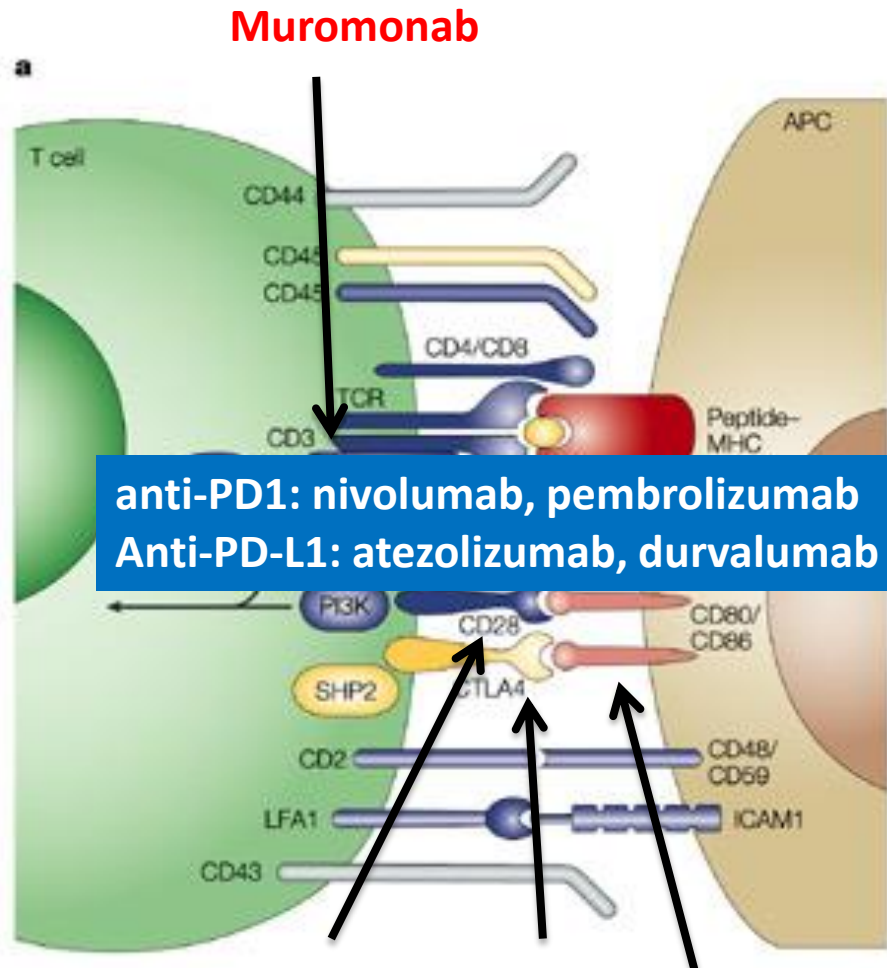


"Determination of the first dose for multispecific monoclonal antibodies: practical examples"

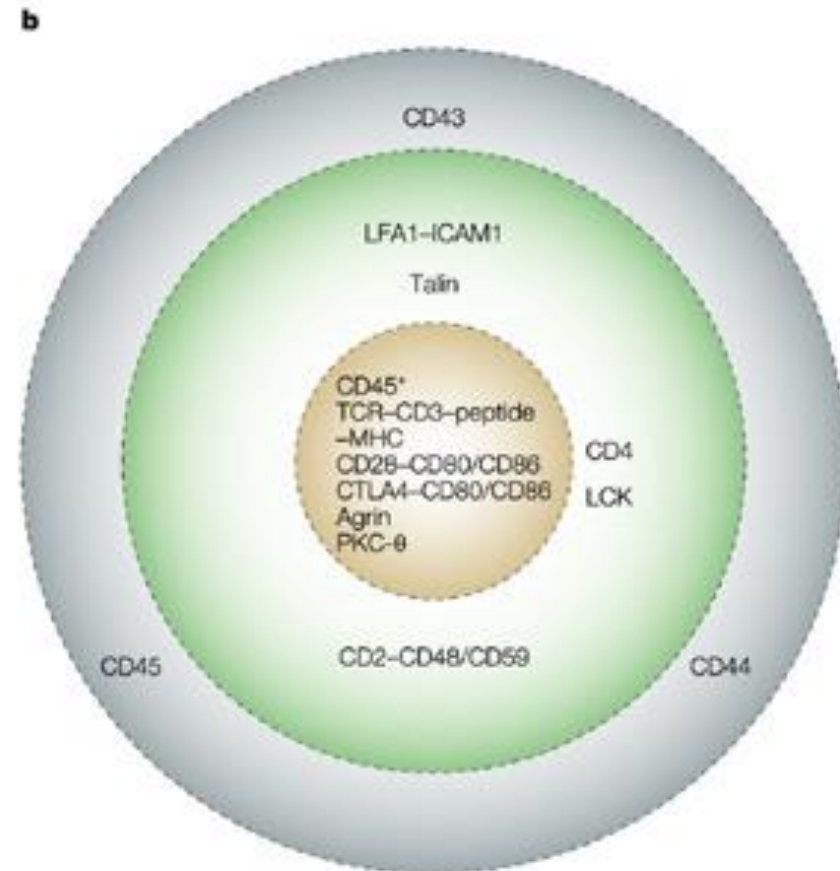
Marc Pallardy
INSERM UMR 996
Faculty of Pharmacy, University Paris-Sud
France

EUFEMED preconference May 2017

The immunological synapse = target for biologics

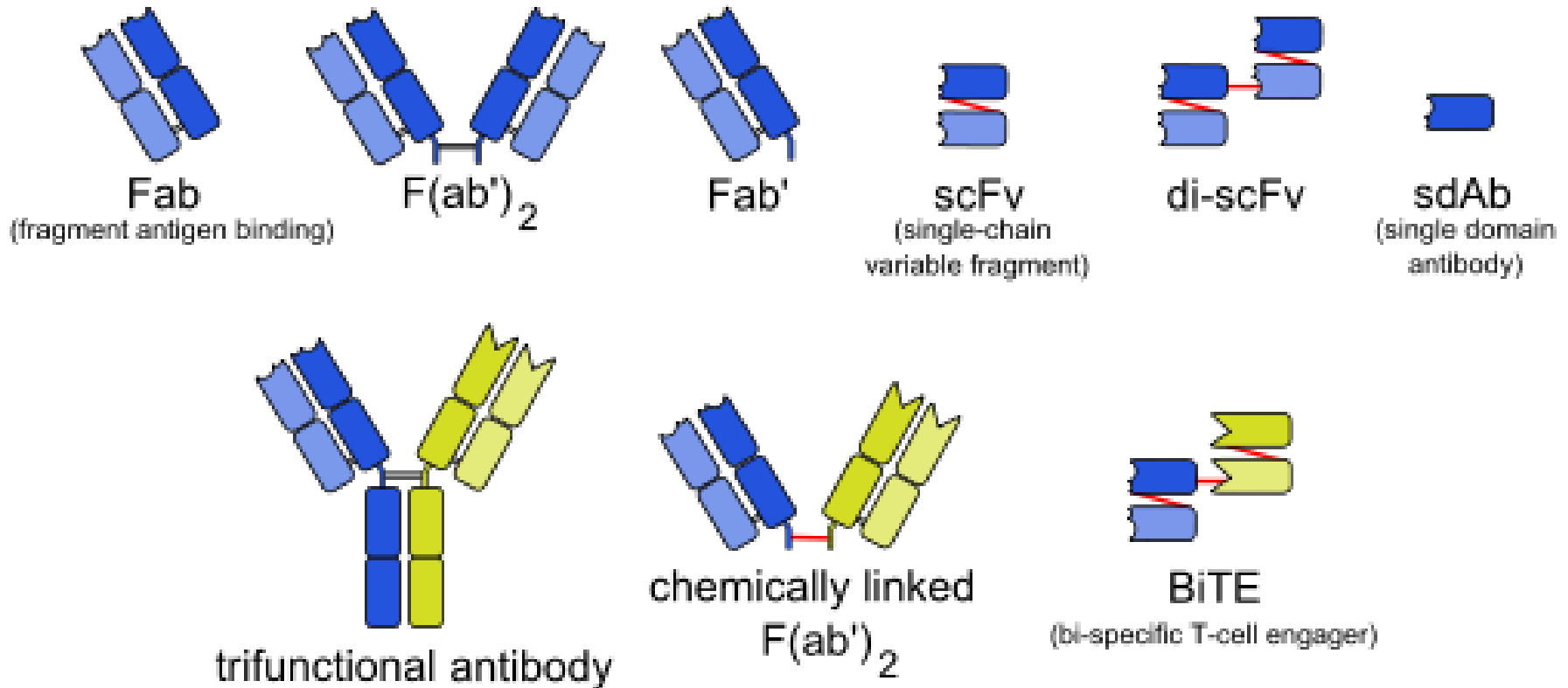


TGN1412 Ipilimumab Abatacept
Tremelimumab (CTLA4-Ig)



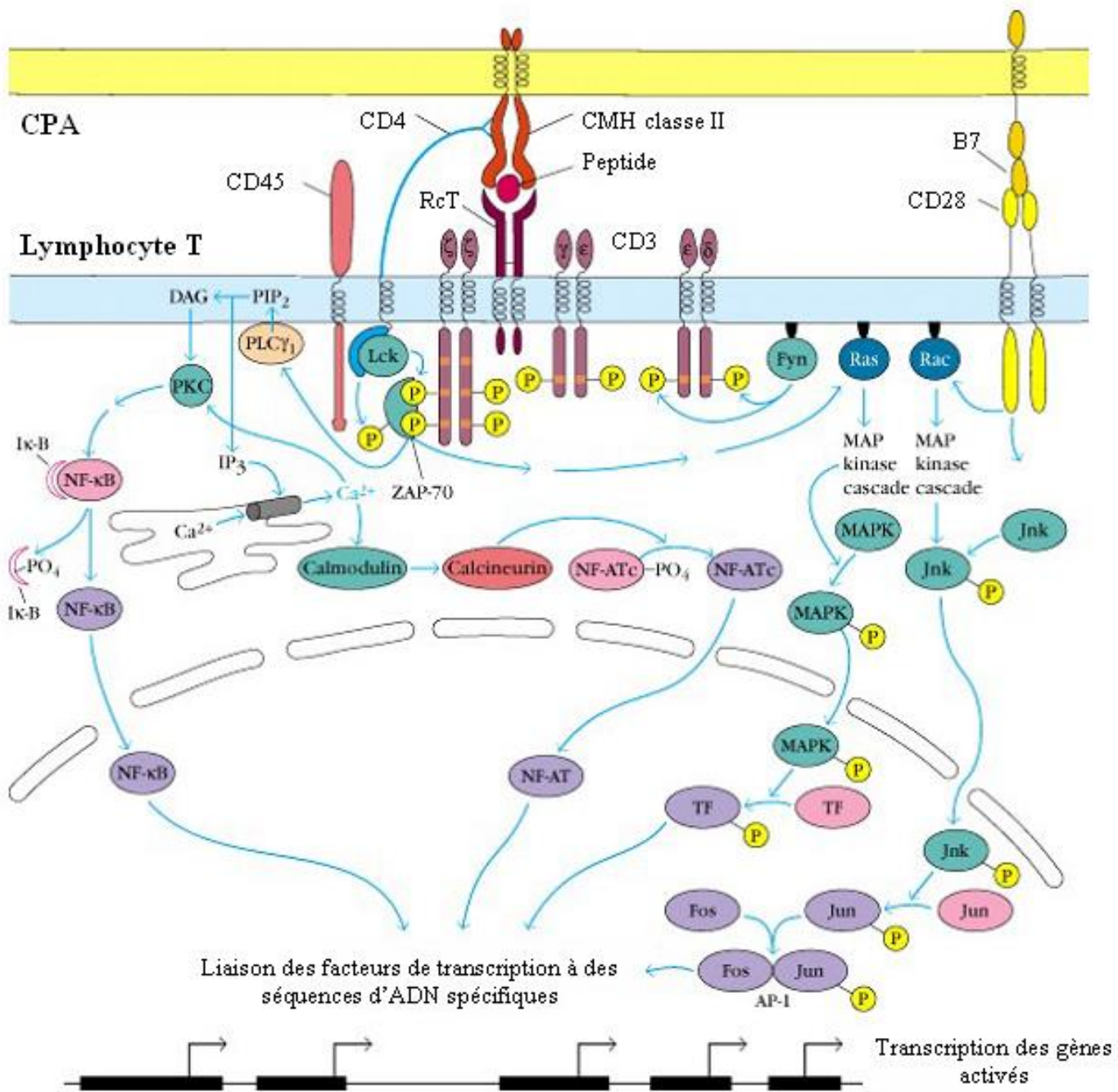
Different types of monoclonal antibodies

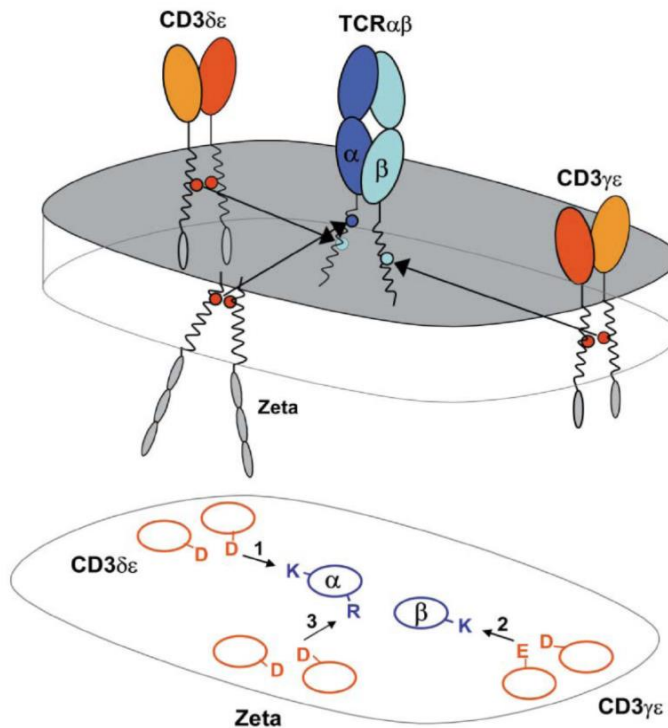
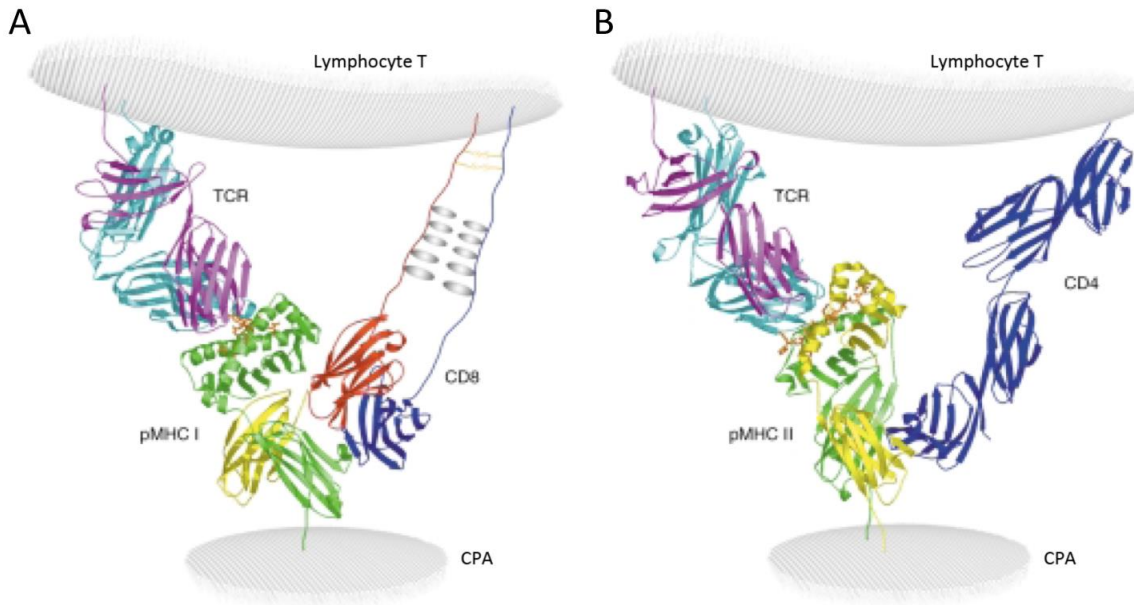
- Fab, F(ab')₂ fragment, Fab' fragment, single-chain variable fragment, di-scFv, single domain antibody
- Bispecific antibodies (trifunctional antibody, Chemically linked F(ab')₂, Bi-specific T-cell engager)



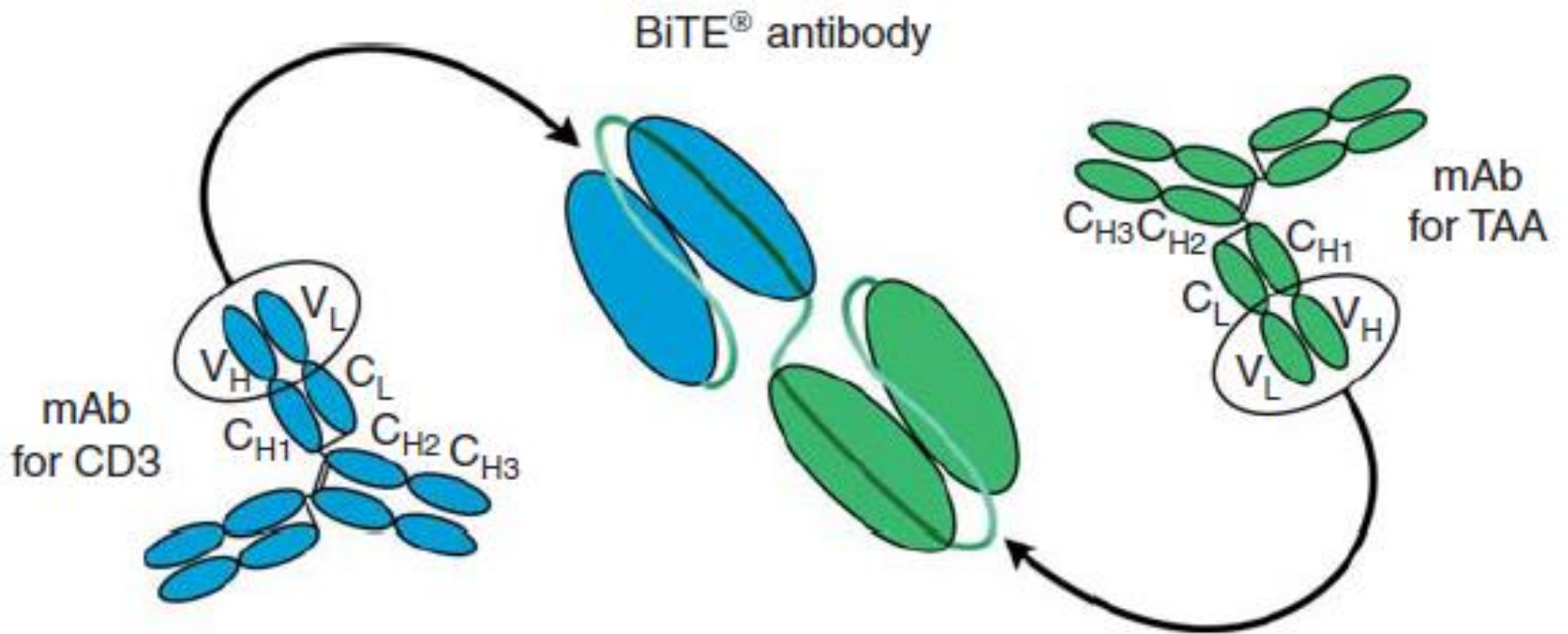
Anti-CD3/Anti-CD20; Anti-CD3/Anti-CD19.....

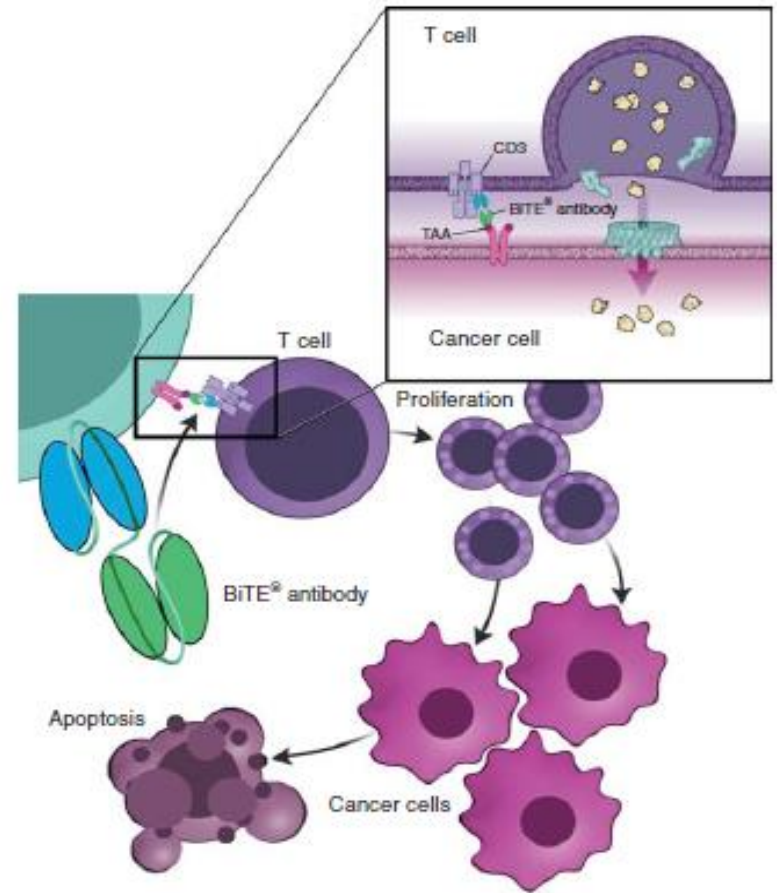
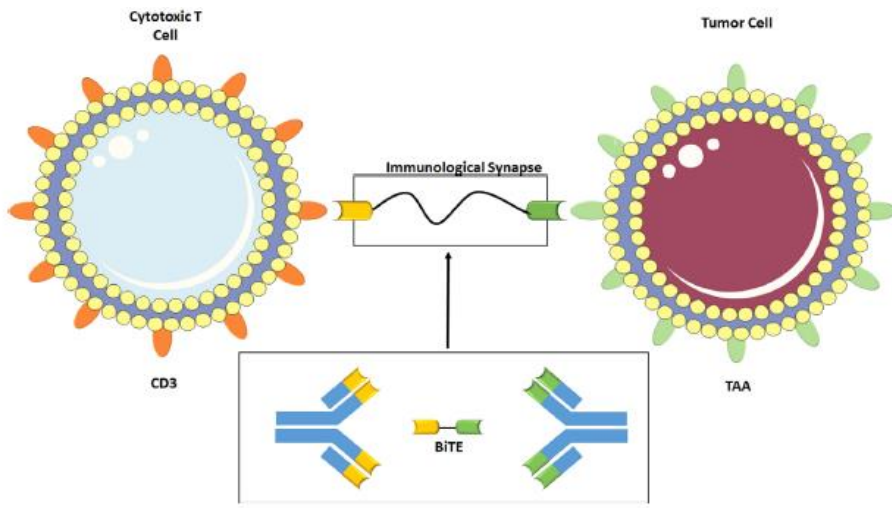
T-cell receptor signalling pathways





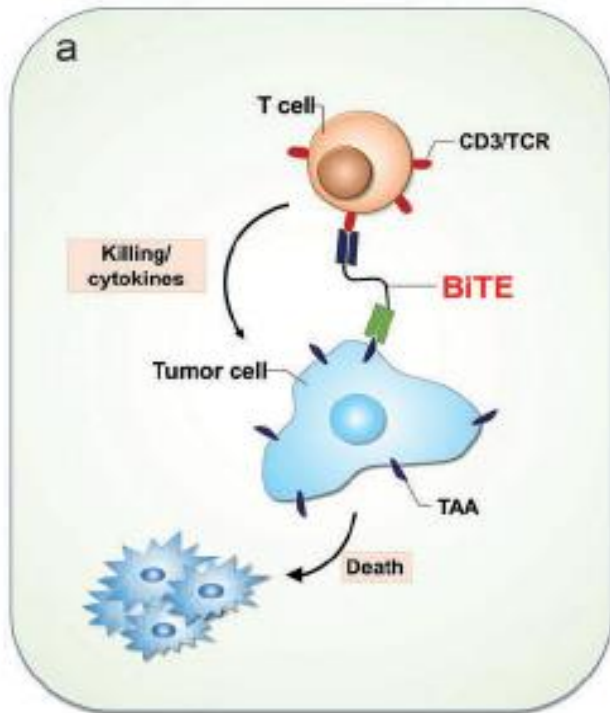
- Anti-CD3s are not fully working the same way as an antigen
- Not all anti-CD3s have the same mechanisms



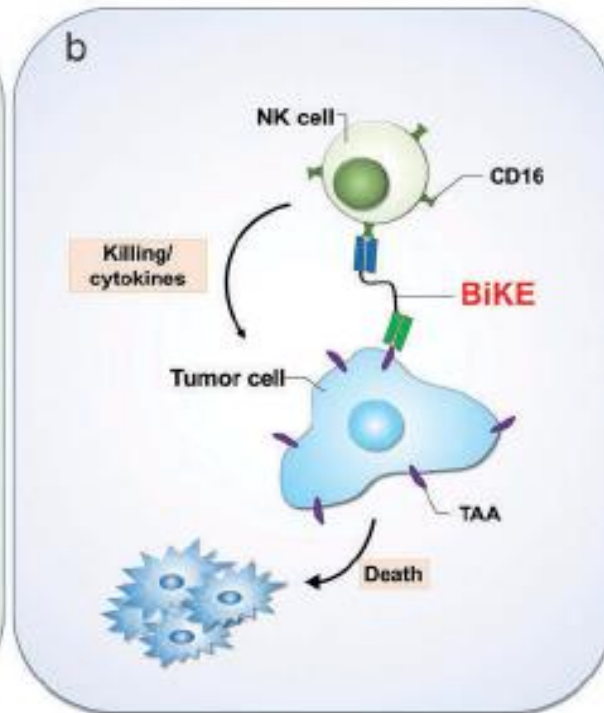


Stiegmaier L (Exp Op Biol Ther, 2015)

T-cells



NK-cells



NK-cells

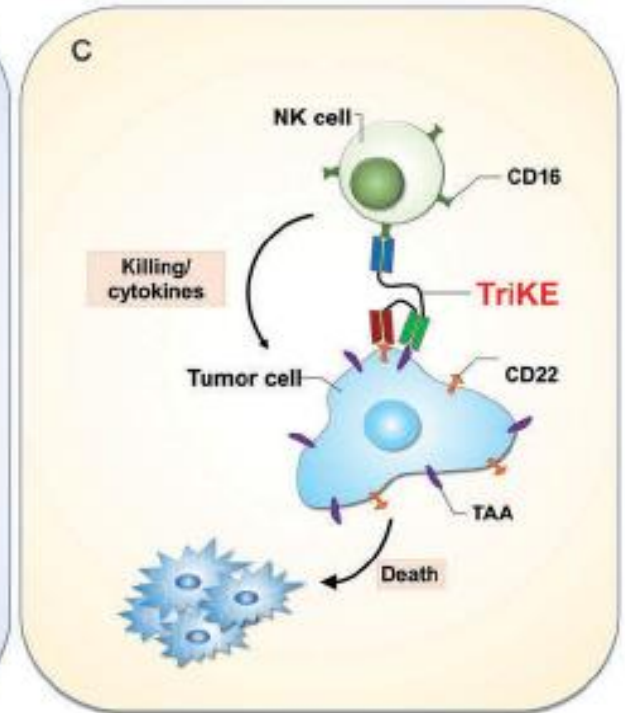


Table 1. Multivalent antibodies currently ongoing in clinical trials.

Name	Format	Target	Development stage
Blinatumomab (MT103)	BiTE	CD19xCD3	FDA approved in 2014
Solitomab (MT110, AMG110)	BiTE	EpCAMxCD3	Phase I
AMG330	BiTE	CD33xCD3	Phase I
AMG211	BiTE	CEAxCD3	Phase I
CD16xCD33	BiKE	CD16xCD33	Preclinical development
CD16xCD133	BiKE	CD16xCD133	Preclinical development
EpCAM16	BiKE	EpCAMxCD16	Preclinical development
AFM13	BiKE	CD30xCD16A	Phase I
CD16xCD19xCD22	TRIKE	CD16xCD19xCD22	Phase II
Catumaxomab (Removab®)	Trifunctional Ab (Triomab®)	EpcamxCD3xFc	Phase II/III
Ertumaxomab (Rexomun®)	Trifunctional Ab (Triomab®)	HER2xCD3xFc	Phase II
Bi20/FBTA05 (LymphomunTM)	Trifunctional Ab (Triomab®)	CD20xCD3xFc	Phase I/II
3F8/GD2Bi	Trifunctional Ab (Triomab®)	GD2xCD3xFc	Phase I/II
EGFRBi	Trifunctional Ab (Triomab®)	EGFRxCD3xFc	Phase I
Bispecific protein therapy			
ULBP2-BB4	Bispecific protein	NKG2D/NKG2D ligands	
UNKG2D-CD3 scFv	Bispecific protein	UNKG2D-CD3	
Anti-MICA therapy	mAb	MICA	Preclinical development
Monalizumab	mAb	NKG2A	Phase II, phase I and II
αCD16	mAb	CD16	
Lirilumab	mAb	KIR2DL1,2,3	Randomized phase II, phase I, Phase II
MGD006	DART	CD123xCD3	Phase I

Abbreviations: Ab: antibody; BiTE: bispecific T-cell engager; BiKE: bispecific killer cell engager; CEA: carcinoembryonic antigen; DART: dual-affinity re-targeting molecules; EGFRBi: epidermal growth factor receptor bispecific antibody; EpCAM: epithelial cell adhesion molecule; MICA: MHC class I-related chain A; mAb: monoclonal antibody; scFv: single-chain variable fragment; TriKE: trispecific killer cell engager; ULBP: UL16-binding protein.

2017: More than 40 bi-specific in clinical development

Side effects and “biologics”

What did we learned **or not** from the clinic ?

- **From the clinic**

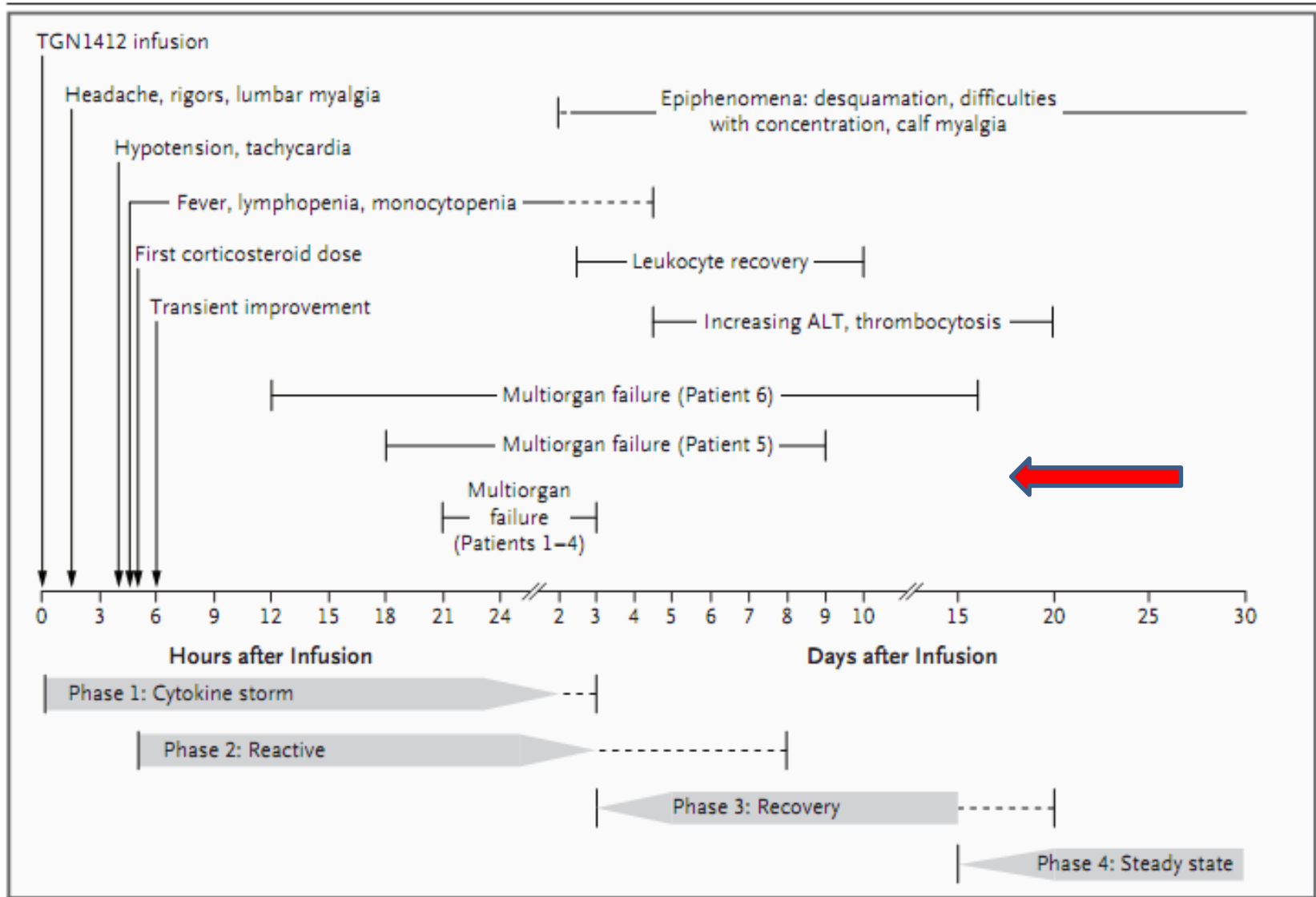
- Anti-CD3 (Muromonab): Cytokine Release Syndrome (CRS)
- Anti-CD52 (Alemtuzumab): CRS
- TGN1412: severe CRS
- IL-2: Vascular Leak Syndrome
- Alzheimer vaccine (AN1792): meningoencephalitis in 6% of immunized patients
- Ipilimumab and severe colitis
- Natalizumab and PML (progressive multifocal encephalopathy)...

The TGN 1412 story

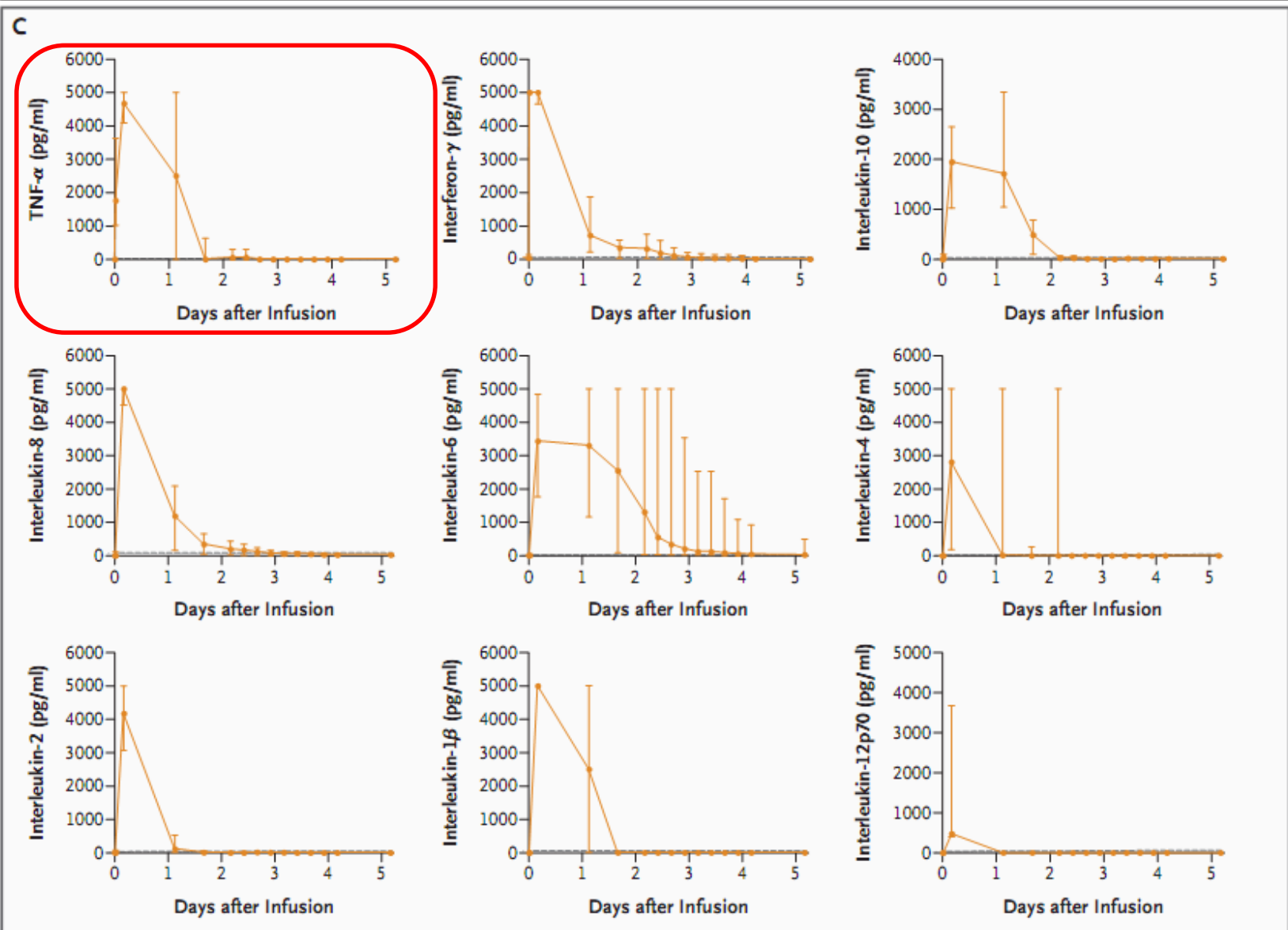
- March 2006
- Phase I clinical trials with a superagonist anti-CD28 monoclonal antibody
- **Potential therapeutic indication: auto-immune diseases**
- 6 human volunteers received the product
- **Major Cytokine Release Syndrome** with lungs affected
- Hospitalisation in intensive care
- Not observed in toxicology studies using cynomolgus monkeys



Summary Timeline of the Main Events after Infusion of TGN1412



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



**What are the main problems
with bi-specific ?**

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:
 - Severe (grade 3) CRS occurred in 2% of 189 patients who received blinatumomab for approved indication (n=3)
 - 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)
- **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
- Neurotoxicology (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 (2)

- **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 occurred 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 \neq from classical therapeutic antibodies
- Consider reducing the number of cells expressing the target before BiTE administration (not possible for all therapeutic indications)

Case studies

Anti-CD20/Anti-CD3 bi-specific

- **Therapeutic indications**

- B-cell non-Hodgkin lymphoma

- **Pharmacodynamics**

- Complete set of in vitro studies using cells from human and monkey
- B cell lysis and **0,7%** CD20 occupation provokes 60% cell lysis
 - T-cell lysis is much more efficient than ADCC (Rituximab)
- **EC50s between monkey cells (45 pM) and human cells (63pM)**
- Proof of concept with human B cell lymphoma in vitro and xenograft mouse models with human tumor cells.

- **Pharmacokinetics**

- In cynomolgus monkeys, clearance was dependent on both dose and level of circulating B cells: **target mediated drug disposition**
- Clearance is reduced after the second administration compared to the first administration
- Consistent with the reduction of B cells after the first administration and a subsequent reduction of target-mediated drug disposition.

- **Toxicology**

- Cynomolgus monkey was considered the appropriate animal species for toxicity testing
- ICH S9 2-week GLP repeat dose toxicity study to support phase 1 entry

- **In vitro assays**

- tissue cross-reactivity in human tissues
- Assessment of cytokine release in both human and cynomolgus monkey blood: whole blood
 - difference in potency between human and cynomolgus monkey of up to 70-fold (for the most expressed cytokine = IL-6).

- **Observations**

- Dose-dependent and transient reduction in all peripheral lymphocyte subsets
- Maximal increases in cytokine release measured 4 hours post-dose
- Less than 100 µg/kg induced clinical signs linked to cytokine release
- Changes in soluble mediators consistent with an acute phase response
- Transient activation of the coagulation system, secondary to post-dose elevations of multiple cytokines
- B-cell depletion
- High incidence of ADAs detected 14 days post-dosing

Starting dose (1)

- **Model:**
- In vivo PK/PD model, in which cytokine release (IL-6, IFN-gamma, and TNF-alpha), is used as a pharmacodynamic endpoint and correlated with drug exposure.
 - Cytokine release, particularly IL-6 and IFN-gamma, were identified as relevant pharmacodynamic readouts, as they reflect both the mode of action and the safety of the product.
 - Baseline B-cell count was applied as an additional constraint to the PK/PD model since cytokine release is dependent on CD19 expression = **B cells in “normal” animals.**
- Serum PK data scaled from monkey to human according to allometric principles
- Indirect response model was used to describe cytokine release in blood after administration of the compound (blood and then tissue).
- To account for species differences in cytokine release, the dose is corrected for the lower potency in cynomolgus monkeys using an in vitro factor of 70-fold for IL-6
- **ISSUE:**
 - The PK/PD model only considers B-cell counts in circulation. B cells in tumors can contribute to cytokine release into the circulation
 - No data to allow an estimate of the effect of tumor load on cytokine release in plasma.
 - A 10-fold safety factor is applied to the dose predicted from the PK/PD model due to this uncertainty.

Starting dose (2)

- **EIH dose**

- Predicted from the PK/PD model according to the following clinical constraints:
 - more that 90% of the patients below 600 pg/mL IL-6
 - IL-6 was shown to be the most sensitive studied cytokine following administration
- This threshold is seen as a clinically tolerable based on clinical experience with other bi-specifics
- 50 B cells/ μ L blood at dosing time.
- **Proposed EIH dose was 200 fold lower than the estimated therapeutic dose ranges**

Anti-CLEC12A/CD3 bi-specific

- **Therapeutic indications**

- Acute myelogenous leukemia (AML)/CLEC12A/leukemic stem cell-associated antigen
- CLEC12A is expressed on AML leukemic stem cells, but not on normal haematopoietic cells
- CLEC12A+ expressed on granulocyte-monocyte lineage in bone-marrow and on dendritic cells
- Species cross reactivity is limited to humans (both for the CD3 arm and the CLEC12A arm)
- No eligible animal model
- In vitro human data
 - Lysis of HL60 tumour cells by MCLA-117 using purified human T cells in presence of human serum.
 - Killing of AML blasts isolated from bone marrow or peripheral blood samples by the product and autologous effector T cells present in the non-manipulated sample at very low E:T ratio
- **Killing of CLEC12A expressing cells with EC20 values for T cell activation being in the range 10-85 ng/mL, and in the range 6-25 ng/mL for cell lysis of HL-60 cells.**

- **Pharmacokinetics**

- Human Immune System (HIS) mice = immuno-deficient, pre-conditioned BALB/c mice in which human CD34+ haematopoietic progenitor cells derived from umbilical cord blood are transplanted. Both human CD3 and CLEC12A are expressed on a large majority of myeloid subsets in HIS mice
- Initial study in the BRGSF-HIS mouse **showed only limited T cell activation** following IV injection of 1 mg/kg and no antibody was detected in the serum at 2, 7 or 14 days after injection
- **No PK studies in cynomolgus monkeys**

- **Toxicology**

- No animal toxicology studies conducted

- **In vitro assays**

- tissue cross-reactivity in human tissues
- Assessment of cytokine release in both human : whole blood and PBMCs
 - Whole blood: strong stimulation of IL-6 release at 100 ng/mL and higher, with significant increases in IL-10, TNF α and IFN γ release becoming evident at concentrations between 300 and 3000 ng/mL

Starting dose

- **Model**

- Based on the MABEL from Pharmacological data
- Calculation of MABEL based on co-incubation studies using HL-60 cells and human PBMC
- The most sensitive of these readouts (T cell activation as measured by CD69 increase on CD8 T cells) has been used

- **ISSUE**

- No assessment of neutropenia

- **EIH dose**

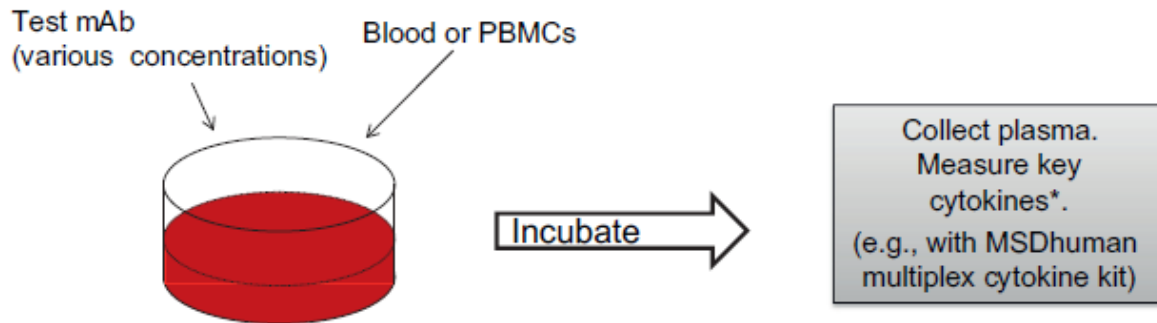
- MABEL = mean EC20 = 10 ng/mL
- A starting dose of 25 μ g was proposed for the product infused intravenously
- Assuming that the product will be distributed only in the plasma compartment (about 2.5 liters in an adult; 10 ng/mL x 2500 mL = 25 μ g)
- Efficacious estimated concentrations for AML cell killing (about 100-1000 ng/mL)

Questions/Issues

- **Measurement of CD3 binding affinity**
 - low, medium and high binding affinity depends on the company's definition and the assay being used (e.g., SPR, cell based affinity)
- **CRS**
 - The appropriate format of these assays may be different from the typical formats requested
 - Regulatory bodies are continuing to request the same set of assays
 - Are they relevant for bi-specific
 - What is the utility of cytokine release assays in the absence of exogenous target expressing cells
 - Should cytokine release in human bioactivity (potency) assays been used for human first-in-dose setting for this modality?
- **PK modeling**
 - Predicting the PK is challenging and the conventional approach may not be appropriate: allometric scaling of preclinical parameters to predict human PK
 - Target-mediated drug disposition models (TMDD) that mechanistically describe binding to two or more targets can be proposed
- **FIH dose selection**
 - Considerations for MABEL dose setting for bi-specific, what are the key considerations for the in vitro assays ?
 - FIH determined with MABEL calculated with in vitro model EC20 too conservative, patient will not benefit from the treatment ?
 - PK/PD modelling approaches

Cytokine Release assay using peripheral blood mononuclear cells or blood

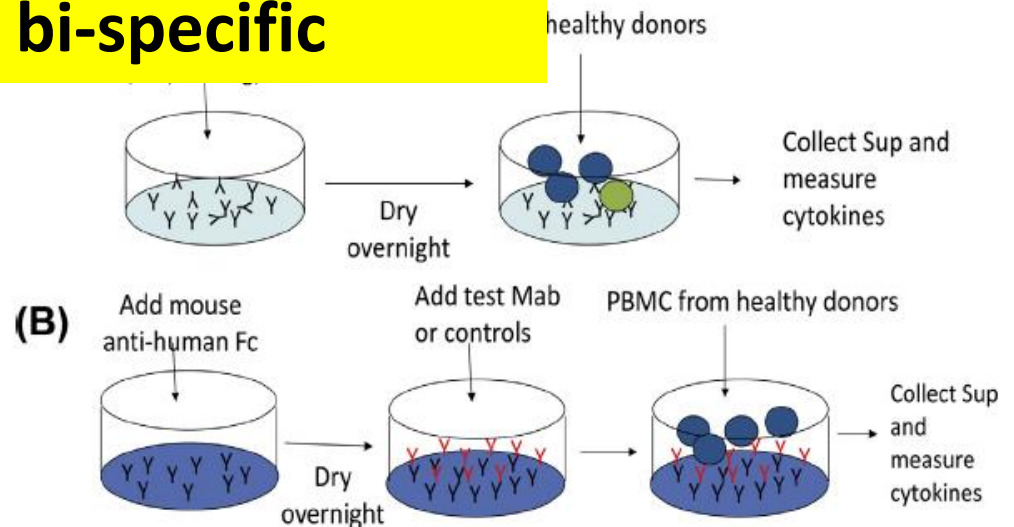
Soluble phase



Multiple Donors
Positive controls
Negative controls

Not fully adapted to bi-specific

Solid phase



Back-up slides

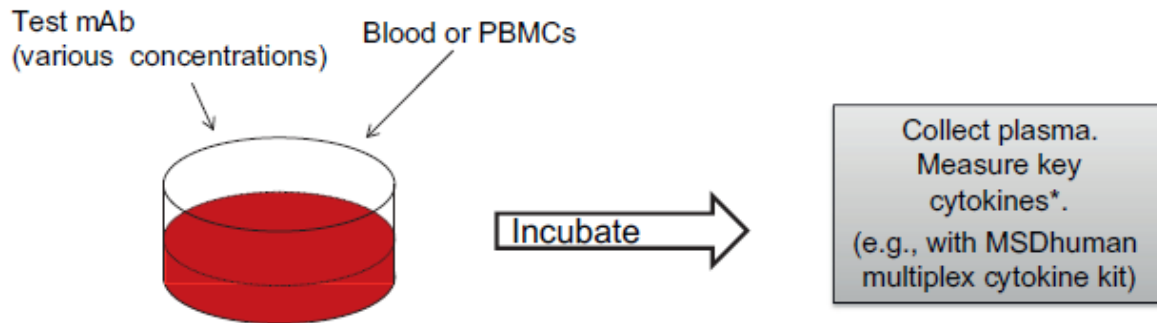
Biotherapeutics ?

- **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

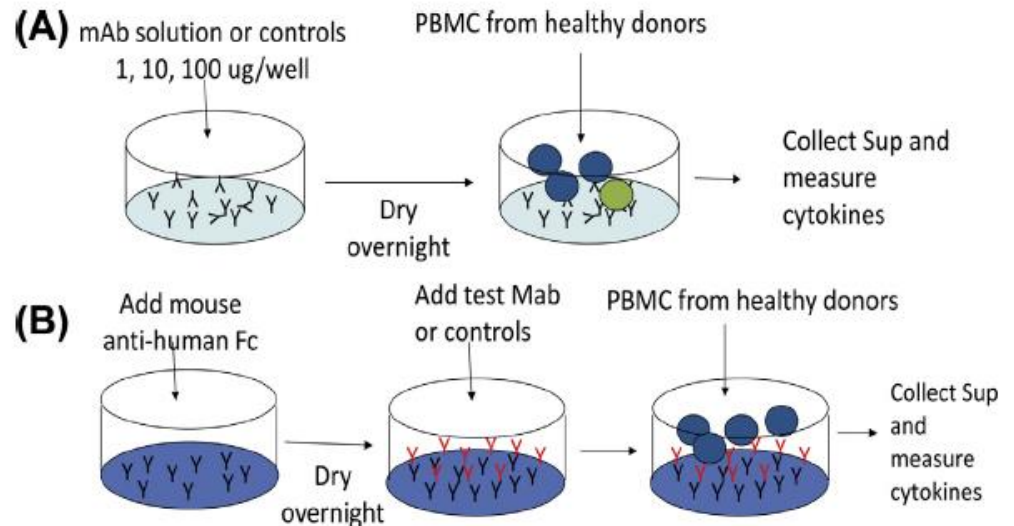
Cytokine Release assay using peripheral blood mononuclear cells or blood

Soluble phase

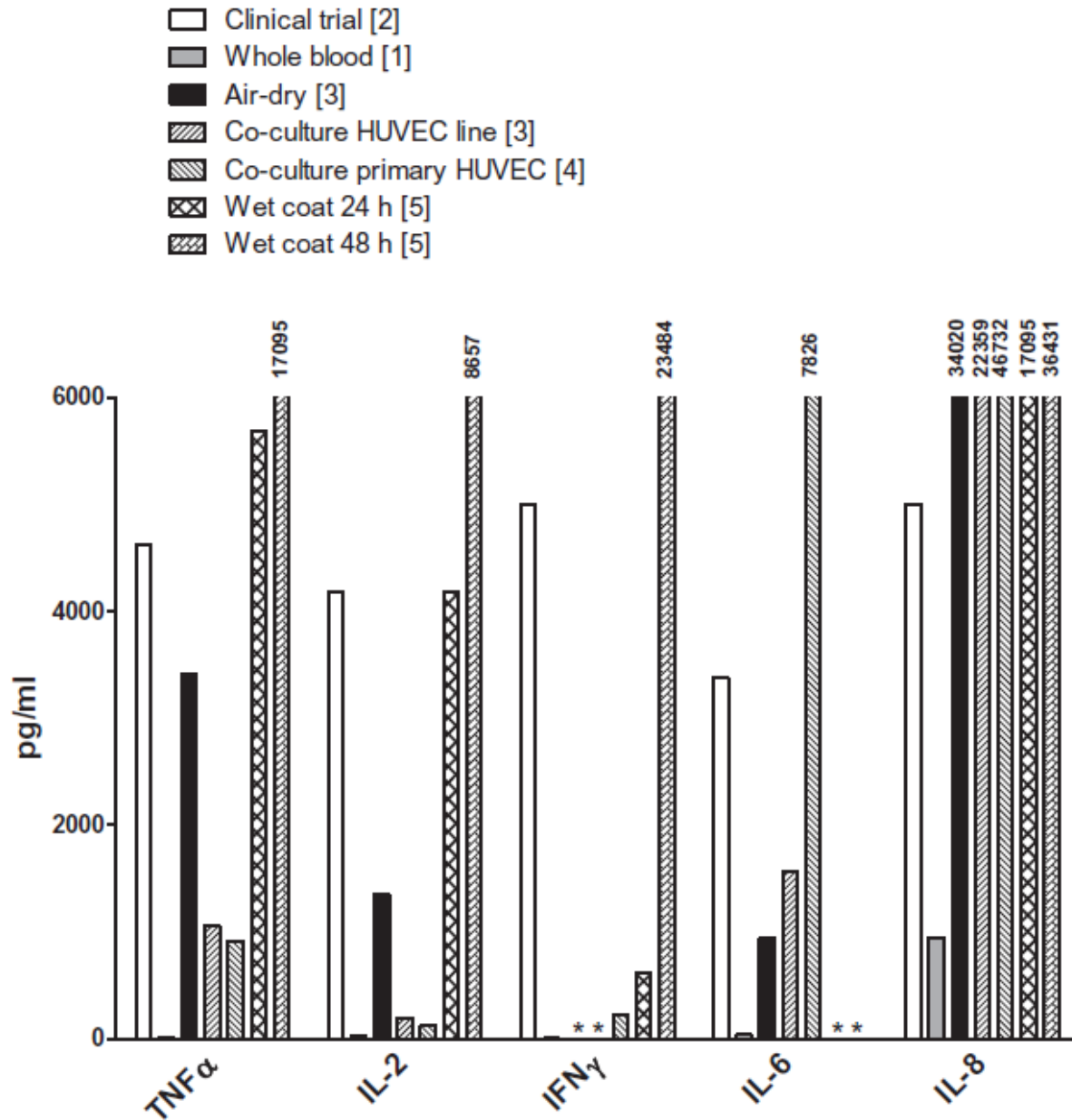


Multiple Donors
Positive controls
Negative controls

Solid phase



Cytokine levels obtained with the different CRAs using TGN 1412



Toxicity evaluation: 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 inspired 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***