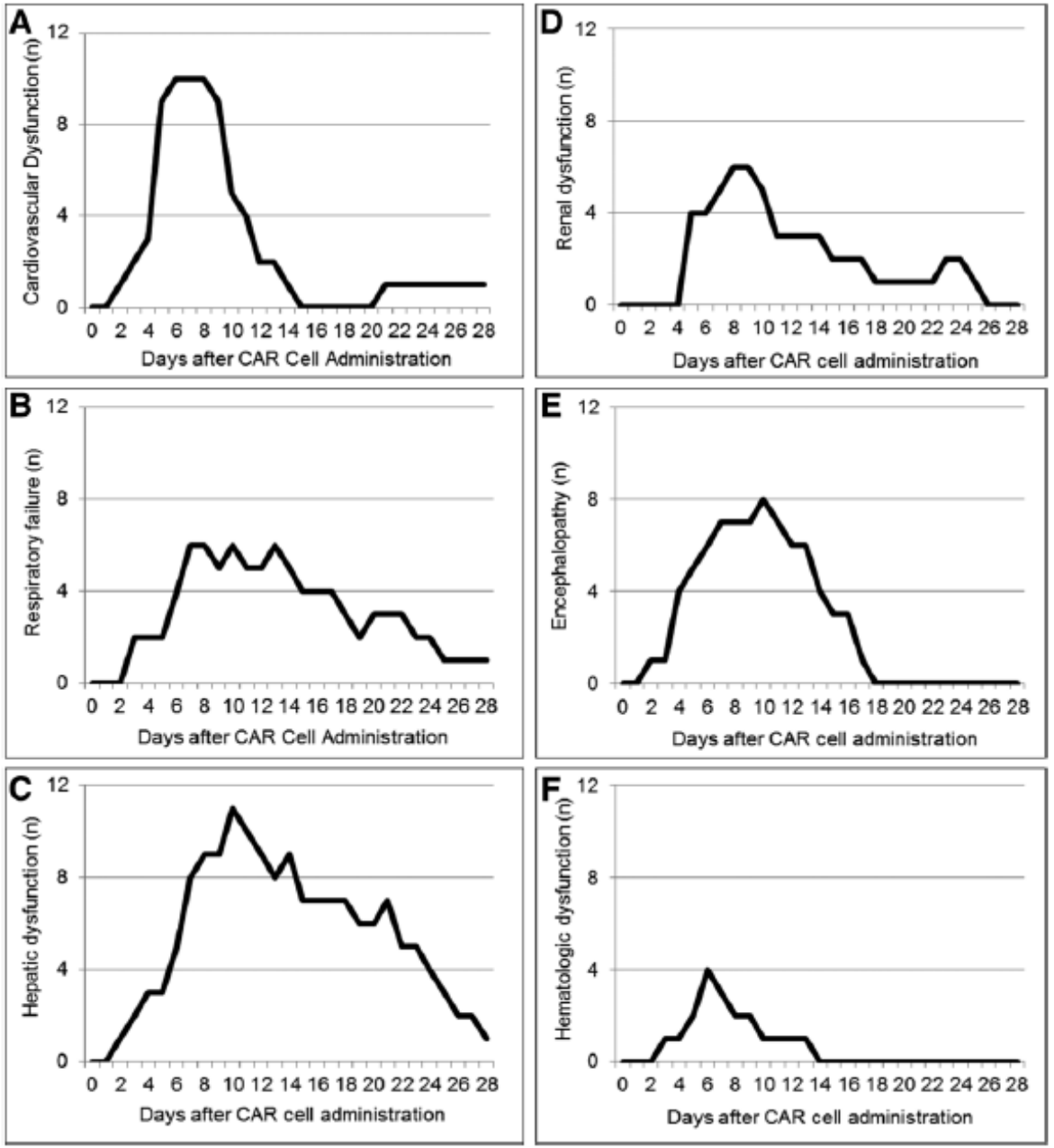
CRS and CAR T-cells



Serum cytokine level after cell infusion

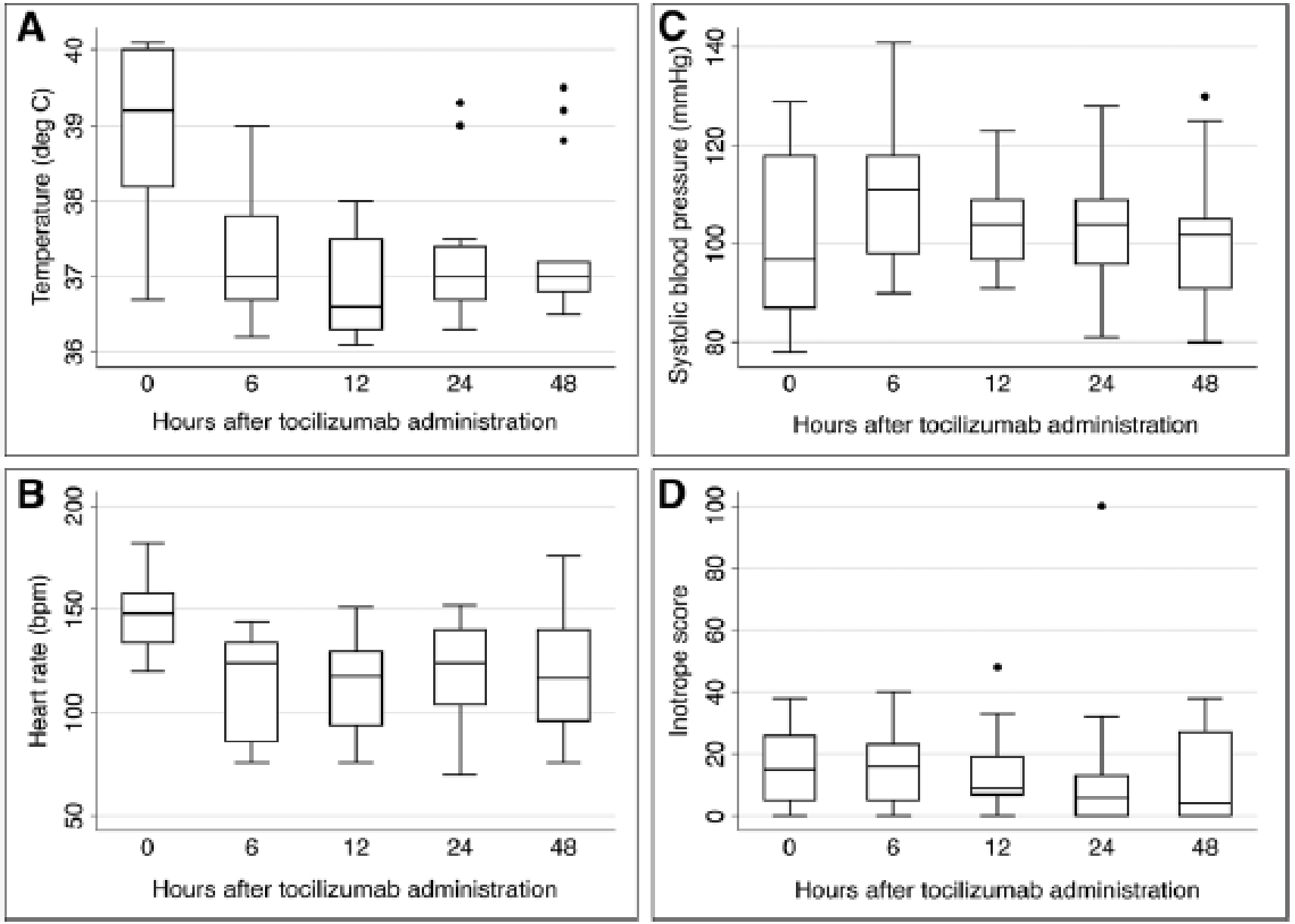
Morgan RA et al, 2010

Time-course of organ dysfunction after CAR T-cell therapy



Fitzgerald et al, 2017

Tocilizumab administration



Fitzgerald et al, 2017

Catumaxomab

- BsAb targeting both CD3 and EpCAM
- Approved in 2009 for the treatment of malignant ascites with IP administration
- Kill tumor cells with a mechanism involving ADCC (NK cells), T-cells and phagocytosis
- In FIH study by IV route
  - Fatal acute liver failure
  - Cytokine release-associated systemic toxicity even at low doses

Blinatumomab

- **Blinatumomab :** engager of T-cell activity via binding to CD19 and CD3 (BiTE).
- Therapeutic indications: refractory B-precursor acute lymphoblastic leukemia (approved in 2014).
- CRS was found as the dose-limiting toxicity in early clinical trials
  - Modification of the administration schedule and incremental dosage increase was used to mitigate this issue
- Large phase 2 study:
  - Adverse effects have emerged as an issue in Blinatumomab therapy, about 99% of patients in this trial experienced some grade of adverse effects
  - Often attributable to cytokine release (pyrexia, headache, peripheral edema) or destruction of the B cells (lymphopenia)
  - Severe (grade 3) CRS occurred in 2% of 189 patients who received blinatumomab for approved indication (n=3)

Blinatumomab (2)

- **CRS has been observed in other trials and seems to correlate with disease burden; CRS decreases with number of cycles of treatment**
  - Steroid pre-treatment with dexamethasone has been identified as an effective manner of controlling CRS
- Neutoxicology (encephalopathy)
  - grade 3, 12% of patients: presence of CD19+ cells in the brain
- **Blinatumomab shows effectiveness at very low clinically achievable doses (20-30 µg/day) compared to similar conventional antibody therapies**
- The drug also features a small protein size at 55 kDa and rapid clearance
  - Such clearance necessitates a continuous infusion, good ability to easily reach the site of action

Blinatumomab (3)

- **No animal models**
  - Surrogate molecule (muS103new) binding to murine CD3 and CD19 was used for non-clinical toxicology and safety pharmacology
  - CRS and B-cells lysis were observed in mice but these results were not used for FIM administration
- A « trial and error » approach was applied
  - Three pilot phase 1 conducted before the pivotal clinical studies
  - CNS effects and CRS were observed
  - AEs were dose-dependent and occured mainly at the beginning of the treatment
- **How to mitigate the risk ?**
  - Step-dosing regimen plus steroids pretreatment **(reduced cell numbers)** were then used to better manage AEs
  - If baseline B-cell levels are low, AEs related to the immune system appeared less prevalent
    - If baseline B-cell levels are high and target dose level low = one-step regimen (ALL)
    - If baseline B-cell levels are high and target dose level high = two-step regimen (NHL)

Lessons

- It is possible to enter clinic without relevant animal models
- Very low levels of compound are needed to have pharmacological activity ≠ from classical therapeutic antibodies
- Consider reducing the number of cells expressing the target before BiTE administration (not possible for all therapeutic indications)

Neutralizing antibodies and Immunosuppression

- **Natalizumab (Tysabri®)**
  - Humanized antibody directed to the integrin α4 subunit
  - **Therapeutic indications: Multiple sclerosis**
    - 3 months after approval (November 2005), 2 cases of progressive multifocal leukoencephalopathy (1 death; 3rd case of PML in a clinical trial with Crohn patients
    - Due an infectious agent: JC (John Cunningham) virus (infects oligodendrocytes)
    - June 2006: reapproval in the US and approval in Europe
  - Between July, 2006, and November, 2009
    - 28 new confirmed cases of PML (8 fatals)
  - **Now risk estimated at 1/100; increase if previous immunosuppression**
    - **Also observed with Efalizumab (withdraw from the market), rituximab, tocilizumab**
- **Anti-TNF-α therapy: Rheumatoid arthritis, Crohn disease**
  - Rate of pulmonary complications = 0,29% (n=271000 patients followed)
  - Augmentation of the risk of tuberculosis
  - Infections have been documented: pneumocytis listeriosis, systemic candidiasis, aspergillosis, CMV, varicella-zoster virus

Immunogenicity

Immune reaction specific for epitopes of the therapeutic product used for treating patients

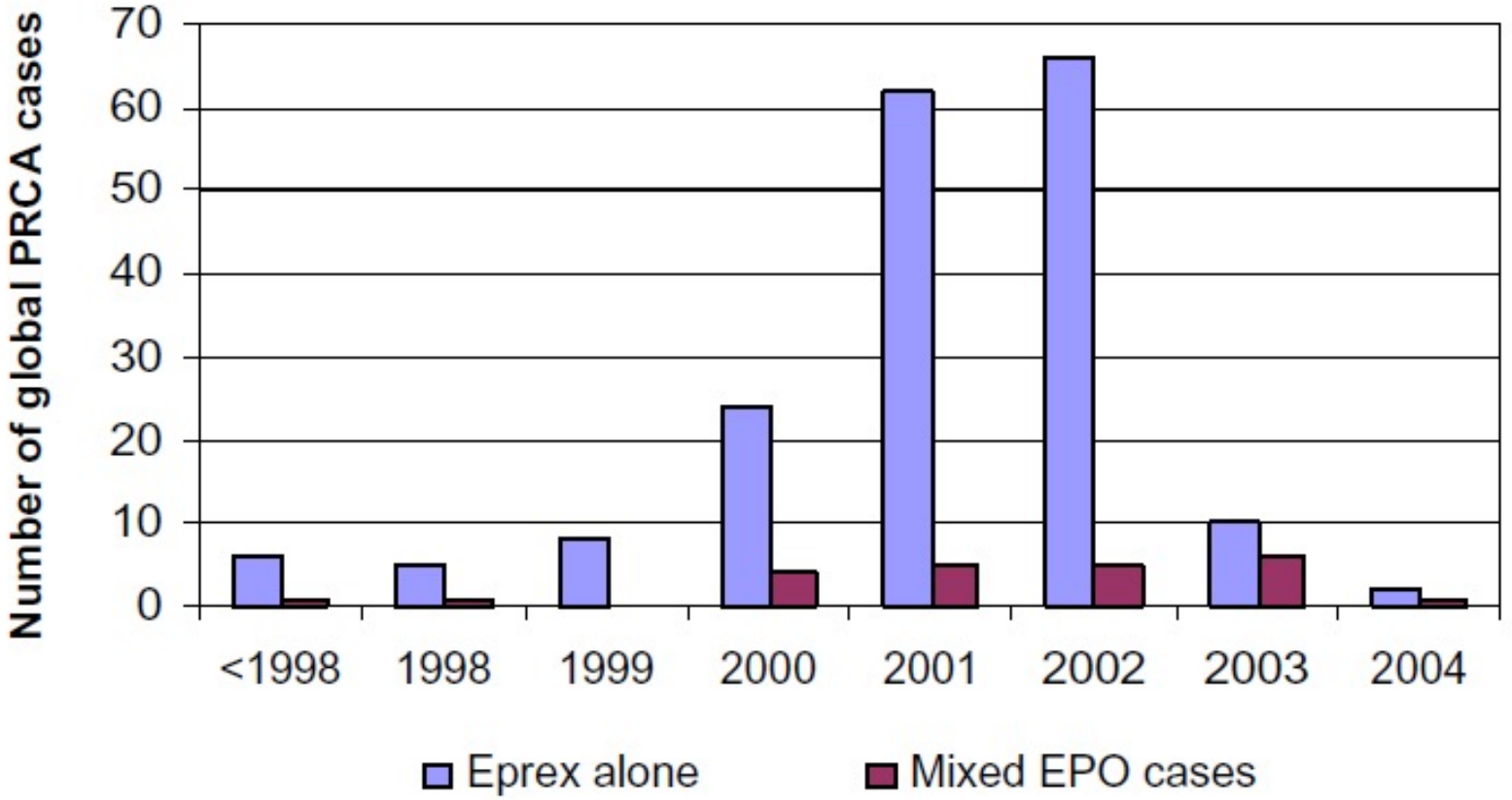
- Can lead to three major effects:
  - *Neutralisation of the biological activity of an endogenous protein (Erythropoietin and PRCA)*
  - Allergic reaction
    - Anaphylaxis (Cetuximab)
    - Immune-complexes
    - Skin reaction: Delayed Hypersentivity Reaction (DHR)
  - *Loss Of Clinical Response (Therapeutic antibodies, factor VIII)*

Mechanisms of Immunogenicity

- **Patients can be immunized following a break in immune tolerance**
  - The case of therapeutic proteins containing an identical sequence to the endogenous product
  - Need an additional signal or specific conditions of administration
    - Erythropoietin
    - Factor VIII
    - IFN beta…
- **Patients can be immunized to proteins containing foreign epitopes**
  - Therapeutic antibodies (murine, chimerics (human/mouse), humanized antibodies, human antibodies)

Immunogenicity and break in immune tolerance

Erythropoietin (EPO)
pure red cell aplasia(PRCA)

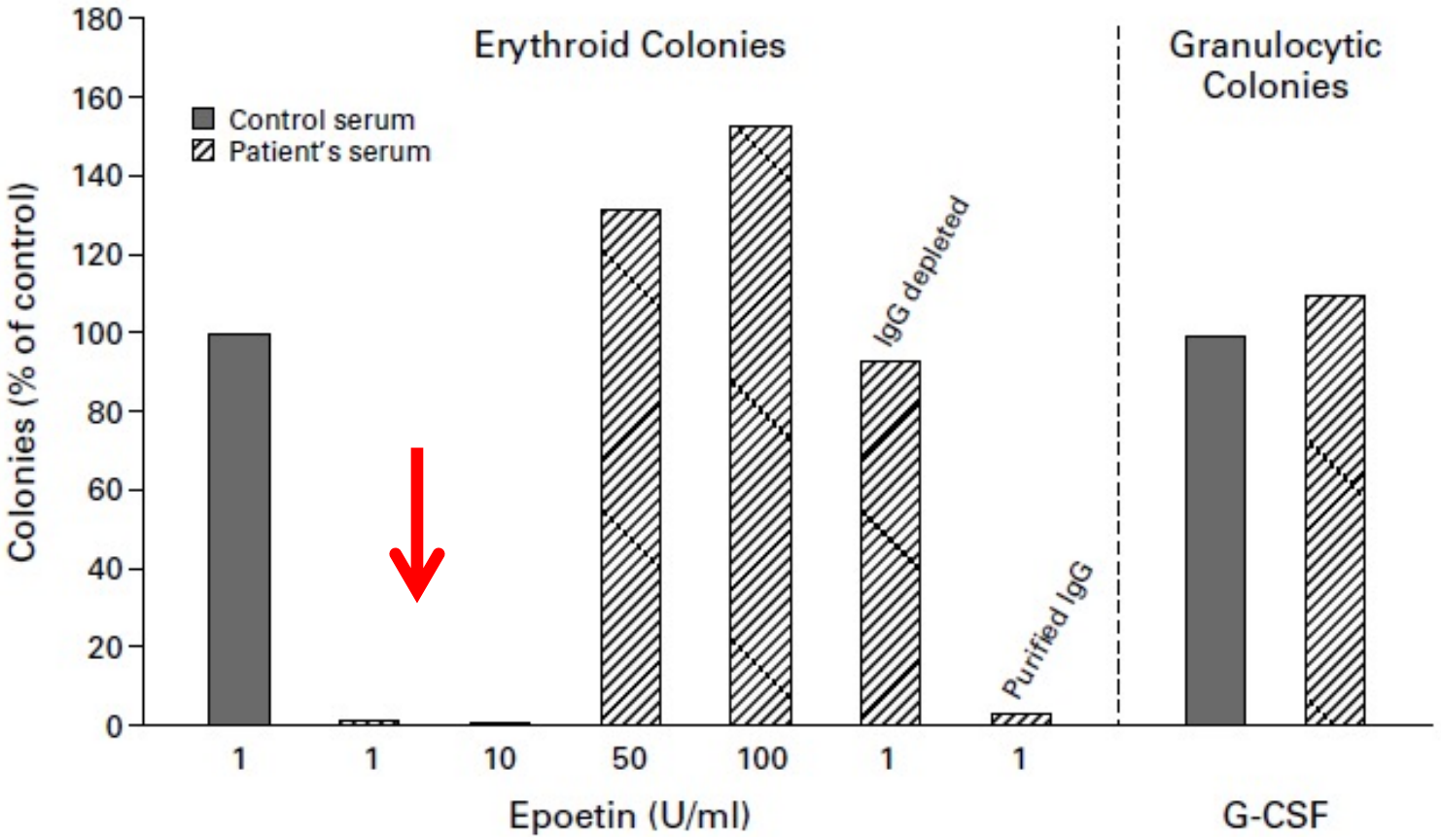


Administration of recombinant EPO in dialyzed patients suffering from kidney disease

Global case number of antibody mediated PRCA from 1998 to August 31st 2004

Mc Dougall et al, Nephrol Dial Transplant (2005)

PRCA was mediated by antibodies



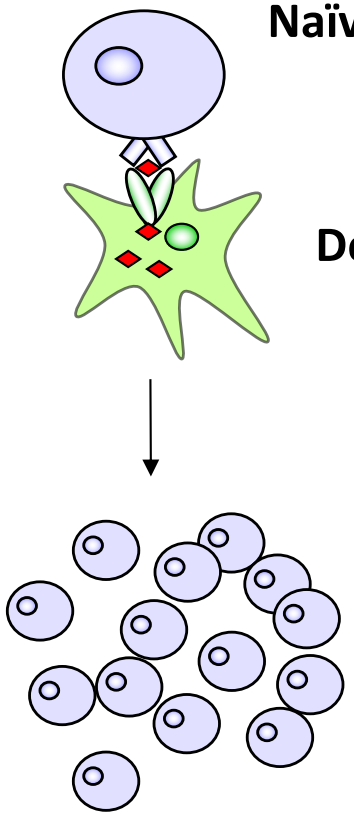
Inhibition of Erythroid-Colony Formation by Serum from Patient 1

Casadevall et al, N Engl J Med 2002

Possible causes

- Upsurge of PRCA is associated with a formulation change introduced in 1998 when human serum albumin (HSA) as protein stabilizer was exchanged with polysorbate 80
- The likely hypothesis is a break in immune tolerance to EPO provoked by the new formulation administered S.C
- New impurities recognized as a danger signal by dendritic cells allowing an effector immune response to be mount vs a tolerogenic immune response ?
  - Leachates from uncoated rubber stoppers have been found in the formulation
  - Aggregate formation during handling and storage due to the exchange of HSA by polysorbate 80 as stabilizer

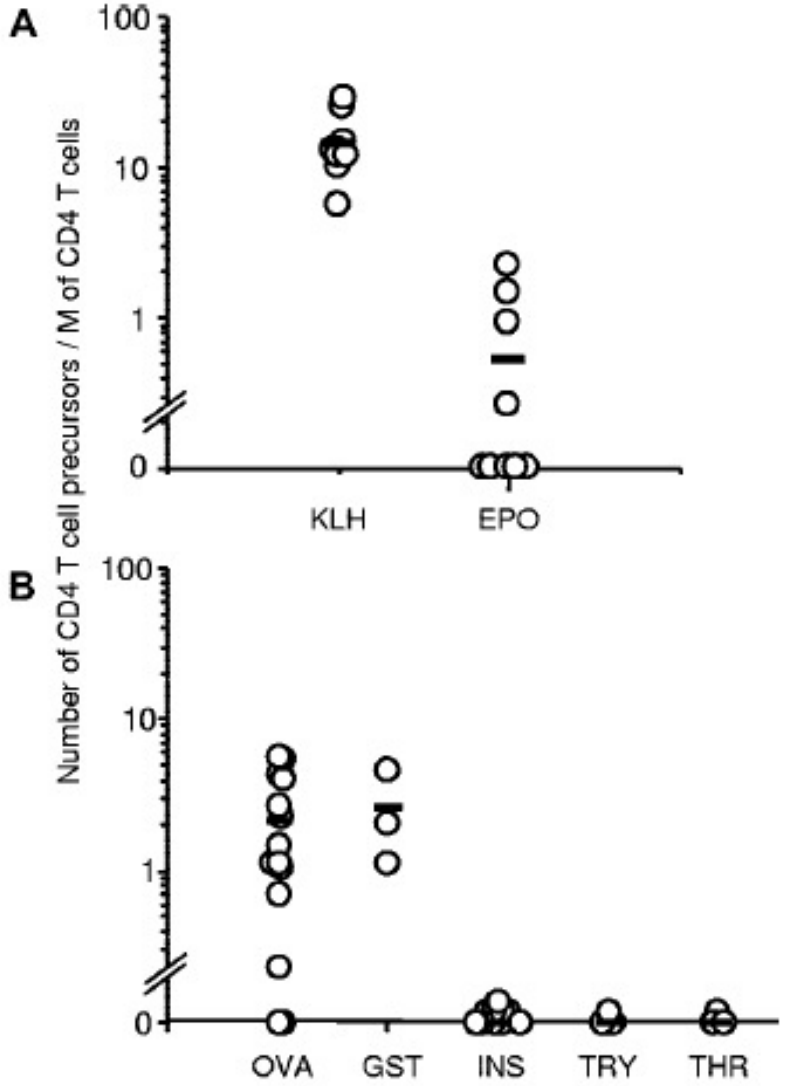
Pre-existing naïve T-cell repertoire for EPO ?



Naïve CD4+ T-cells

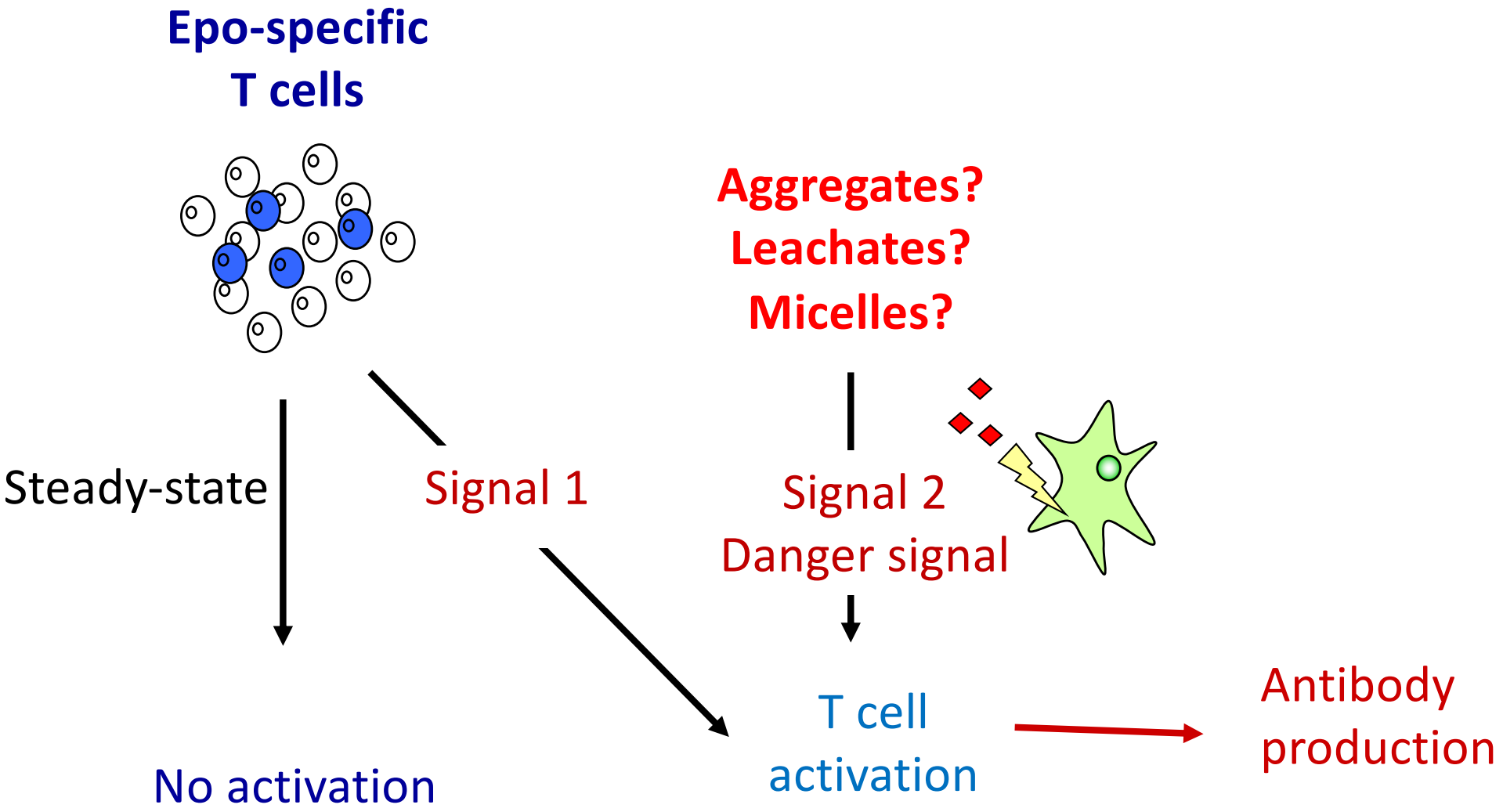
Dendritic cells

Repeated autologous DC-T cell stimulation
Read-out IFN-gammaELISPOT



Frequencies of preexisting CD4 T cells specific to EPO in the blood of normal donors

Maillere B et al, Blood (2011)

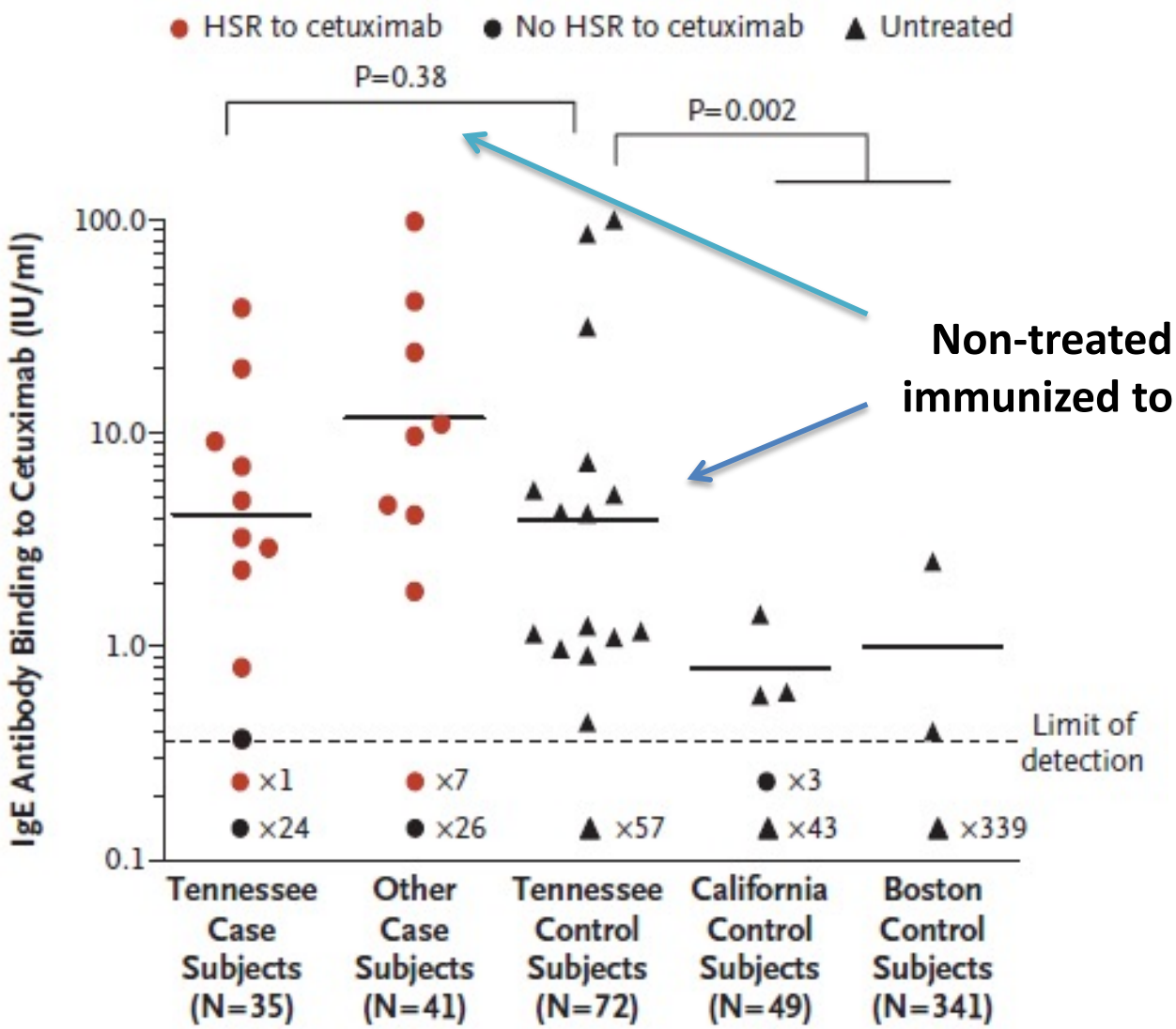


Delluc et al, Blood, 2010

Cetuximab and anaphylaxis

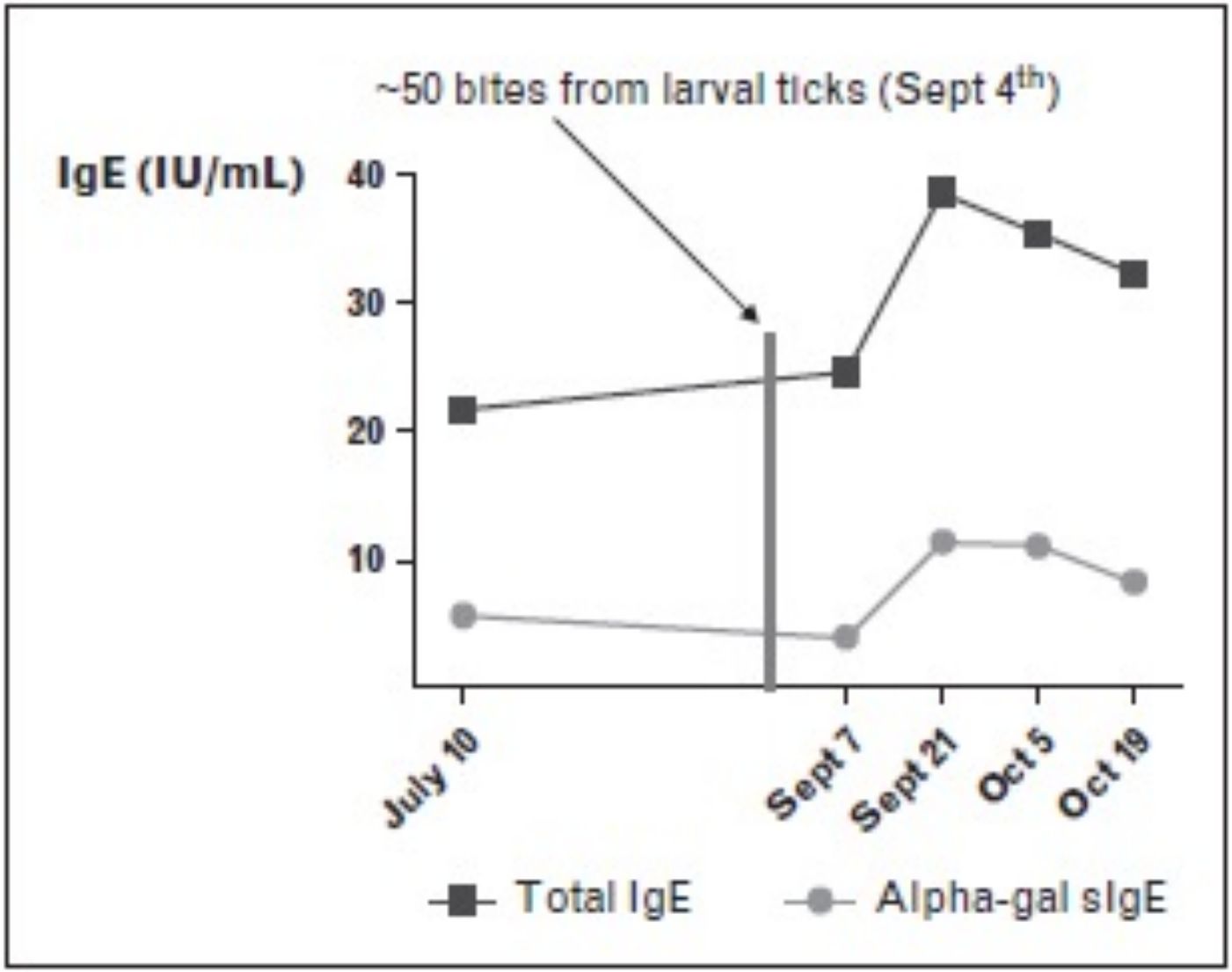
- Cetuximab (Erbitux™)= chimeric mouse–human IgG1 monoclonal antibody against the epidermal growth factor receptor (EGFR)
- **Indicated for the treatment of colorectal cancer and squamous cell carcinoma of the head and neck**
- A high prevalence of hypersensitivity reactions to cetuximab has been reported in some areas of the United States
  - North Carolina, Arkansas, Missouri, Virginia, and Tennessee (O.Neil et al, J Clin Oncol 2007)
  - 22% of patients who were treated with cetuximab in Tennessee and North Carolina had severe hypersensitivity reactions **during the first IV injection**

IgE antibodies binding to cetuximab in serum samples
76 case subjects and 462 control subjects



Non-treated subjects are immunized to cetuximab !!!

Chung et al, NEJM, 2008



Change in total IgE and alpha-gal-specific IgE over the weeks

(approximately 50 bites from larval ticks in a patient)

Commins et al, Curr Opin Allergy Clin Immunol 2013

Pre-existing antibodies

- Presence of antibodies specific for the therapeutic proteins in untreated patients
  - ✓ Crossreactive antibodies elicited by foreign antigens
  - ✓ Anaphylactic shock (IgE mediated)

- Anaphylactic reactions to Cetuximab
  - ✓ A chimeric Mab anti-EGFR: colorectal and head and neck cancer
  - ✓ Severe hypersensitivity reactions in 3% of patients (up to 22%)
  - ✓ Pre-existing antibodies: symptoms at the first injections of Cetuximab
- **The antibodies are specific for galactose-α-1,3-galactose (α-Gal)**

- ✓ α-Gal : present in the Fab part of the cetuximab heavy chain
- ✓ abundantly expressed on cells tissues of nonprimate mammals
- ✓ IgE result from allergy to tick bites or to meal (Beef, pork)

	Type of Cetuximab†		
Hypersensitivity reaction	SP2/0‡	CHO‡	α-gal
Anaphylaxis related to cetuximab			
1	41.6	0.35	13.8
2	38.8	0.35	35.2
3	20.2	0.35	12.6
4	11.1	0.35	2.9
5	4.9	0.35	2.0
6	4.2	0.35	2.7

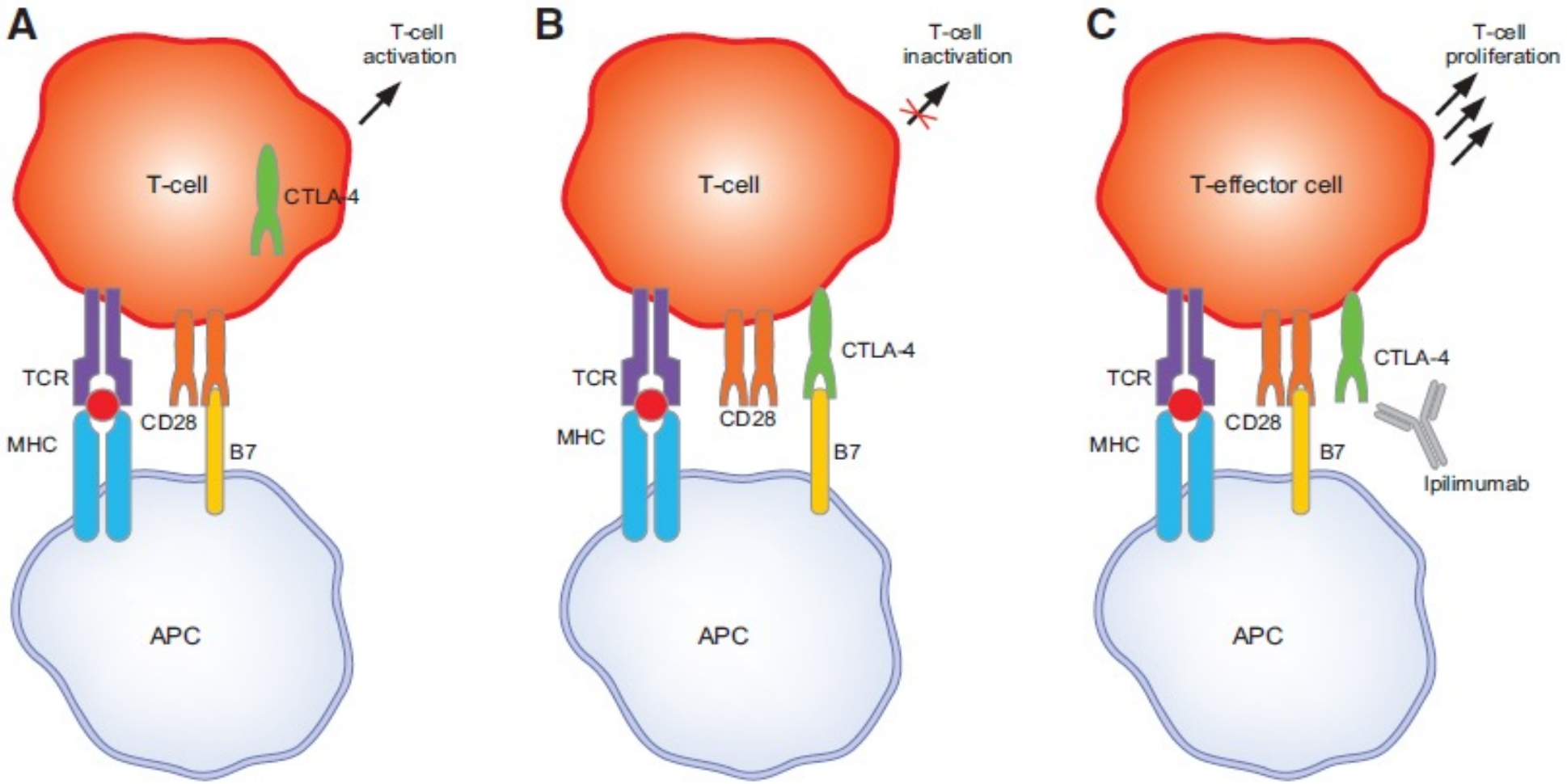
Cheung, NEJM, 2008

Autoimmunity ? or “immune-related reactions” ?

Autoimmunity linked to the mode of action of Ipilimumab (Yervoy®)

Goal: boost the immune system against cancer cells

Therapeutic indications: Melanoma



Fecher et al, Oncologist (2013)

Ipilimumab (Yervoy®), tremelimumab
Autoimmune colitis, Vitiligo

- Fully human IgG1 monoclonal antibody approved for treatment of late stage metastatic melanoma
- Blockade of CD152/CTLA-4 (negative regulator of T-cell activation) with ipilimumab can potentiate anti-tumour immunity
- Side/toxic effects
  - Any grade diarrhea incidence is approximately 30% to 35%
  - Incidence of grade 5 diarrhea or enterocolitis is 5% to 8%, bowel perforation can be observed
  - Vitiligo doesn't involve disruption of the treatment and is associated with therapeutic response
  - Mechanisms may involve breaking of immune tolerance and/or lack of control of autoimmune T-cell

AEs following Nivolumab monotherapy in melanoma patients

Any treatment-related AE leading to discontinuation of study drug	3.0	2.1
Treatment-related select AEs	49.0	3.6
Skin	34.0	0.7
Pruritus	17.2	0.2
Rash	12.7	0.3
Vitiligo	7.8	NA
Rash maculopapular	4.5	0.2
GI	13.4	1.2
Diarrhea	12.7	0.5
Colitis	1.0	0.7
Endocrine	7.8	0.3
Hypothyroidism	4.2	0
Hyperthyroidism	2.1	0.2
Hypophysitis	0.2	0.2
Hepatic	4.2	1.0
AST increased	2.8	0.3
ALT increased	1.9	0.7
γ-glutamyltransferase increased	0.2	0.2
Hepatitis	0.2	0.2
Liver function test abnormal	0.2	0.2
Pulmonary	1.9	0
Pneumonitis	1.7	0
Renal	1.4	0.3
Blood creatinine increased	0.5	0
Renal failure acute	0.2	0.2
Tubulointerstitial nephritis	0.2	0.2

Nivolumab 3 mg/kg every two weeks
N = 576 patients

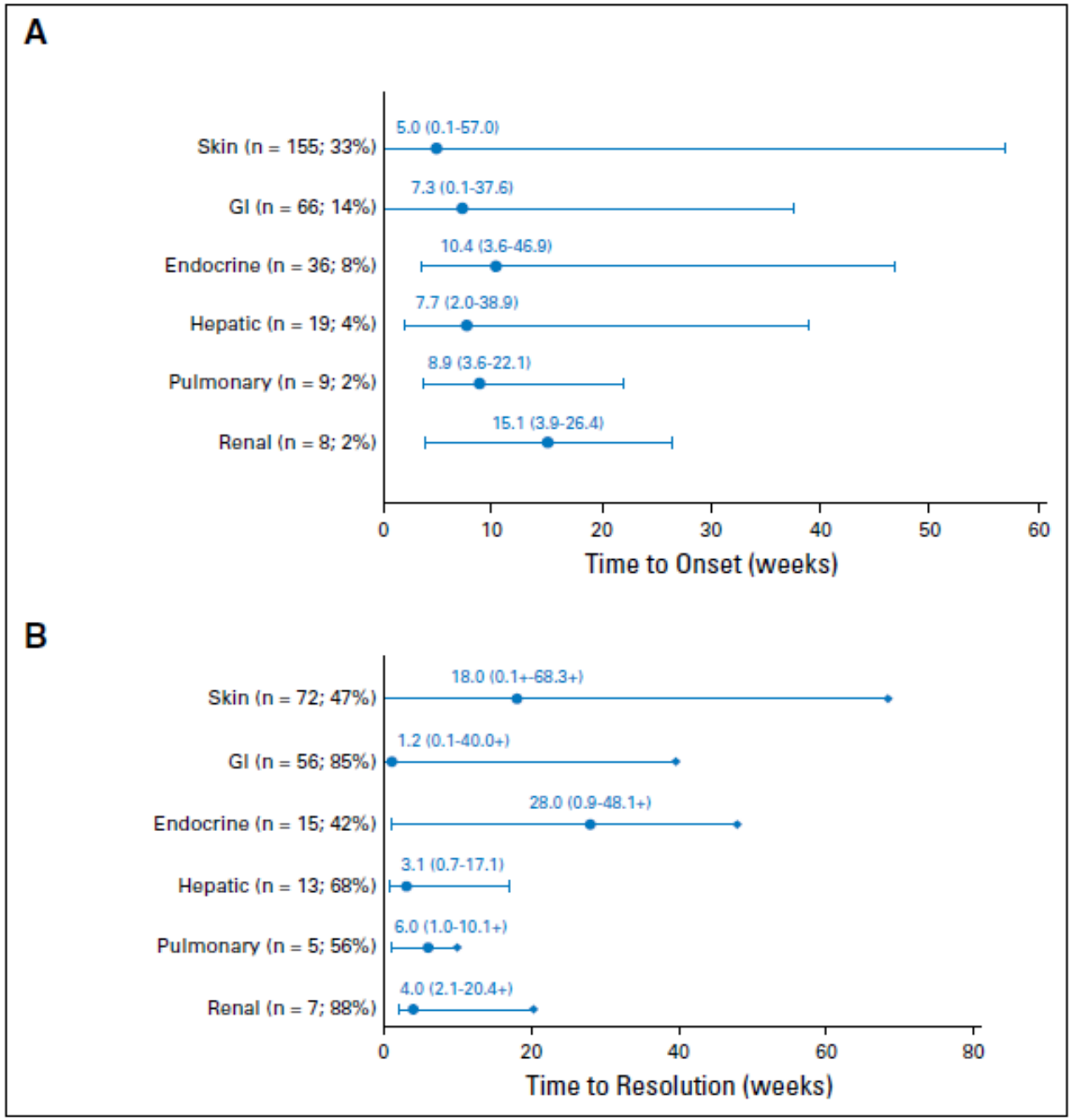
10% of patients, grade 3 or 4 related AEs

Select AEs (49% of patients)
skin related, GI, endocrine, Hepatic; grade 3 to 4 select AEs occurred in 4% of patients

AUTO-IMMUNITY ?

Weber et al, 2016

Time to onset and resolution of treatment-related select adverse events (AEs) of any grade, with or without use of immune-modulating agents



Weber et al, 2016

Dose-related effects ?

- Risk of severe grade adverse events increased from 7 to 25% with an increase in the dose of ipilimumab from 3 mg/kg to 10 mg/kg
  - Due to increase in the episodes of diarrhea
- When nivolumab dosing is increased from 0.3 mg/kg to 10 mg/kg, no increase in toxicity
- Pembrolizumab toxicities were also similar at doses of 10 mg/kg every 2 or 3 weeks compared to its FDA-approved dosage of 2 mg/kg every 3 weeks
- Dose-dependency ?
  - anti-CTLA-4 YES ?
  - anti-PD-1/anti-PDL-1 NO ?

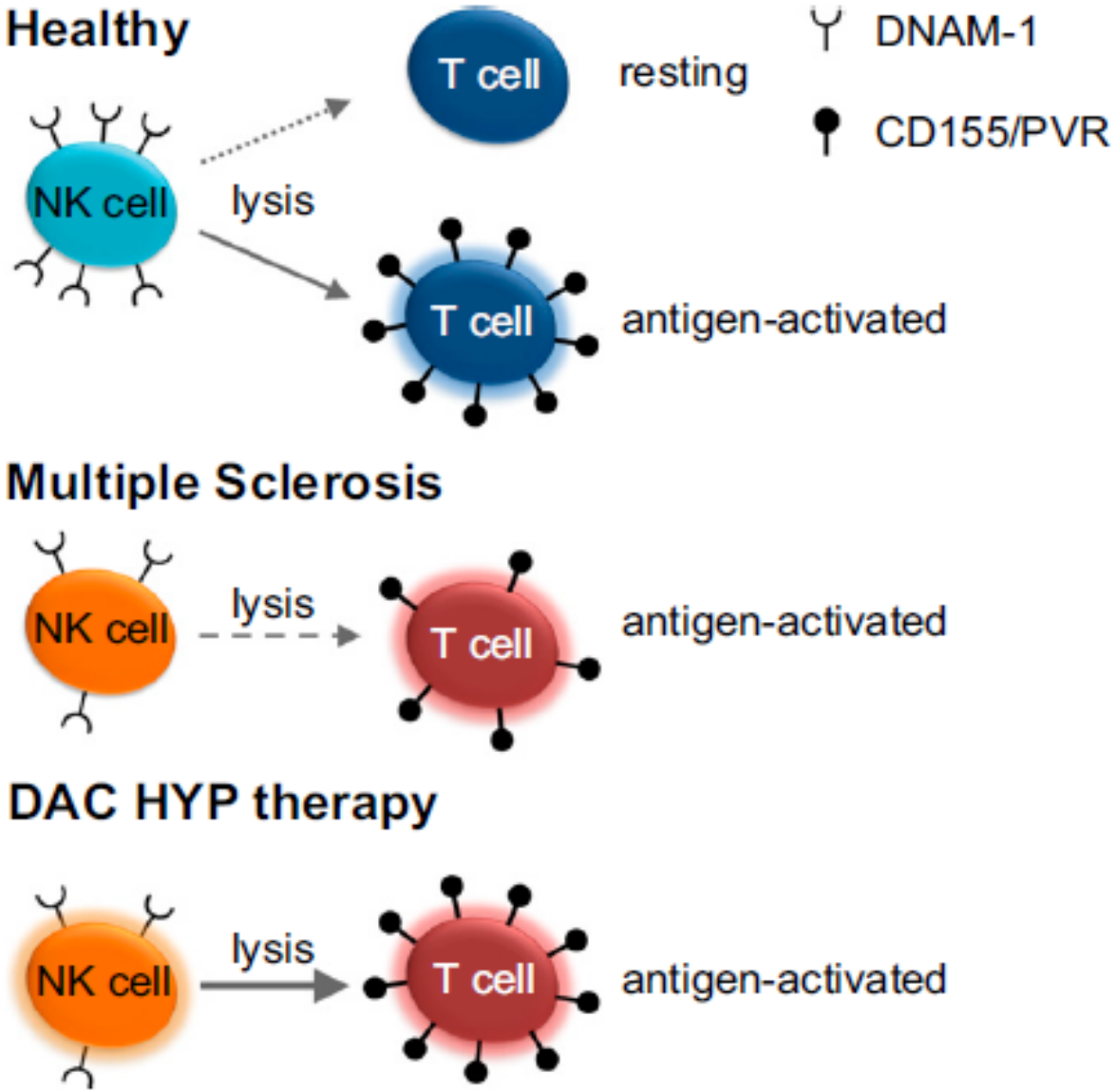
Kumar et al, 2017

Combination Therapy ?

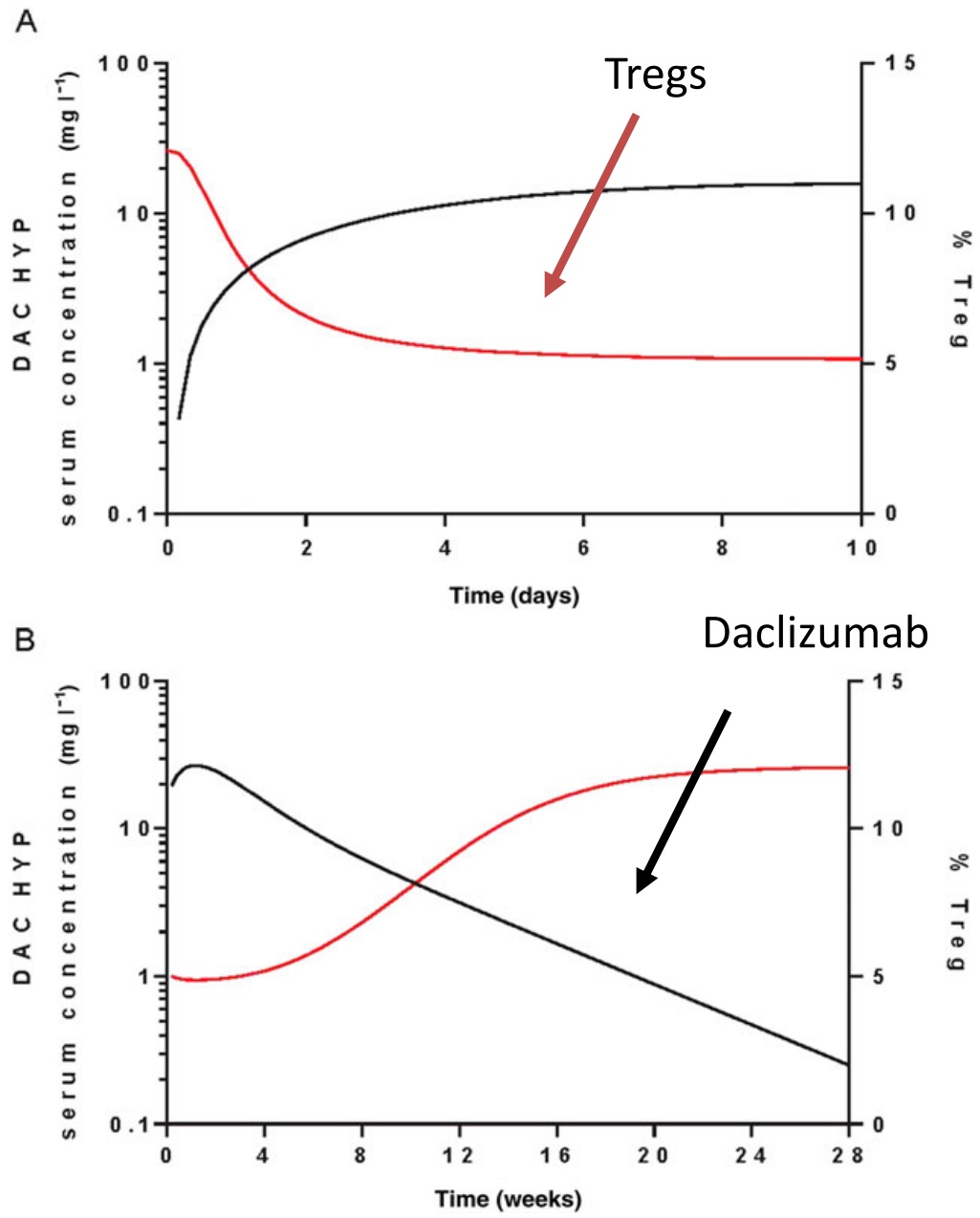
- Anti-CTLA-4 and anti-PD-1/anti-PD-L1 antibodies have distinct mechanisms: combination therapies in a variety of malignancies
- The incidence of severe adverse events due to the combination of ipilimumab and nivolumab is reported to be around 55%
- This number is significantly higher than either agent individually and leads to discontinuation of treatment in one-third of patients (Larkin et al., 2015)

Daclizumab (anti-CD25) and Multiple Sclerosis and Liver effects

Mechanisms ?



Grossa CC et al, 2016



Lei Diao, 2016[1]

Simulated Treg percent following

A) the first daclizumab HYP s.c. 150 mg dose

B) the last daclizumab HYP s.c. 150 mg dose every 4 weeks at steady-state

Toxicity evaluation/prediction: how to proceed with biopharmaceuticals ?

- Existing guidelines (ICH S6, ICHS8) are not well covering the topic of toxicity and biologics
- Neutralization of an immune mediator in normal animals may not reflect what will happen in humans
  - *The factor is not circulating at steady-state, the target is not expressed on the same cells in animals and humans…*
- **Be inspire by the literature and the science**
  - Necessity of "cross-fertilization" between toxicology/research/pharmacology
  - The most critical factor in understanding patient safety is *to understand the full spectrum of the pharmacological effects*
  - *Sometime very tricky, evolves very rapidly (CAR T-cells, bi-specific antibody…)*
  - *Need to investigate where the target is expressed*
- This can only be accomplished by examining the entire weight of evidence across all sources.
- Knowledge of interspecies differences regarding the biology of the target is mandatory
- ***Need of in vitro models using human cells***

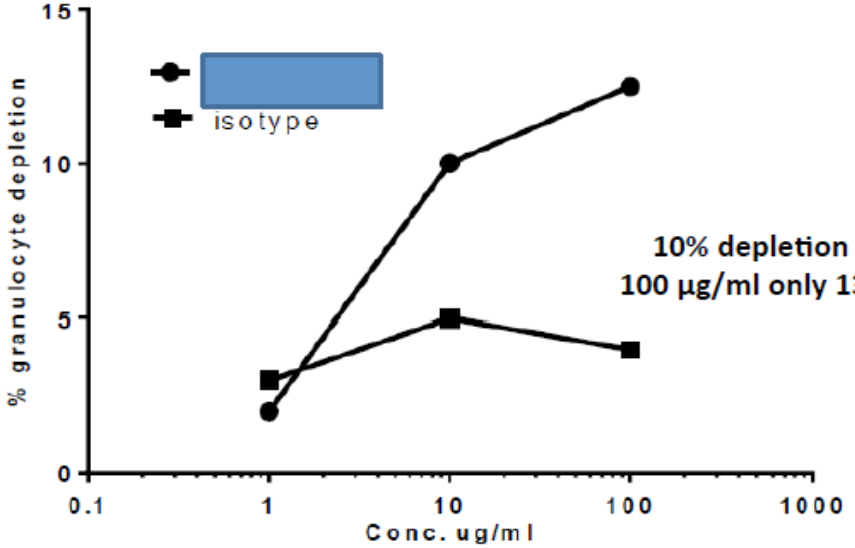
Daclizumab/Multiple sclerosis/liver effects

- Severe autoimmune hepatitis occurred during daclizumab treatment and led to death in one patient
- These hepatotoxic events are unpredictable despite monitoring
- Difficult to identify or exclude patients who are under higher risk

Use human cells

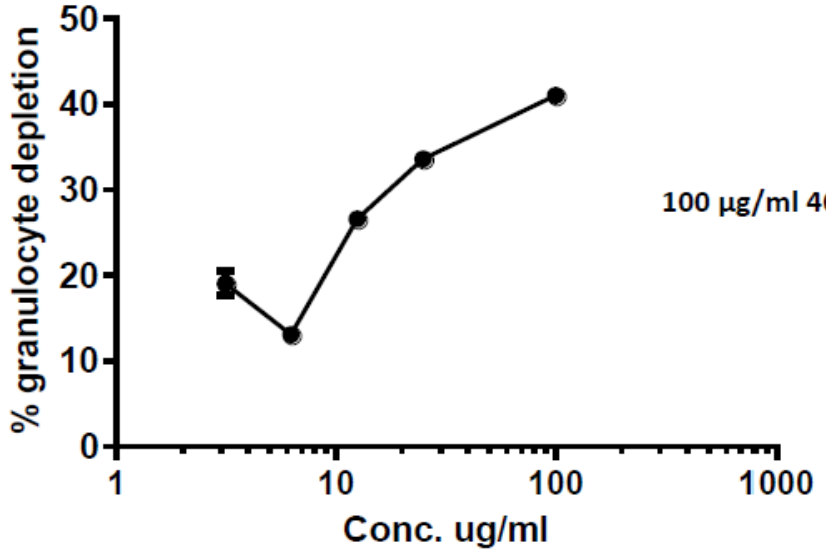
humanized anti-X antibody

Monkey neutrophil assay



10% depletion at 10 µg/ml
100 µg/ml only 13% depletion !

Human neutrophils assay

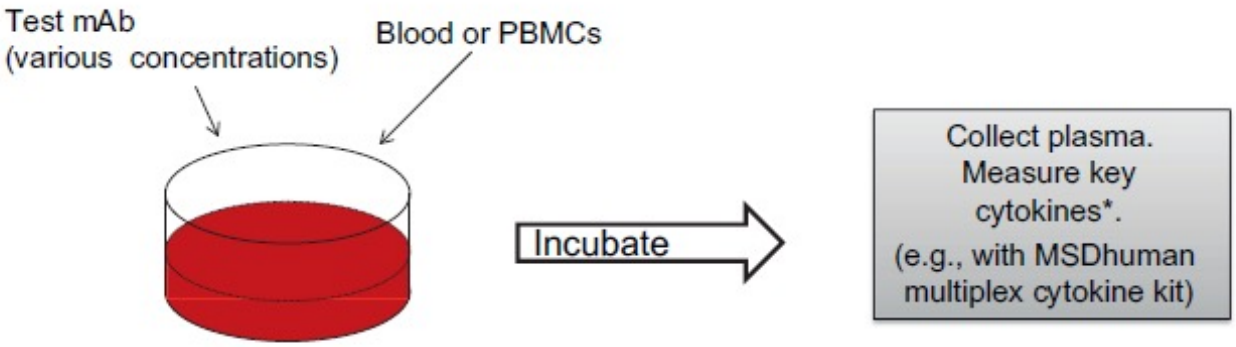


100 µg/ml 40% depletion !

A minimal Active Dose (PAD) can be established at 3 µg/ml
Affinity to Fcγ receptors is comparable between human and monkey

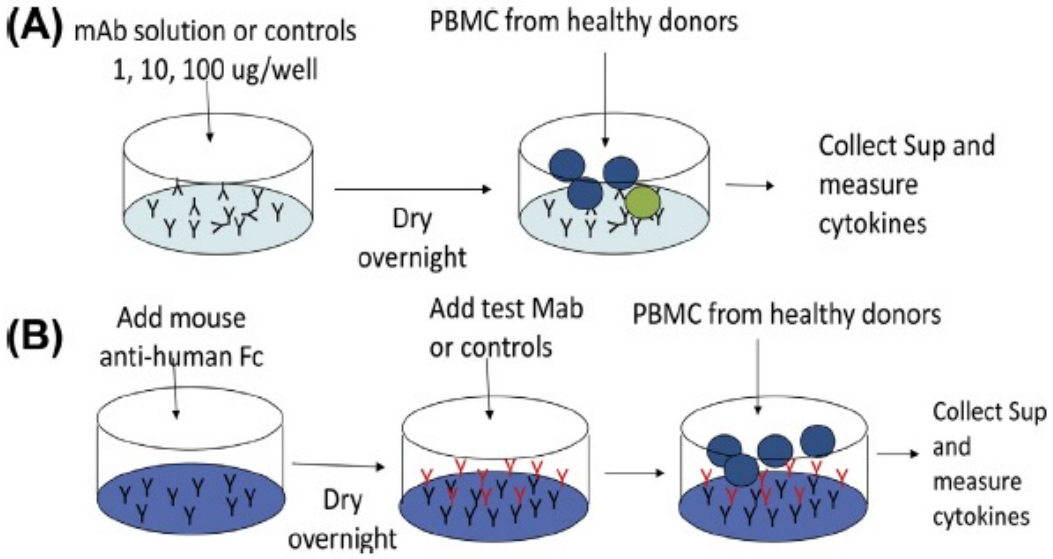
Cytokine Release assay using peripheral blood mononuclear cells or blood

Soluble phase



Multiple Donors
Positive controls
Negative controls

Solid phase



Conclusions

- “Biologics can exert their toxic effects through multiple mechanisms leading mainly to:
  - Cytokine release syndrome
  - Infections : case of neutralization of endogenous product
  - Immunogenicity
  - Auto-immunity ? Target-organ immune-related effect ?
- In vitro models can be of great help to mitigate the risk
  - Use human cells