

# Übertragbarkeit von Ergebnissen aus Tierversuchen



**Ulrich Kalinke, Ph.D.**

TWINCORE, Zentrum für Experimentelle  
und Klinische Infektionsforschung

# Entwicklung neuer Therapeutika

**Vorklinische Entwicklung**

**Phase I: Ersterprobung  
im Menschen**

**Clinical Development**

**Multizentrische Studien  
innerhalb der EU**

**Marktzulassung**

**Relevante Spezies?**

**Seit 2004: Genehmigung  
klinischer Studien auf Ebene  
der jeweiligen EU Länder**

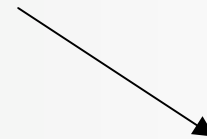
**Für die gesamte EU**

# Arzneimittelzulassung: Zwei zuständige Behörden in Deutschland



## PEI (Paul-Ehrlich-Institut)

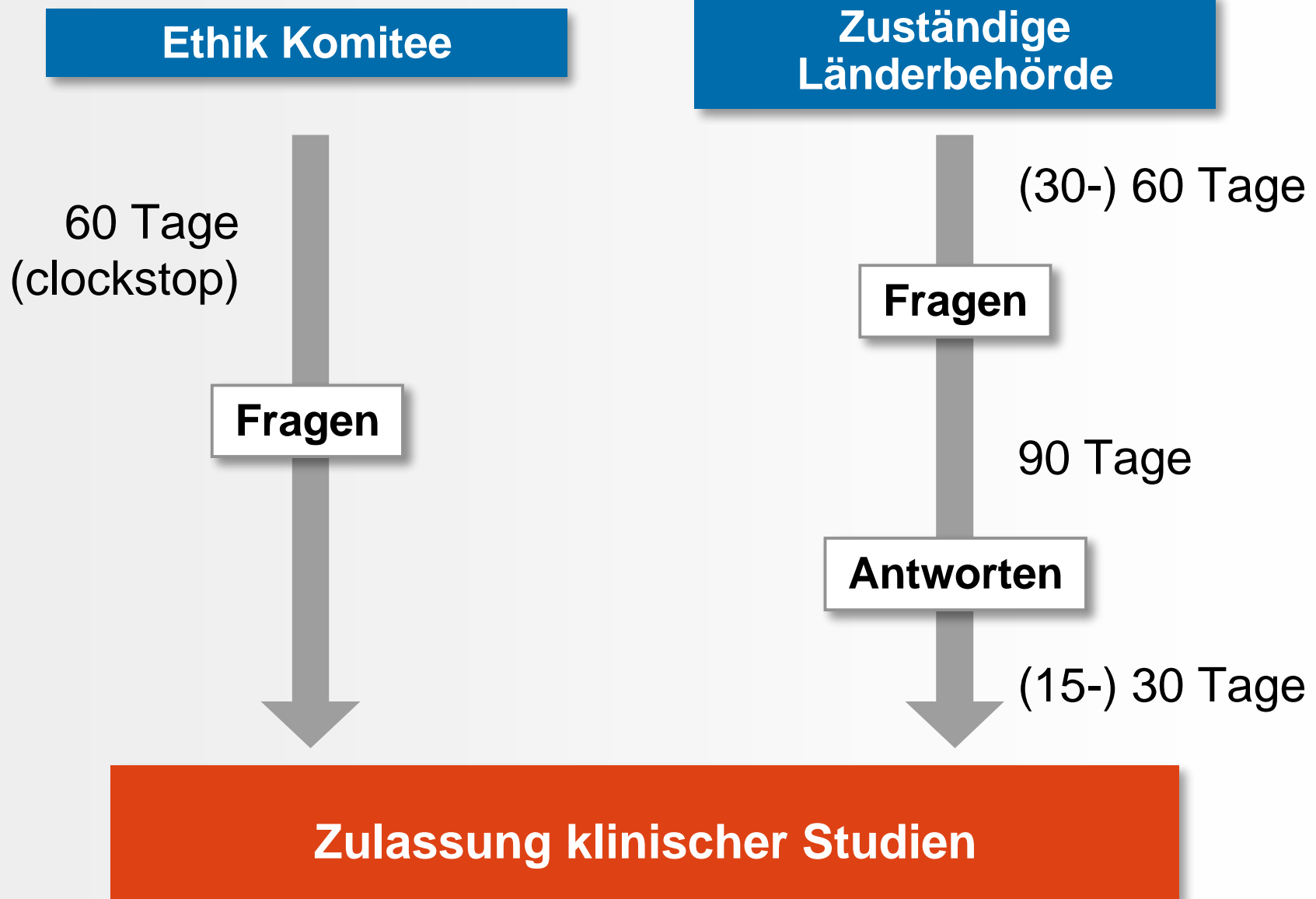
Viele biologische Arzneimittel wie Impfstoffe und monoklonale Antikörper



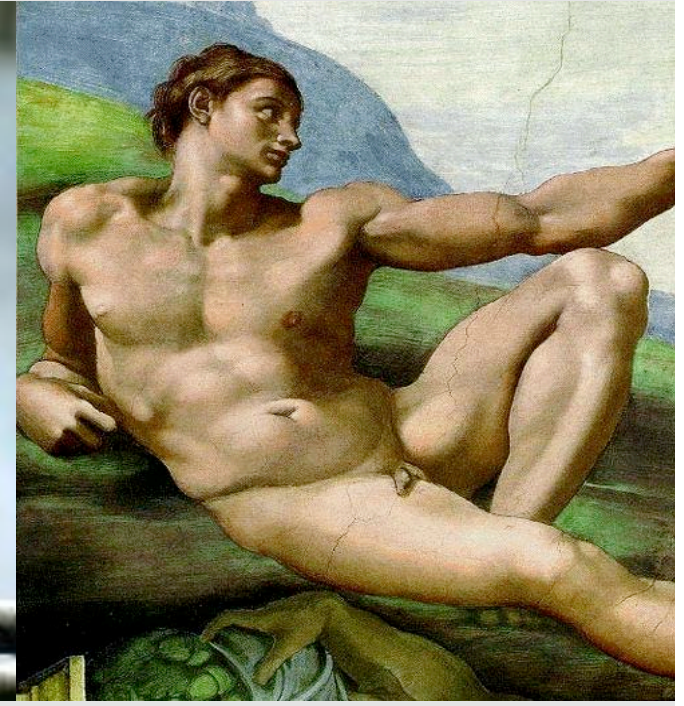
## ➤ BfArM (Bundesinstitut für Arzneimittel und Medizinprodukte)

Alle anderen Produkte

# Zulassung klinischer Studien in der EU



# Viele biotechnologische Produkte sind spezies-spezifisch



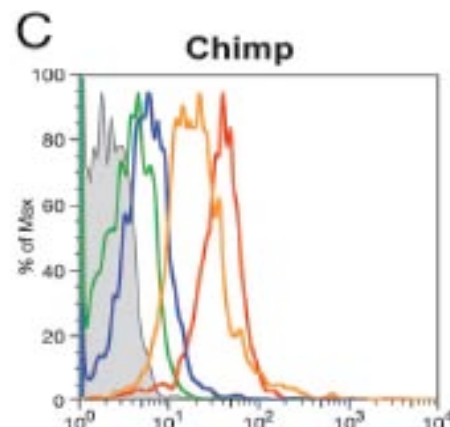
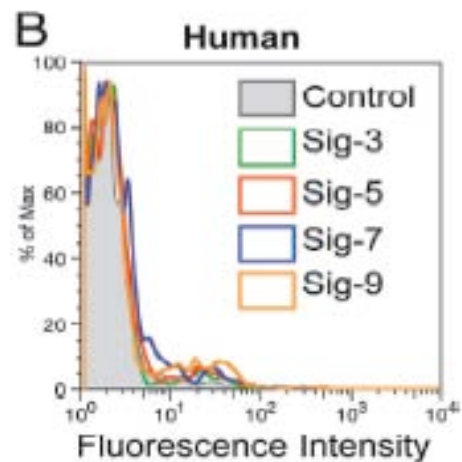
Unter biologischen Systemen sind insbesondere das **zentrale Nervensystem** und das **Immunsystem** noch spät in der Entwicklung einem starken evolutionären Druck ausgesetzt worden

# „Späte“ Evolution des Immunsystems

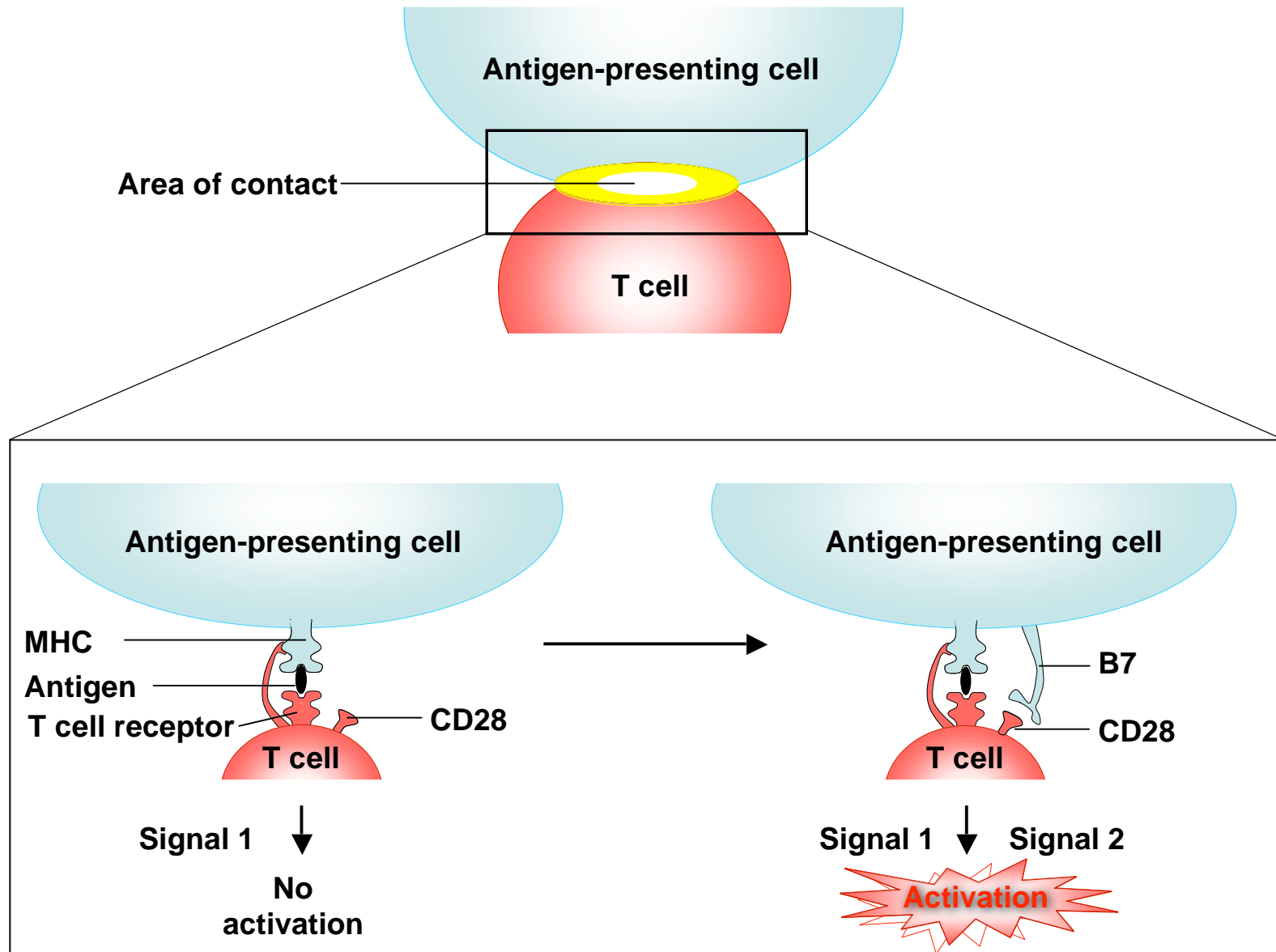
## Loss of Siglec expression on T lymphocytes during human evolution

Dzung H. Nguyen<sup>\*†</sup>, Nancy Hurtado-Ziola<sup>\*†‡</sup>, Pascal Gagneux<sup>\*</sup>, and Ajit Varki<sup>\*5</sup>

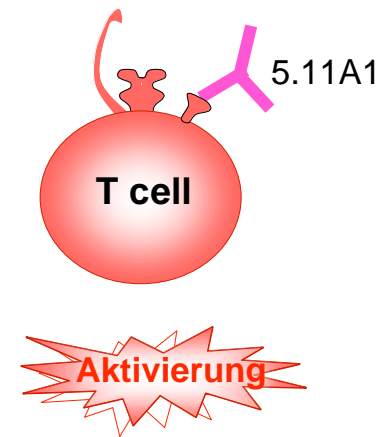
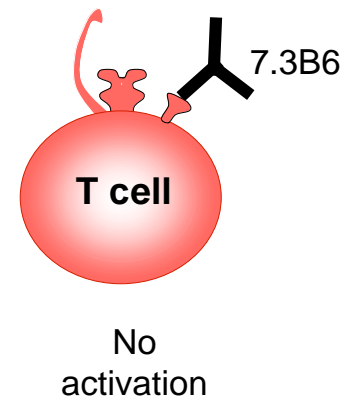
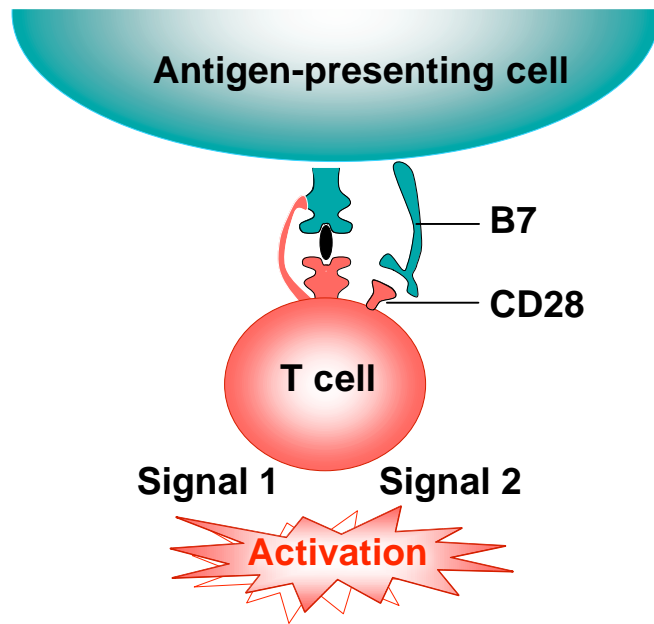
PNAS | May 16, 2006 | vol. 103 | no. 20 | 7765-7770



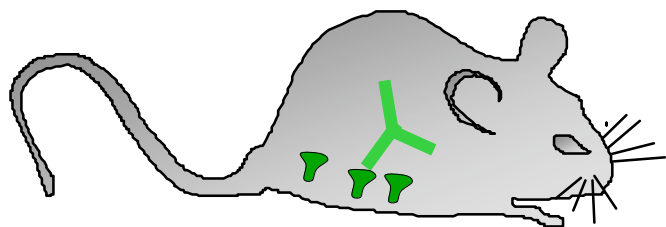
# Zwei Signale sind für die T-Zellaktivierung notwendig



# Superagonistische und normale anti CD28-Antikörper



# Superagonistische anti-CD28-Antikörper können Autoimmunerkrankungen verhindern!



## Behandlung von Autoimmunerkrankungen in Ratten

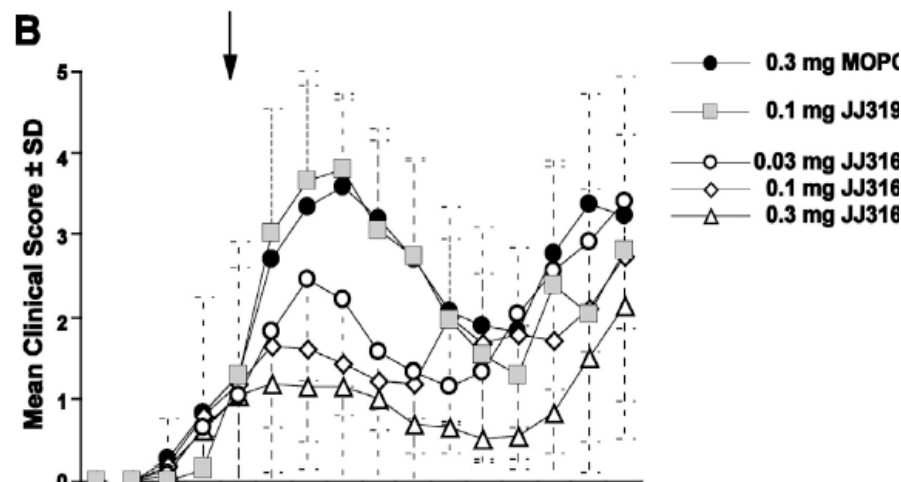
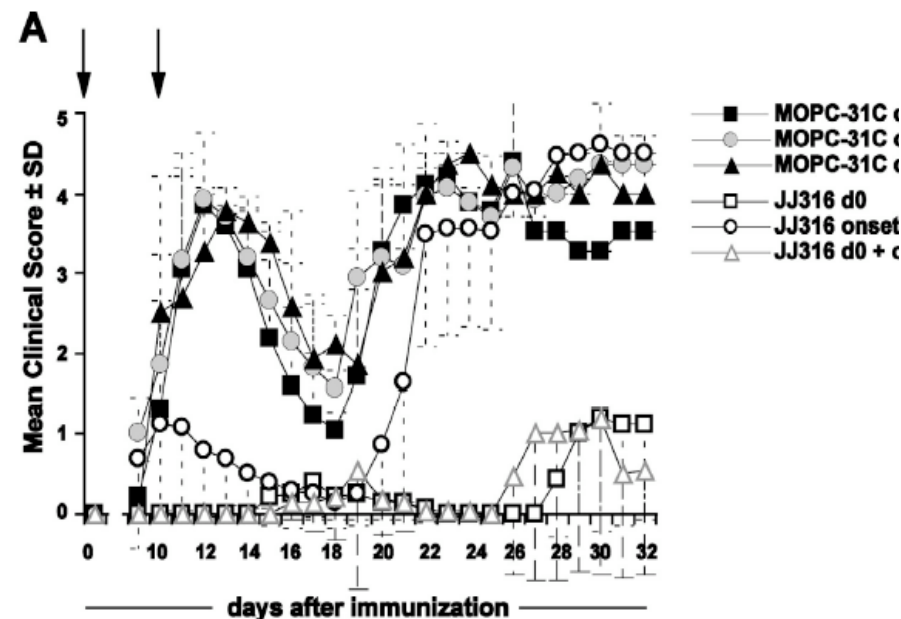
- Kollagen-induzierte Arthritis
- Adjuvant Arthritis
- Experimentelle autoimmune Encephalomyelitis

Selective targeting of regulatory T cells with CD28 superagonists allows effective therapy of experimental autoimmune encephalomyelitis

Niklas Beyersdorf,<sup>1</sup> Stefanie Gaupp,<sup>2</sup> Karen Balbach,<sup>1</sup> Jens Schmidt,<sup>2</sup>  
Klaus V. Toyka,<sup>2</sup> Chia-Huey Lin,<sup>1</sup> Thomas Hanke,<sup>3</sup> Thomas Hünig,<sup>1</sup>  
Thomas Kerkau,<sup>1</sup> and Ralf Gold<sup>2,4</sup>

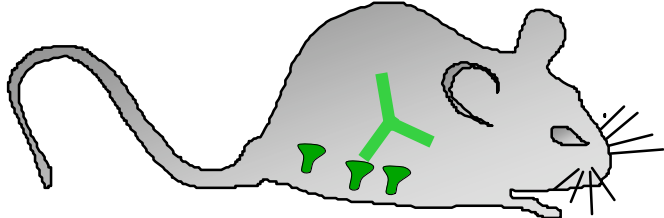
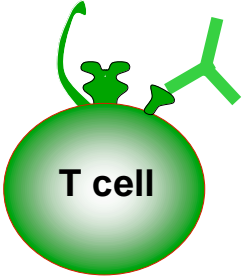
JEM © The Rockefeller University Press \$8.00

Vol. 202, No. 3, August 1, 2005 445-455 www.jem.org/cgi/doi/10.1084/jem.20051060

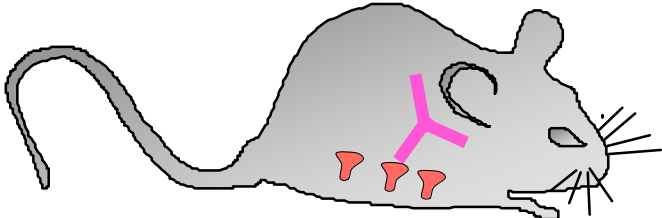
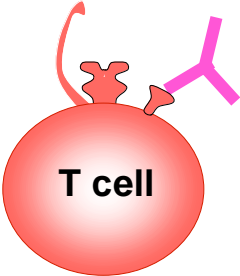


# Surrogat-Antikörper, transgene Mäuse, relevante Spez

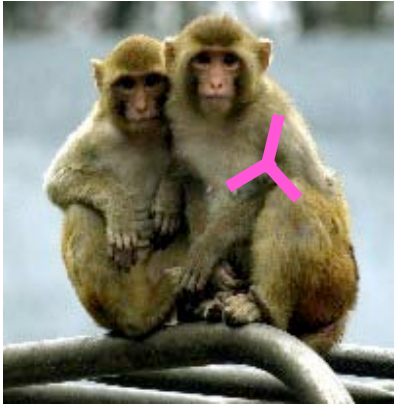
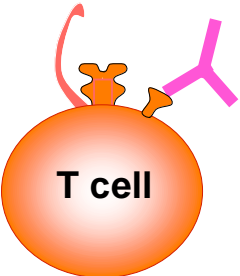
Rat



Human



Non-human primate



# Der TGN1412-Zwischenfall

- Behandlung von 8 Freiwilligen (6 Verum, 2 Placebo) innerhalb von 2 Stunden
- Nebenwirkungen (Zytokinsturm) manifestierten sich innerhalb weniger Stunden nach Behandlung



Stern no. 17, 20.04.2006



## IS IT A DREAM?

People who have near-death experiences are more likely to find REM sleep intruding on reality. [www.nature.com/news](http://www.nature.com/news)

## Can super-antibody drugs be tamed?

As it becomes clear that the London clinical trial disaster was indeed the fault of the drug itself, **Michael Hopkin** looks at what went wrong, and whether there is any future for 'superagonist' antibody therapies.

There was no warning from animal tests, but last month the experimental antibody drug TGN1412 put six British men in intensive care. The resulting investigation ruled out any failure of experimental and regulatory procedures — a relief for those involved, but a damaging blow for the field. Immunologists are now left asking what went so badly wrong in the trial, and whether the fearsome potency of 'superagonist' antibody therapies will outweigh their promise.

The six volunteers at London's Northwick Park Hospital were probably struck by a huge immune reaction called a cytokine storm — a flood of inflammatory molecules released by cells called helper T cells, which shut down their organs in hours (see *Nature* 440, 338–339; 2006). The UK Medical and Healthcare products Regulatory Agency, which approved the trial, announced last week that it has found no evidence of contamination in the treatments, which means the devastating effects were almost certainly caused by TGN1412 itself.

With hindsight, it might be no surprise that the compound, dubbed a 'superagonist' antibody by its creators, could run amok in the immune system. Around 20 antibody therapies are currently approved or nearing approval, most of which mimic natural human antibodies against specific viruses or cancer-cell types. But TGN1412 is different. It was designed to circumvent the usual checks and balances that prevent T cells from overreacting in the course of their normal duties.

Usually, T cells respond to



Gathering storm? Antibody therapies aimed at large swatches of the immune system might be too hot to handle.

**"With hindsight, it might be no surprise that the compound could run amok in the immune system"**

receptor (TCR) and the CD28 receptor. TCR binding is specific to the antigen in question, whereas CD28 binding acts more like a 'go' switch for the T cell; both are normally required (see graphic, overleaf). But the makers of TGN1412 found a way to switch on the CD28 green light without TCR binding, activating T cells across the board.

T cells fall into three broad categories: killers, which des-

elements of the immune system. Roughly half of killer T cells, and virtually all helper and regulatory T cells, express the CD28 receptor.

When Thomas Hünig, an immunologist at the University of Würzburg and researchers at TeGenero, the spin-off company he co-founded to develop TGN1412, began testing the antibody on animals, they found that the drug seemed only to activate regulatory T cells (Beyersdorf, N. *et al. J. Exp. Med.* 202, 445–455; 2005). This made it an enticing candidate for treating autoimmune diseases such as rheumatoid arthritis and type-1 diabetes. The researchers hoped that TGN1412 could make immunosuppressive cells soothe sites of overinflammation, while the rest of the immune system carried on as usual.

It is still not clear why, when TGN1412 affected only regulatory T cells in animals, the same almost certainly did not occur in the human trial. It is likely that in humans the super-antibody activated helper T cells en masse, triggering a cytokine storm.

"We were shocked and surprised to see what happened in humans," Hünig told *Nature*. In preclinical trials, monkeys got a dose 500 times that given to the human volunteers, and the monkey CD28 receptor is identical to the human one, says Hünig. This means that the effects in the monkey trial should have been comparable.

One possible source of the difference between the animal and human trials is that the 'tail' of the antibody molecule at the opposite end from the CD28-binding site may not be the same in humans and monkeys. Antibody tails can undergo a process called crosslinking, which amplifies an immune

# Regulatorische Aktivitäten nach dem TGN1412 Zwischenfall

- April 2006: MHRA publishes interim measures for mAbs ([www.mhra.gov.uk](http://www.mhra.gov.uk))
- May 2006: UK Expert Scientific Group on Phase One Clinical Trials (ESGPOCT) meets for the first time
- May 2006: PEI publishes potential criteria for classification of high-risk compounds (Schneider, Kalinke, Löwer (2006): TGN1412 – A Regulator's perspective. **Nature Biotechnology** 24, 493-6.)
- July 2006: ESGPOCT publishes interim report ([www.dh.gov.uk](http://www.dh.gov.uk))
- July 2006: French AFSSAPS publishes concept paper ([www.afssaps.sante.fr](http://www.afssaps.sante.fr))
- Dec 2006: ESGPOCT publishes **Final Report**
- Dec 2006: PEI starts drafting an internal "high-risk IMP" SOP
- Dec 2006: PEI approves first high-risk IMPD according to new SOP
- Jan 2007: EMEA announces
  - CHMP guideline on First-in-Man Clinical Trials for Potential High-Risk Medicinal Products
- March 2007: PEI formally implements internal SOP
- Sept 2007: **Guideline on Strategies to Identify and Mitigate Risks for First-in-Human Clinical Trails with Investigational Medicinal Products**

# TGN1412—a regulator's perspective

To the editor:

As your editorial in the April issue (*Nat. Biotechnol.* 24, 368, 2006) highlighted, the tragic and unexpected severe adverse events in the recent phase 1 clinical trial of the agonistic anti-CD28 antibody TGN1412 illustrate the risks inherently associated with first-in-human studies of new monoclonal antibodies (mAbs). For reasons of data reproducibility and better estimation of safety margins, phase 1 trials are often carried out in healthy volunteers. The adverse effects associated with the TGN1412 phase 1 trial indicate that the predictive value of animal models requires re-evaluation and that, in certain cases, standard clinical protocols may need refinement or redesign. As researchers at the Paul Ehrlich-Institut (PEI) in Langen—the German regulatory authority that had approved a trial protocol similar to the one approved and carried out at London's Northwick Park Hospital in the United Kingdom on March 13—we provide here our perspective on the TGN1412 case and the likely consequences for future regulatory oversight of mAbs. Even though the exact reasons and the underlying mechanisms associated with the severe acute adverse responses to TGN1412 are still under evaluation, it is clear that the scientific and regulatory principles for mAb development and approval of clinical trials require re-evaluation. We propose the introduction of three new definition criteria for mAbs entering the clinic to ensure that the next generation of new and very potent immunomodulatory mAbs is developed at the lowest possible risk to human subjects.

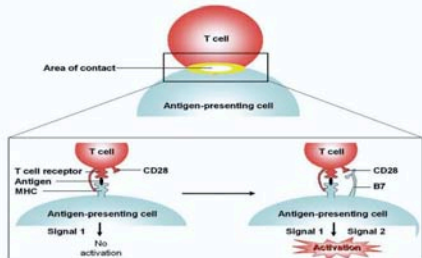
In any drug development program, moving from preclinical animal testing to clinical trials in humans is a critical juncture. New drugs are either studied in patients who might profit from the treatment or in healthy volunteers. Testing of healthy adults, usually male subjects, is expected to yield more reproducible results than studies in patients because disease-related and other treatment-related factors can highly influence the pharmacokinetics and pharmacodynamics of the new drug under study and thus could affect the estimation of safety of use. In

particular, for immunomodulatory and/or immunosuppressive therapeutics, it is often argued that a healthy immune system is less vulnerable to therapeutic interference because of functional physiological compensation mechanisms and therefore the risk of experiencing unexpected effects is reduced.

For biologic therapeutics, the classic principles of pharmacology have to be reconsidered and applied case by case. For mAb therapeutics, pharmacokinetics is also a function of antigen dose and can therefore largely be influenced by the pathological state. A case in point is Xolair (omalizumab), an anti-IgE mAb for the treatment of severe bronchial asthma, the pharmacokinetics of which is strongly influenced by IgE levels in the patient<sup>1</sup>. As a result of these factors, data

from healthy volunteers might be of less relevance and not always easily transferred to patients with the target indication. With the development of new generations of mAbs that are highly specific for distinct (conformational) target epitopes and that therefore have a highly specific mechanism of action, the biological activity and efficacy in the immune system of a healthy volunteer might be entirely different from that in the pathologically altered immune system of a patient. Nevertheless, up to now, studies in healthy volunteers have been an integral part of the clinical development program for many products.

Not all types of mAb products undergo phase 1 safety testing in healthy volunteers. For example, the cytotoxic mAbs that are



**Figure 1** Antigen-presentation and T-cell activation. The T cell receives a co-stimulatory signal 2 (the corresponding antigen structure, on the T cell side of the antigen B7 (CD80 and CD86) complex results in a high co-stimulatory signal. T-cell activation (triple proliferation), thus can

only occur if a strong co-stimulatory signal 2 is provided. Issues were raised during the assessment procedures and after these were satisfactorily addressed by TIGene, both the MHRA and PEI approved the protocol independently. As the protocol was approved three weeks later by the PEI than by the MHRA, TIGene pressed ahead with their phase 1 trial in the United Kingdom rather than Germany.

A battery of preclinical data, both on efficacy and safety, is requested by regulators before a drug can proceed to testing in humans. Regulatory guidelines from the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) and guidelines issued by the European Committee for Medicinal Products for Human Use (CHMP) are available that provide clear guidance for the preclinical development of new medicines. They were implemented into the regulatory framework to provide grounds for a high safety standard in the development of new medicines. For biologics, study requirements are assessed on a case-by-case basis<sup>2</sup>.

The requirements concerning the nature and timing of nonclinical studies with respect to clinical trials include anticipated duration and exposure to the study drug, its characteristics, the target disease, the route of administration and the intended future use in special populations such as women of child-bearing potential<sup>3</sup>. For first studies in humans, the nonclinical studies should allow an adequate determination of dose, taking into account nonclinical pharmacokinetic, pharmacological and toxicological evaluations. Usually, in the EU, repeated dose toxicity studies in two species (one non-rodent) for a minimum duration of two weeks are required to support a single, first human dose<sup>4</sup>. In this respect, the choice of an adequate nonclinical model system is central for biologics and especially for mAbs. Nonclinical safety pharmacology and toxicological studies should be performed in a relevant animal species, and nonclinical studies in species where the biologic has no pharmacodynamic effect are considered irrelevant and are usually not required. For mAbs, a relevant model is a species where a homologous target structure is expressed. Such homology should include not only primary structure (amino acid sequence homology) but also homology of domain organization and pharmacodynamic effects and comparison of the binding affinity of the mAbs in the model species with the

lines, transgenic models expressing the human antigen or surrogate models. For these last models, a surrogate mAb of animal origin recognizing the nonhuman analog antigen in that particular species is tested, usually murine mAbs in mice or rats.

Given the fact that, in the case of TGN1412, all of these tests failed to anticipate the severe systemic inflammatory responses observed in the six human subjects receiving the drug, what can regulators learn from these events? First, TGN1412 is a 'first-in-class' product that had never been tested in humans before the UK trial. In this light, and given the lack of clinical precedent, it is preferable that similar products should be studied sequentially, at least for the first dose. In other words, the classic 'cohort' design, where a cohort of patients is tested at the same time so as to study different dosing steps within the dosing escalation strategy, requires modification.

Second, despite the use of a battery of murine, nonhuman primate studies and even ex-vivo human cell assays, the immunological models used in TGN1412 preclinical testing were of insufficient predictive power to anticipate the serious adverse events in humans. Regulators thus are likely to require further scientific clarification of the relevance of such preclinical models in future, even if the sequence homology of the drug target is very high and a close evolutionary relationship exists between the species. This is likely to require more detailed research concerning the differences in the immune systems of human and nonhuman primates.

As mAbs expand into new indications and address new targets with novel biologics, regulators are likely to be increasingly stringent concerning the standards and the amount of preclinical data required to allow a molecule to proceed into human testing, especially for mAbs that stimulate or modulate the immune system through mechanisms that are novel or show human-specific properties. At present, mAbs currently (or soon to be) on the market target approximately 15 proteins (including vascular endothelial growth factor and its receptor, epidermal growth factor receptor, tumor necrosis factor  $\alpha$ , CD32, CD33, CD11a, CD20, CD25, CD4, integrin and I $\beta$ )<sup>5</sup>. However, a profusion of new targets is being explored, and experts estimate that currently more than 150 new mAbs are being developed. What's more, the next generation of mAb therapies is likely to target highly restricted epitopes or even subregions of drug targets, for which no

implemented to define high-risk mAbs that require extended preclinical development before human testing and for which a sequential phase 1 design might be the only feasible option.

**Criterion 1. The mAb employs a new mechanism of action.** This would include the following two antibody types: first, mAbs interfering with 'master switches' of the immune system, such as CD28 or CTLA-4; and second, mAbs that act as inducers and/or modulators of pleiotropic cytokines, including pro-inflammatory and anti-inflammatory ones, such as interferon- $\gamma$ , interferon- $\alpha$  and IL-10.

**Criterion 2. The mAb addresses a target that lacks appropriate animal models.** Relevant therapeutics would include the following three antibody types: first, mAbs binding (sub)epitopes that are present only in humans; second, mAbs for which no surrogate model exists; and third, mAbs interfering with signaling pathways that show human-specific properties.

**Criterion 3. The mAb comprises a new type of engineered structural format.** This would include the following two antibody types: first, engineered Fc parts; and second, divalent antibodies and other new constructs.

For these 'high-risk' products, data obtained from related mAb products will clearly not be sufficient for regulatory decisions (aside from class effects) each product will need to be assessed on a case-by-case basis. At the same time, regulators will have to balance their concerns with practical considerations: for example, animal models of certain mAb targets may be extremely difficult, or even impossible, to develop. A case in point is the difficulty of generating a model for bispecific antibodies with altered Fc parts that are intended to interact with certain human Fc receptor polymorphisms. At least in part, such scenarios are already the reality, as monospecific mAbs with engineered Fc parts and bi-/tri-specific mAbs are under development.

It should be emphasized that the TGN1412 case, although a human tragedy for the volunteers and their families, should not lead to the conclusion that mAbs are 'high-risk' drugs *per se*. There are even examples of mAbs that influence other 'master' switches in the immune system: products targeting tumor necrosis factor- $\alpha$ , such as Remicade (infliximab), Enbrel (etanercept) and Humira (adalimumab), have all been approved; others targeting CTLA-4, such

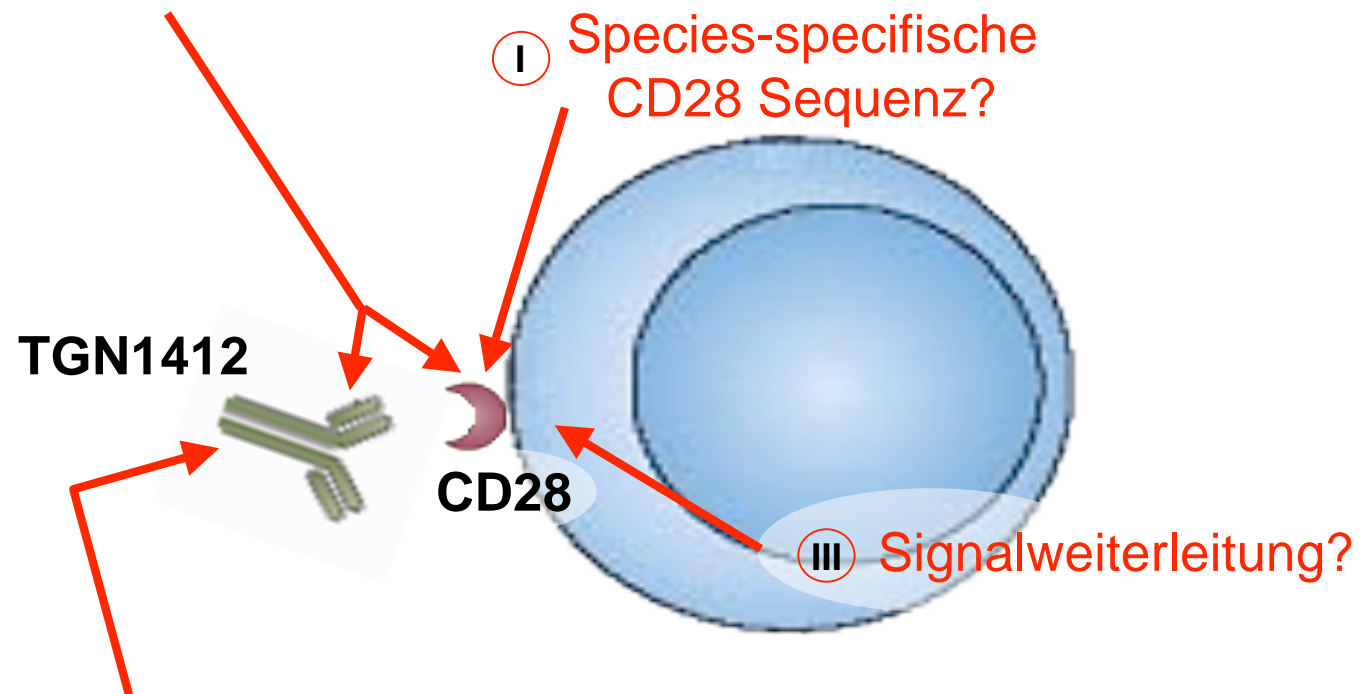
**Criterion 1. The mAb employs a new mechanism of action.** This would include the following two antibody types: first, mAb interfering with 'master switches' of the immune system, such as CD28 or CTLA-4; and second, mAbs that act as inducers and/or modulators of pleiotropic cytokines, including pro-inflammatory and anti-inflammatory ones, such as interferon- $\gamma$ , interferon- $\alpha$  and IL-10.

**Criterion 2. The mAb addresses a target that lacks appropriate animal models.** Relevant therapeutics would include the following three antibody types: first, mAbs binding (sub)epitopes that are present only in humans; second, mAbs for which no surrogate model exists; and third, mAbs interfering with signaling pathways that show human-specific properties.

**Criterion 3. The mAb comprises a new type of engineered structural format.** This would include the following two antibody types: first, engineered Fc parts; and second, divalent antibodies and other new constructs.

# Ursachenforschung

II Rezeptorbesetzung?



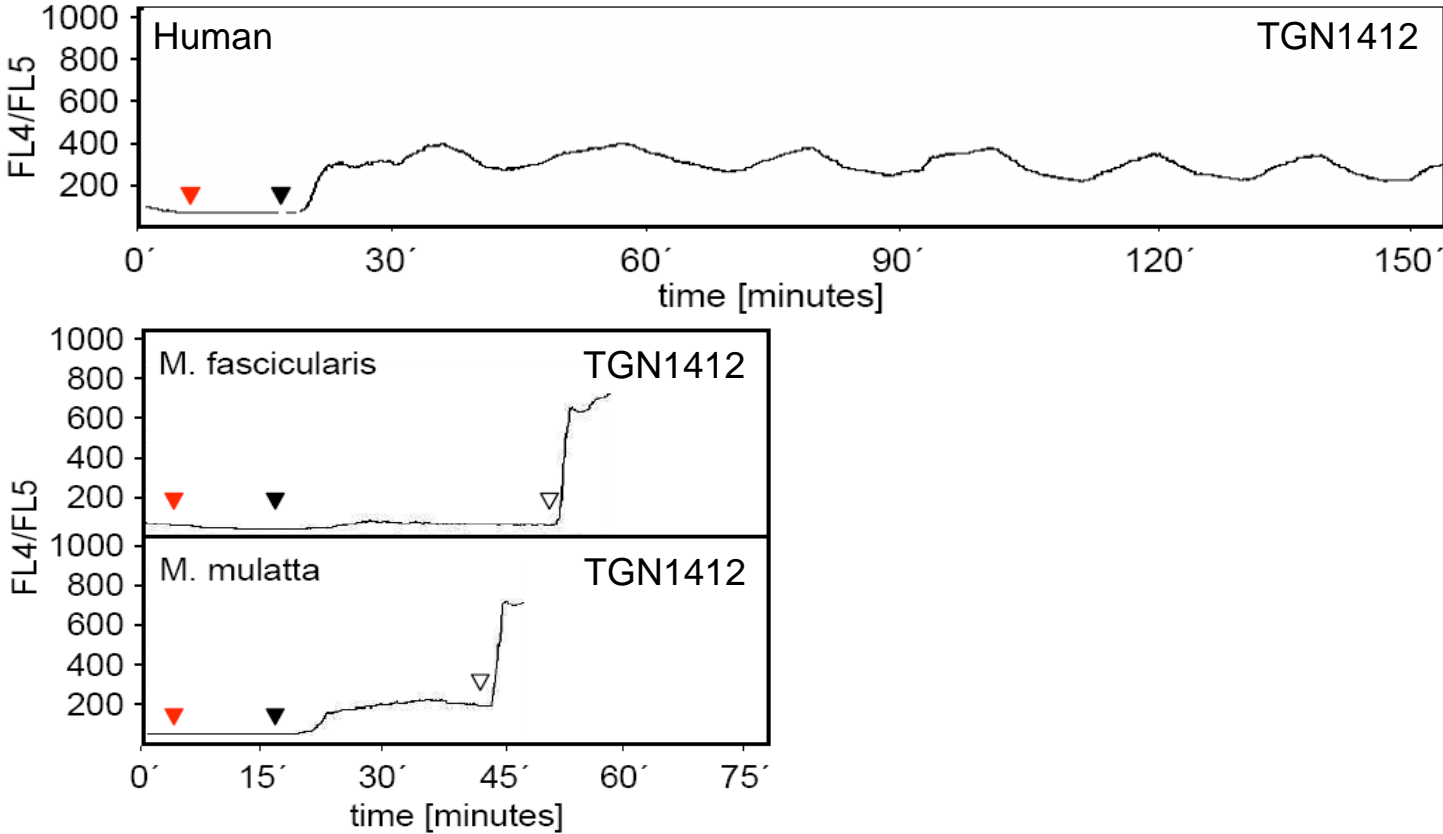
IV FC Rezeptor-vermittelte Effekte?

# Sequenzvergleich von CD28 im Menschen und in *M. fascicularis* und *M. mulatta*

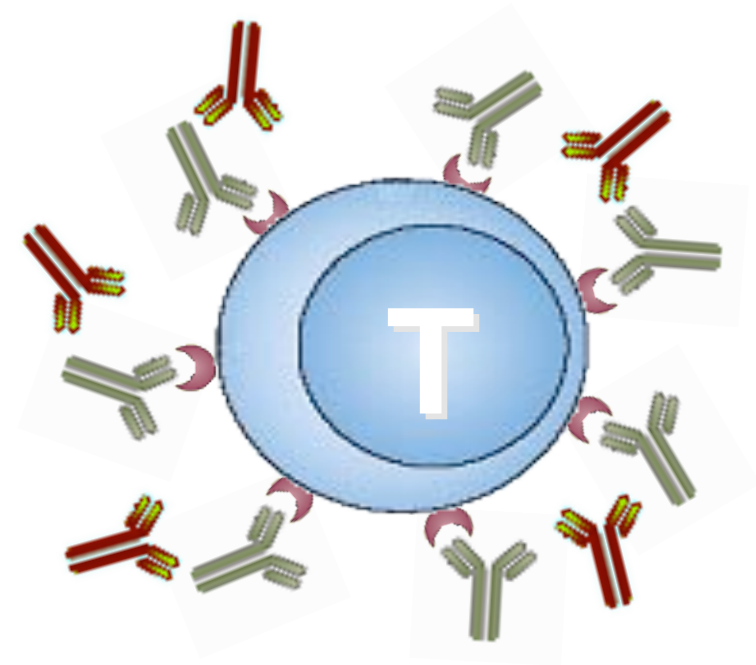
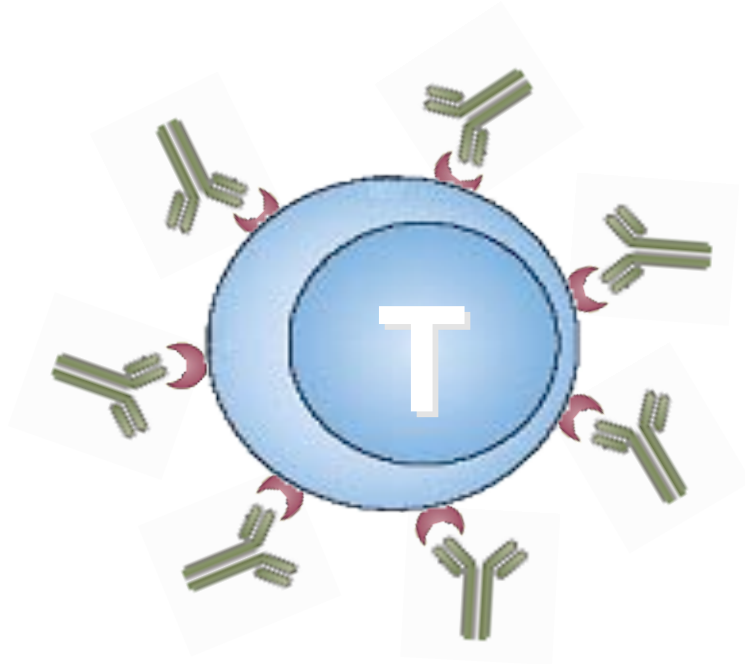


H. sapiens	1	MLRLLALNLFPSIQVTGNKILVKQSPMLVAYDNAVNLSCKYSYNLFSREFRASLHKGLD	
M. fascicularis	1	-----L-----	
M. mulatta	1	-----L-----	
<hr/>			
H. sapiens	61	SAVEVCVVYGNYSQQLOVYSKTGFNC DGKLGNESVTFY LQNLV NQTDIYFCKIEVMYPP	
M. fascicularis	61	-----	
M. mulatta	61	-----	
<hr/>			
H. sapiens	121	PYLDNEKSNGTIIHVKGKHLCPSP LFPGPSKEFWVLVVVGGVLACYSLLVTVAFIIFWVIR	
M. fascicularis	121	-----A-----C-----M-----	
M. mulatta	121	-----A-----C-----M-----	
<hr/>			
H. sapiens	181	SKRSRL LHSDYMNMTPRRPGPTRKH YQPYAPPRDFAAYRS	220
M. fascicularis	181	-----	220
M. mulatta	181	-----	220

# T-Zell Aktivierung funktioniert im Affen anders als im Menschen



# In der Kulturschale hat TGN1412 alleine keine Wirkung!



Proliferation

# Sollte man „die Finger“ von potenten Produkten lasse

<b>Generic Name</b>	<b>Trade Name</b>	<b>Structure</b>	<b>Target</b>
TGN 1412	-	Humanized IgG4	CD28
MDX-010	-	Fully human IgG1	CD152 (CTLA-4)
Ticilimumab	-	Fully human IgG1	CD152 (CTLA-4)
Abatacept (CTLA-4-Ig, BMS-188667)	Orenica	Fusion protein with extracellular CTLA-4 domain	CD80/CD86 (B7.1/B7.2)
Belatacept (BMS-224818)	-	Higher affinity mutant of Abalaccept	CD80/CD86 (B7.1/B7.2)
Muronomab	OKT3	Mouse IgG2a	CD3
TNX-355	-	Humanized IgG4	CD4
Daclizumab	Zenapax	Humanized IgG2a	CD25 (IL-2R)
Basiliximab	Simulect	Chimeric IgG1	CD25 (IL-2R)
-	Cyclosporin	Cyclopeptid	IL-2
-	Tacrolimus	Macrolide	IL-2
-	Rapamycin	Makrozyklisches Peptid	IL-2R

# Konsequenzen und Herausforderungen

- Die Relevanz eines vorklinischen Tiermodells muss intensiv hinterfragt werden.
- Nicht-humane Primaten sind nicht mehr als der “goldene Standard” anzusehen  
→ Gibt es ein anderes Tiermodell, das grundsätzlich eingesetzt werden kann, sind “humanisierte Mäuse” dafür geeignet?
- Die Kultruschale ist kein Ersatz für Tierexperimente!
- Falls die Relevanz eines Vorklinischen Tiermodells nicht belegt werden kann, ist es möglich, auch mit gering dosierten Produkten zu arbeiten → Auf welcher Basis soll eine Dosisescalation vorgenommen werden?

# Danksagung



## Division of Immunology

### 3/0 Research

Zoe Waibler

Linda Sender



### Burkhard Schraven

Otto-von-Guericke-Universität

Magdeburg

Germany

### 3/1 Immune Chemistry

Siegfried Giess

Stefan Christians



### Hartmut Hengel

Heinrich Heine Universität

Düsseldorf

Germany

### 3/2 Monoclonal and Polyclonal Antibodies

Christian Schneider

Jan Müller-Berghaus

Bernd Liedert

Leopold Löwer (Präsident DEK)

