Immunotoxicology of Biopharmaceuticals

Preclinical and Translational Perspectives

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Global Discovery Pathology | Translational In-vivo Models Research Platform

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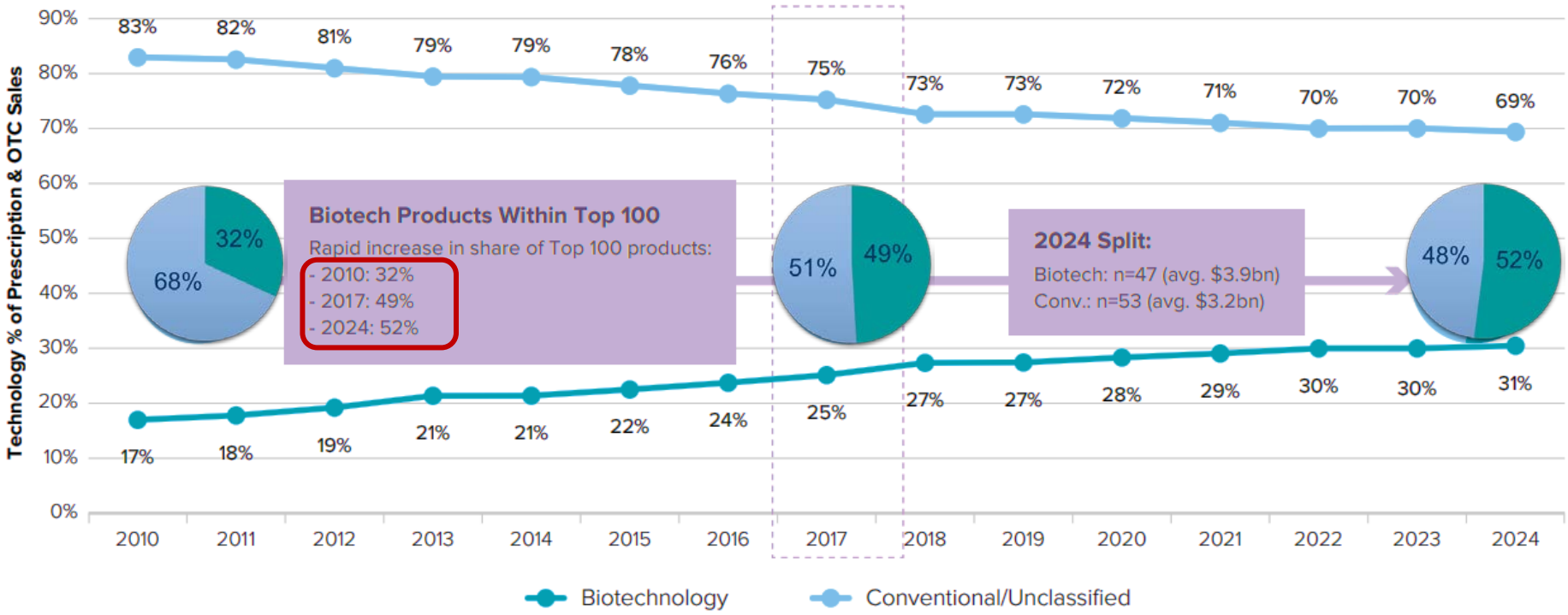
STP-I Symposium October 26-28, 2018

Worldwide Prescription Drug & OTC Pharmaceutical Sales: Biotech vs. Conventional Technology

Source: Evaluate, May 2018



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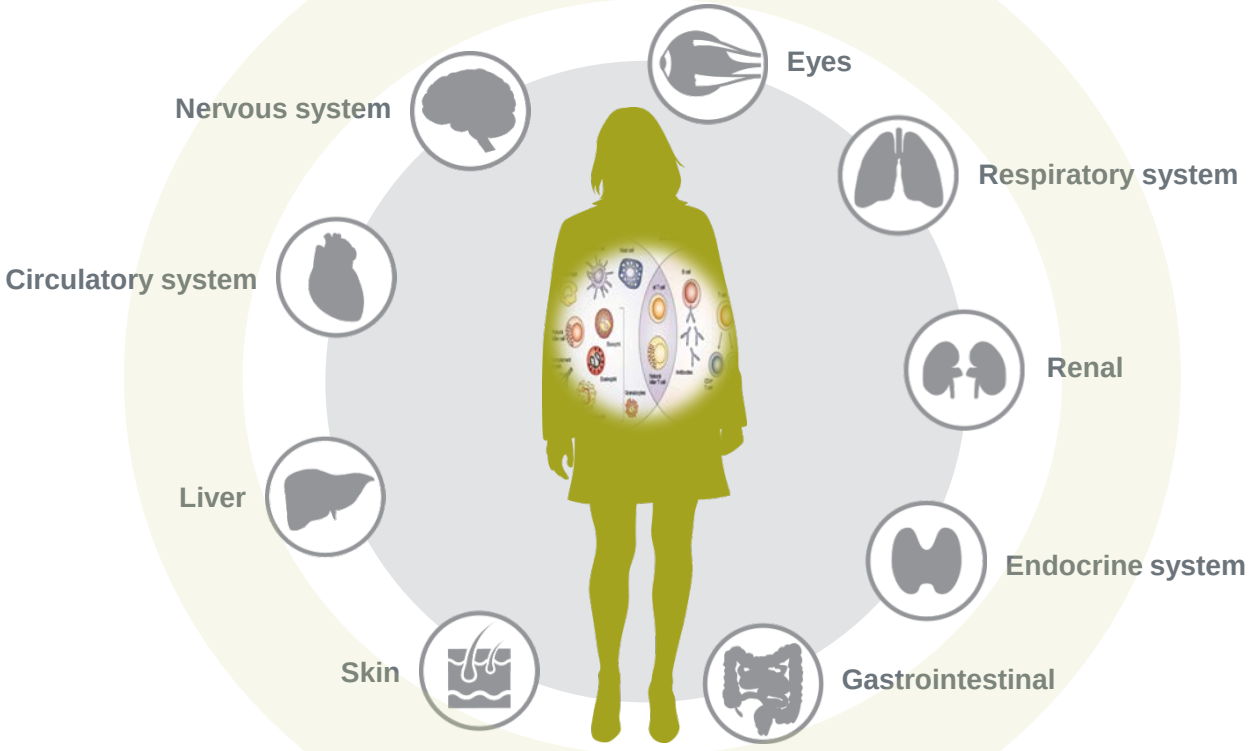
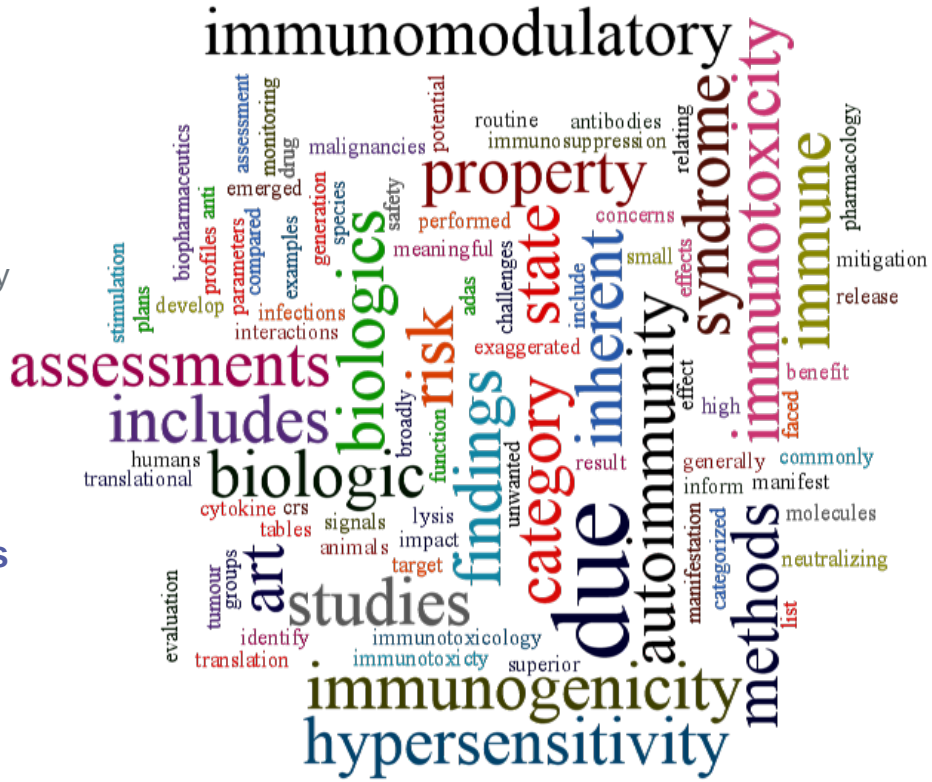
Multisystem Interactions



Outline

1. **Biopharmaceuticals**
   - Large molecules vs. small molecule drugs
2. **Immunotoxicology**
   - Specialized branch of toxicology; studies immunotoxicity
3. **Immunotoxicity of Biologics**
   - On target vs. off target effect? Exaggerated pharmacology
   - Example of immunotoxicity in the clinics
4. **Approaches to Immunotoxicity Assessment**
   - Nonclinical studies
   - Regulatory guidance
   - Immunotoxicity assays
5. **Translational Implications of Immunotox findings in Nonclinical Studies**
   - Concordance between nonclinical and clinical studies
6. **Examples and Case Studies**



Take Home Points

1. Biopharmaceutical drug development is complex and entails many challenges including immunotoxicity; novel biologic targets and innovative (e.g. multi-specific) formats warrant ***tailor-made*** **nonclinical development strategies**

2. Immunotoxicity of biopharmaceuticals may either be related to **exaggerated pharmacology** or **an off target** effect

3. Immunotox assessment of biologics is undertaken on a **'case-by-case' basis using weight of evidence** from standard tox studies and as per regulatory guidance

4. Nonclinical in-vivo/ex-vivo immunotox studies are helpful for identifying the human safety risk of the biopharmaceuticals, but require critical understanding of the target, species relevance for both pharmacology and toxicology, and **multi-disciplinary approach to data analysis and integration**

5. **Toxicologic pathologists have a critical role** in discussion of translatability of nonclinical immunotox findings / Concordance of nonclinical studies and clinical studies

Biopharmaceuticals or Biologics

Drug products manufactured in, extracted from or semi-synthesized from **biological sources**, or produced by **biotechnology/ recombinant DNA technology**

- **Types of biologics:**
  1. Homologs of endogenous proteins (e.g. growth factors, cytokines, enzymes)
  2. Monoclonal antibodies
  3. Receptor constructs (fusion proteins)
  4. Vaccines
  5. Cell based
  6. Nucleic acid based
  7. Viral based

Biologics vs. Small Molecule Drugs

Size & Complexity

	Small molecule drug	Large molecule drug	Large biologic
Size	Aspirin 21 atoms	hGH ~3,000 atoms	IgG antibody ~25,000 atoms
Complexity	Bike 20 lbs	Car ~3,000 lbs	Business jet ~30,000 lbs (without fuel)

Small molecule drug	Biologic
Low molecular weight	**High molecular weight**
Familiar antecedents	**Potentially unique**
Known impurities	**Unfamiliar impurities**
Often orally dosed	**Often parenteral, IV dosing**
Maximal tolerated dose	**Optimal biologic dose**
Meaningful chronic tox	**Uncertain chronic tox**
Species-independent	**Species-specific**
Biotransformed	**Degraded**
Not immunogenic	**Immunogenicity issues**

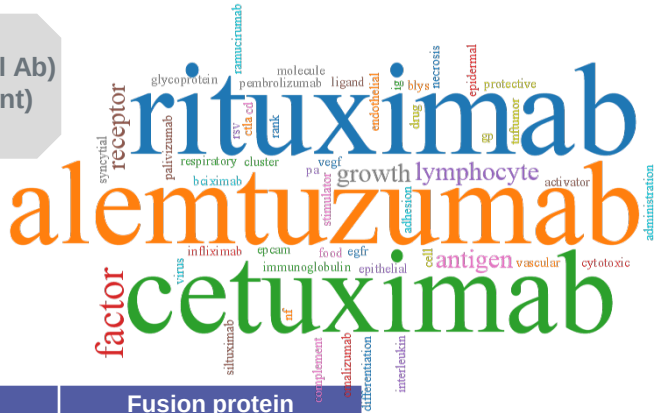
https://www.azbio.org/small-molecules-large-biologics-and-the-biosimilar-debate

What's in a Name?

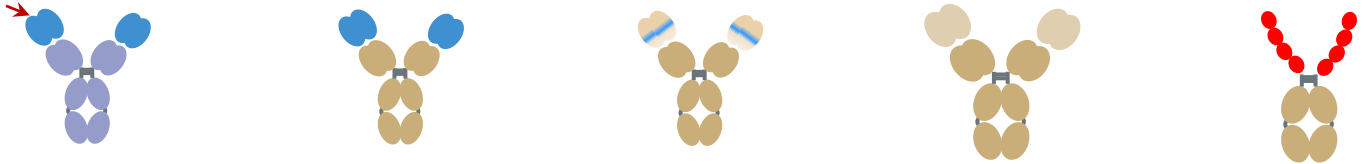
The case of mAbs

Prefix	Fragment target indicative	Fragment origin indicative	Suffix
FREE-	-TU- or -TA- (Tumors) -LI- or -LIM- (Immune syst) -CI- (Cardiovascular) -DE- (endocrine)	-O- (Mouse) -XI- (Chimeric) -ZU- (Humanized) -U- (Human)	-MAB (Monoclonal Ab) -CEPT (Ab fragment)

INO	LI	MO	MAB
RI	TU	XI	MAB
TRAS	TU	ZU	MAB
ADA	LI	MU	MAB



Murine -omab	Chimeric -ximab	Humanized -zumab	Human -umab	Fusion protein -cept



WHO Programme on International Nonproprietary Names (INN)
https://www.who.int/medicines/services/inn/Revised_mAb_nomenclature_scheme.pdf

Safety of Biologics

- **Biologics are considered 'safer'**
  - High molecular specificity
  - Low off target promiscuity
  - Low susceptibility for biotransformation (*usually degraded to constituent amino acids- very low risk of phospholipidosis, lipid peroxidation, mitochondrial toxicity, genotoxicity*)
  - Human or humanized mAbs have similarity to endogenous human proteins (*low or no immunogenicity*)
- **However, many biologics:**
  - Are **immunomodulatory** by nature
  - Have **immunogenicity** issues
  - Impact the **immune system**
  - Have known **adverse effects**

Reslizumab (Cinqair®)

CINQAIR (reslizumab) injection

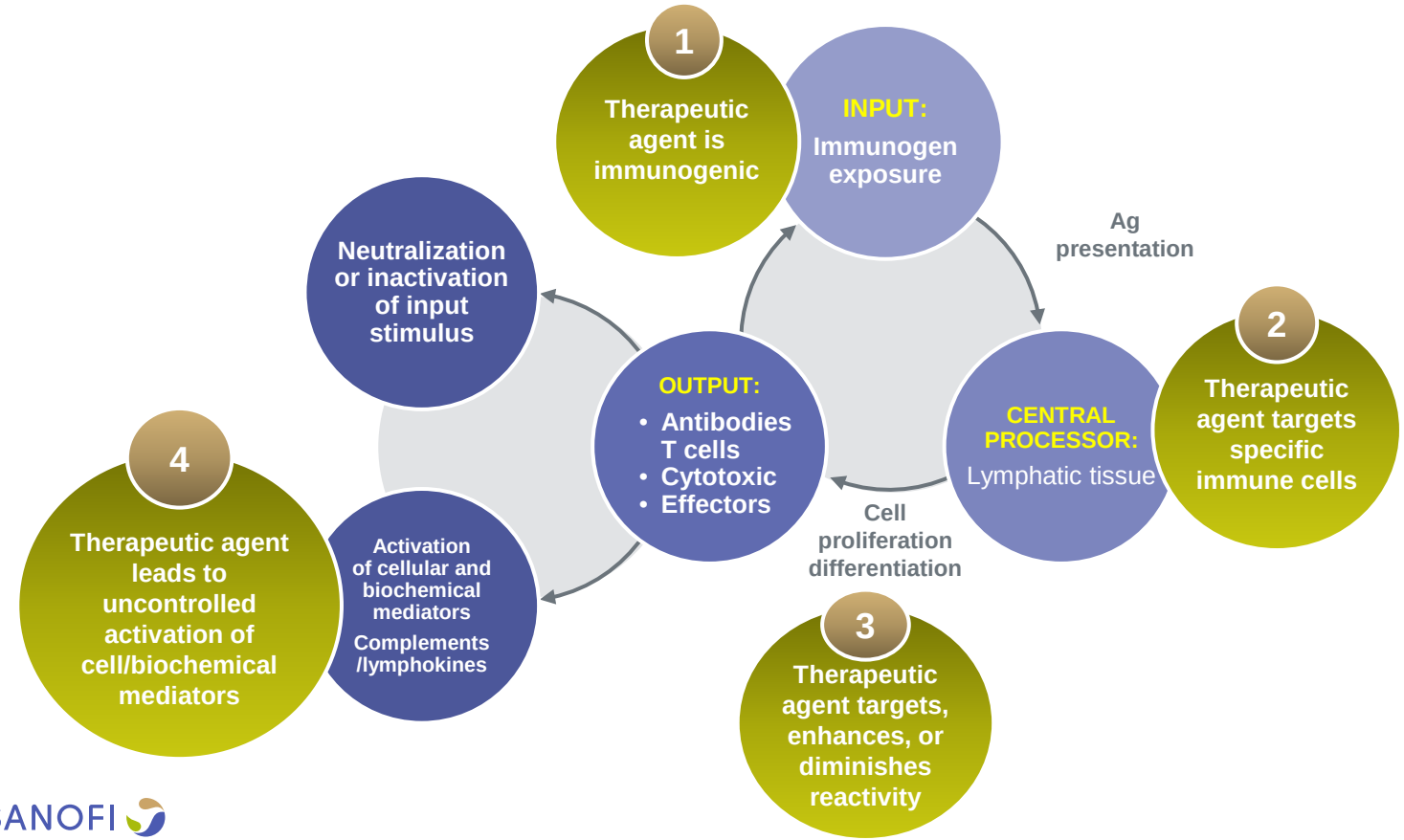
- **Black Box Warning:** Anaphylaxis
  - Occurred in 0.3% of patients in placebo-controlled studies
- **Adverse Effects:** Anaphylaxis, malignancies, increases in creatine phosphokinase (transient), and antibody development
- **Interactions:** No known significant interactions

Daclizumab (Zinbryta®)

Zinbryta™ (daclizumab)

- **Black Box Warning:**
  - Hepatotoxicity including autoimmune hepatitis
  - Other immune-mediated disorders
  - Systemic corticosteroids for treatment of autoimmune hepatitis or immune-mediated disorders
  - Only available through ZINBRYTA REMS Program secondary to risk of hepatic injury
- **Adverse Events:** Skin reactions, autoimmune disease, and infection

Immune System and Immunotoxicants



Immunotoxicology

Immunotoxicology

The study of immune dysfunction resulting from exposure of an organism to a xenobiotic

Immunotoxicity

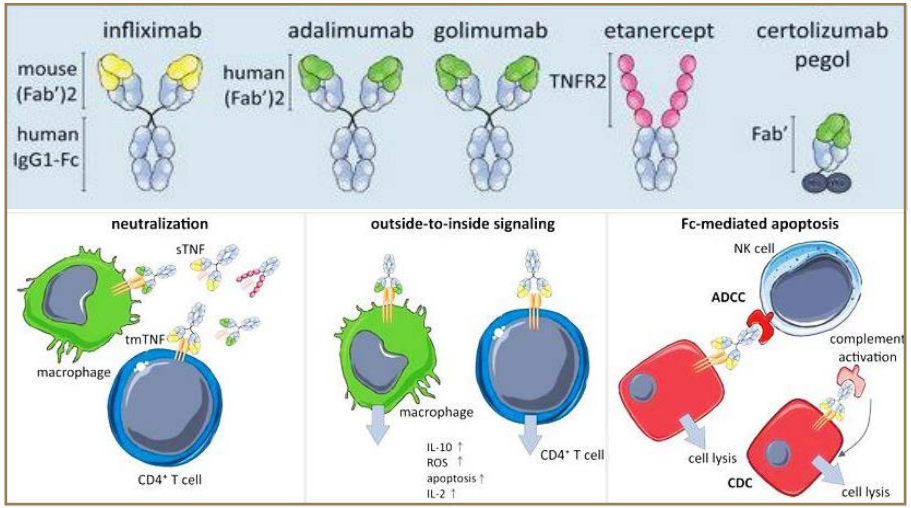
Adverse effect/s on the structure or function of the immune system, or on other systems as a result of immune system dysfunction

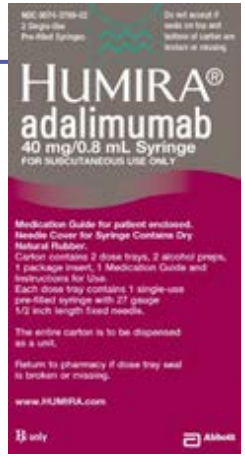
Immunotoxicity of Biologics

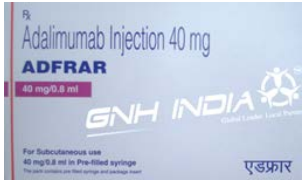
Immunosuppression			Immunostimulation				Immunomodulation	
Reduced immune response Decreased host resistance			**Hypersensitivity**				Enhanced innate response **(Cytokine Storm)**	**Autoimmunity**
Increased infections	**Opportunistic infections**	**Tumors**	**Type I-IV hypersensitivity**				**Immune related adverse events**	
May be exacerbated by age, poor nutrition, stress etc.			Anaphylactic/ atopic reactions **(type I)** **IgE**	Phagocytic **(type II)** **Complement, IgG and IgM**	ICD **(type III)** **Immune complexes, ADA + IgG and IgM**	Granulomatous **(type IV)** **T cell reactions**	**Superagonism**	
			Type I and III are most common in tox studies ADA response in nonclinical tox studies is less relevant to humans					

Common Side Effects of Anti-TNF-a mAbs

- Infusion reaction
- Reactivation of T.B.
- Upper respy. tract infections, opportunistic infections
- Autoantibodies and lupus like syndrome
- Elevated liver enzymes







Adapted/modified from: Dogra and Khullar (2013), Ind J Derm Vener Lepr 79(7):35-46

Autoimmune Diseases Induced by Biologics

A review of 12,731 cases

- **~13000 cases** of autoimmune disease in patients exposed to biologics
- Most commonly in patients with rheumatic disease, cancer and IBD
- Over 50 different biologic induced systemic and organ-specific autoimmune disorders
- Most frequent disorders: **psoriasis (6375), IBD (845), CNS demyelination (80), Interstitial lung dz. (519) and lupus (369)**
- Heterogeneous (**paradoixical**) pharmacologic scenario
- Unclear etiopathogenesis

EXPERT OPINION ON DRUG SAFETY, 2017
VOL. 16, NO. 11, 1255–1271
https://doi.org/10.1080/14740338.2017.1372421

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REVIEW

Check for updates

Autoimmune diseases induced by biological agents. A review of 12,731 cases (BIOGEAS Registry)

Marta Pérez-De-Lis[a], Soledad Retamozo[b,c,d], Alejandra Flores-Chávez[b,e,f], Belchin Kostov[g], Roberto Perez-Alvarez[h], Pilar Brito-Zerón[b,i] and Manuel Ramos-Casals[b,j,*]

Biologic	Molecules/Targets	Cases (n)
TNF-targeted	TNF	9133
B-cell targeted	CD20,CD59, CD19, CD22	741
Anti-cytokine therapies	IL6,IL2, IL12/13, IL1 etc.	285
Checkpoint inhibitors	CTL4, PD1	913
Growth factor targeted	EGFR	549
Other	integrin, CD11a, CD28, C5 etc.	60

A variety of diseases: Lupus, psoriasis, RA, Neutropenia, Thrombocytopenia, CNS demyelinating dz., IBD, ILD, uveitis, peripheral neuropathies,

Consolidated info from Tables 1 and 4

Perez-De-Lis et al. (2017) Expert Opin Drug Safety. 16(11):11255-1271

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Lymphoproliferative Diseases Induced By Biologics

- A larger single-center series of 72 cases of immunomodulatory agent-related lymphoproliferative disorders
- Life-long incidence of lymphoproliferative disorders post solid-organ transplant varies between 1 and 20%
- Similar lymphoproliferative disorders in other patients:
  - Immunosuppression therapy (e.g. autoimmune diseases),
  - Immunosuppression-related disorders (e.g. HIV infection),
  - Age-related immunosenescence

'Other Immunomodulatory agent-related lymphoproliferative disease'
(2017 revised WHO classification)

Classification	IS 42	IM 10	IM + IS 20	All cases 72
Non-Hodgkin lymphoma	35	3	6	44
Hodgkin lymphoma	2	2	2	6
Polymorphic B-cell LPD	3	2	3	8
Non-destructive LPD	2	2	8	12
Unclassifiable	0	1	1	2

Remicade (infliximab)	IM	Monoclonal antibody	Anti-TNFα	14
Humira (adalimumab)	IM	Monoclonal antibody	Anti-TNFα	9
Enbrel (etanercept)	IM	Monoclonal antibody	Anti-TNFα	4
anti-TNFα, not specified	IM	Monoclonal antibody	Anti-TNFα	2
Roactemra (tocilizumab)	IM	Monoclonal antibody	Anti-IL6R	2
Ilaris (canakinumab)	IM	Monoclonal antibody	Anti-IL1β	2
Entyvio (vedolizumab)	IM	Monoclonal antibody	Anti-integrin α4/β7	1
Kineret (anakinra)	IM	Recombinant RA	Anti-IL1R	1
Stelara (ustekinumab)	IM	Monoclonal antibody	Anti-IL12 and anti-IL23	1
Trocade	NA	Collagenase inhibitor	Selective inhibitor of matrix metalloproteinase-1	1

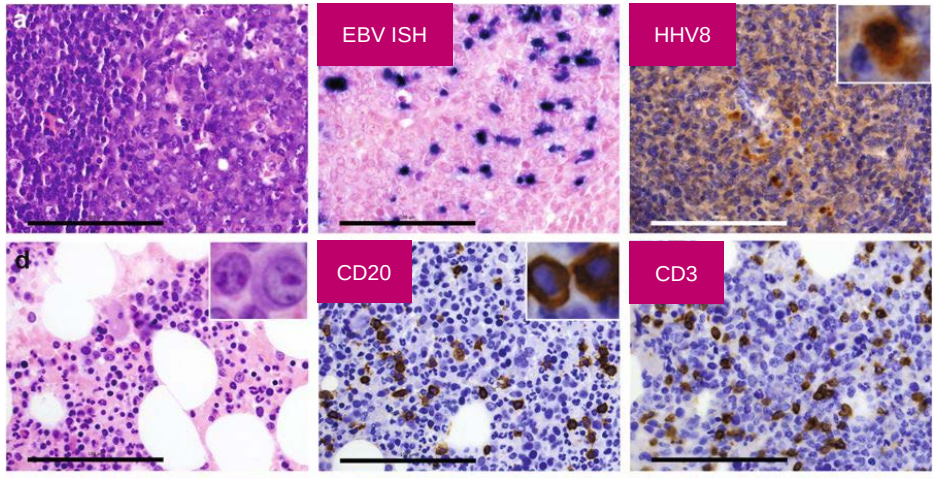
Marcelis et al. Modern Pathology (2018) 31:1457-1469

IS = immunosuppressive, **IM** = Immunomodulatory

Lymphoproliferative Diseases Induced By Biologics

Malignant cases were **more prevalent (88%)** in patients receiving **immunosuppressive** (IS) therapy than those receiving immunomodulatory (IM) therapy (43%)

Case 1	**24-year-old woman with Crohn's disease and SLE**	**Infliximab** for 7 years (+ Azathioprene for 9 years) *IM/IS therapy halted → Dz free at 1 year follow up*	LN biopsy Dx: Nondestructive EBV-driven lymphoproliferative disorder
Case 2	**51-year old woman with RA**	**Adalimumab** (was on IS therapy earlier) *IM therapy halted → Anti-CD20 therapy (Rituximab) → resolution of lesions*	BM biopsy DX: Hodgkin/Reed–Sternberg like B cells (CD20+/CD30+/EBV−) and increased T cells



Marcelis et al. Modern Pathology (2018) 31:1457-1469

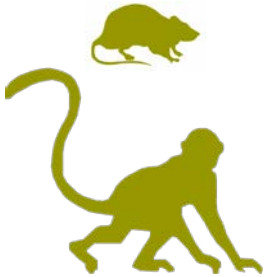
Approaches to Evaluate Immunotoxicity in Nonclinical Studies

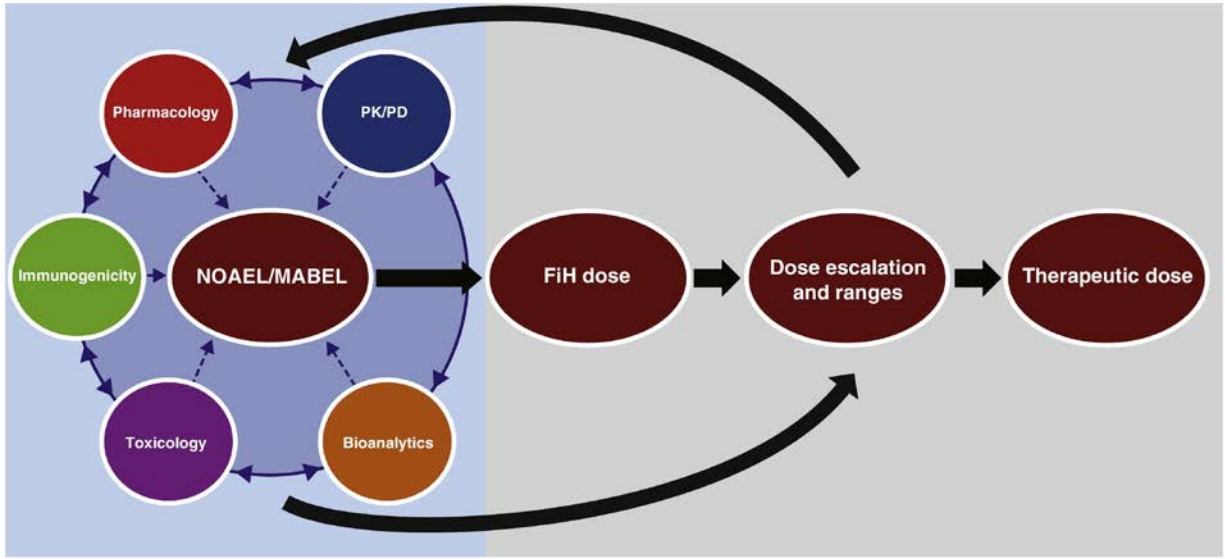
Nonclinical animal studies

Regulatory guidance

Immunotoxicity assays

Nonclinical Studies in Drug Development

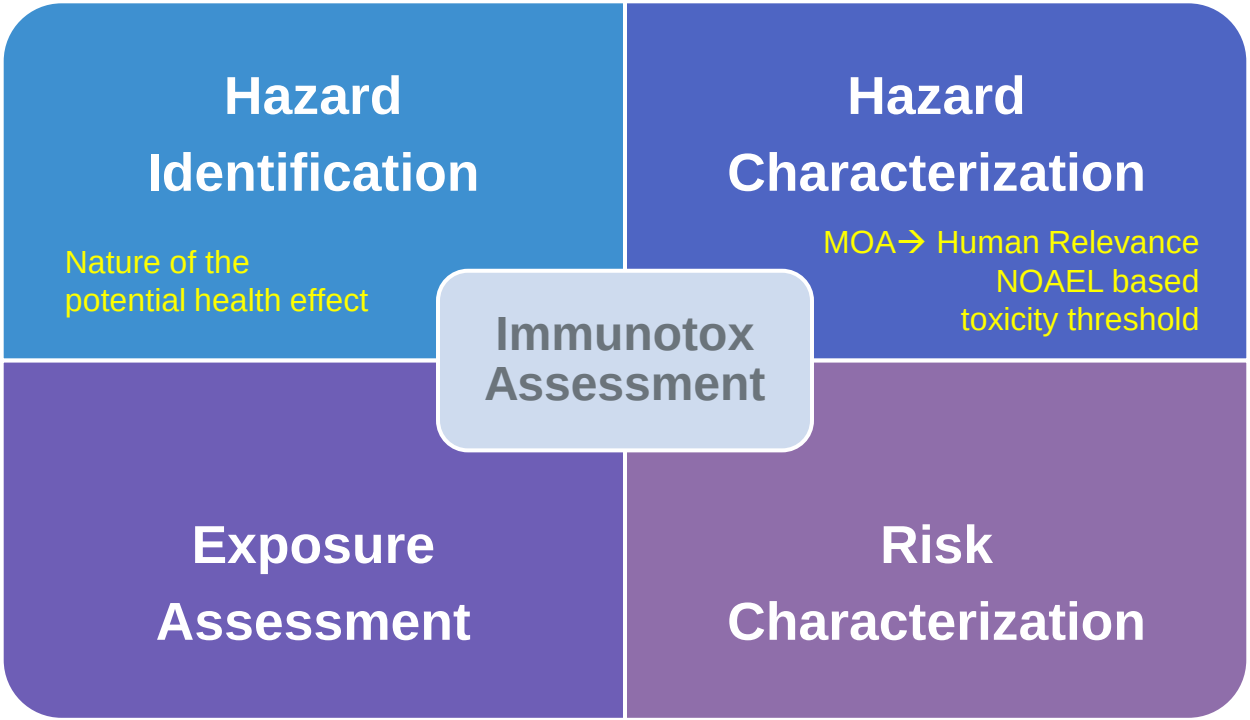




Nonclinical Studies

Drug Discovery Today: Technologies

Framework for Immunotoxicity Assessment



Nonclinical Animal Studies

- Based on regulatory guidance (**NEXT 2 slides**):
  - species selection, surrogate testing, 3R principle etc.

- **'Weight of Evidence'** for Immunotoxicity from Standard Tox Studies (Table below)

Parameter	Specific Component
Hematology	Total and absolute differential leukocyte counts
Clinical Chemistry	Globulin levers and A/G ratio
Gross Pathology	Lymphoid organs/ tissues
Organ Weights	Thymus, spleen (optional lymph node)
Histology	Thymus, spleen, draining lymph node and at least one additional lymph node, bone marrow, Peyer's patches, BALT, NALT

Regulatory Guidance

Guidance	Agency	Year
Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products for Human Use.	FDA	1997
MHLW/JPMA Draft Guidance for Immunotoxicity Testing	Japan	2001
Guidance for Industry: Immunotoxicology Evaluation of Investigational New Drugs	FDA	2002
ICH guideline S8: Notes for Guidance on Immunotoxicity Testing of Human Pharmaceuticals	EMA/FDA/Japan	2005
Guideline on Immunogenicity Assessment of Biotechnology-Derived Therapeutic Proteins	EMA/CHMP/BMWP	2008
ICH guideline M3 (R2): Nonclinical Safety Studies for the Conduct of Human Clinical Trials with Pharmaceuticals, Step 4	EMA/FDA/Japan	2009
ICH guideline S6 (R1): Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals (+ addendum)	EMA/FDA/Japan	2011 2012
Immunogenicity Assessment of Monoclonal Antibodies Intended for *In Vivo* Clinical Use	EMA/CHMP/BMWP	2012
Guidance for Industry: Immunogenicity Assessment for Therapeutic Protein Products	FDA	2014
First-in-man dosing and MABEL		
Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers	FDA/CDER	2005
Guideline on Strategies to Identify and Mitigate Risks for First-In-Human Clinical Trials with Investigational Medicinal Products	EMA/CHMP	2007

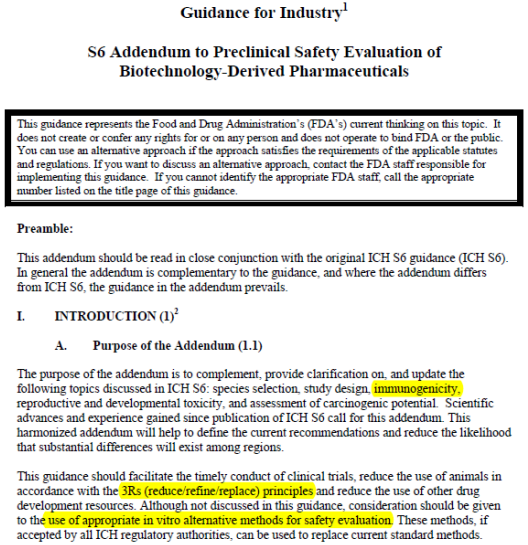
ICH S6(R1):
Preclinical Safety Evaluation of Biopharmaceuticals

- Tox studies may be performed only in **pharmacologically relevant** species
- When testing in two species is feasible, long-term studies may be performed in only one species
- If there are no cross-reactive species, homologous products may be tested as **surrogates** in animals
- If NOAEL not feasible, MTD may be used to guide clinical dose selection
- Immunogenicity (Ab response) in animals should be evaluated and correlated with PK/PD changes
- **For immunotoxicity assessment**, screening studies are recommended but routine tiered or standard testing batteries ARE NOT recommended
- Standard genotox and carc bioassaays are generally not needed
- **Tissue Cross Reactivity (TCR) Studies (*Immunohistochemistry*):**
  - Provide supplemental info on target distribution and potential unexpected binding
  - Human tissue panel assessed with the clinical candidate
  - TCR with animal tissues is not recommended
  - For multispecific Abs, study with individual components is not needed.

ICH S6 (R) emphasizes

3R

In vitro > Rodent > NHPs



Guidance for Industry[1]

S6 Addendum to Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals

This guidance represents the Food and Drug Administration's (FDA's) current thinking on this topic. It does not create or confer any rights for or on any person and does not operate to bind FDA or the public. You can use an alternative approach if the approach satisfies the requirements of the applicable statutes and regulations. If you want to discuss an alternative approach, contact the FDA staff responsible for implementing this guidance. If you cannot identify the appropriate FDA staff, call the appropriate number listed on the title page of this guidance.

Preamble:

This addendum should be read in close conjunction with the original ICH S6 guidance (ICH S6). In general the addendum is complementary to the guidance, and where the addendum differs from ICH S6, the guidance in the addendum prevails.

I. INTRODUCTION (1)[2]

A. Purpose of the Addendum (1.1)

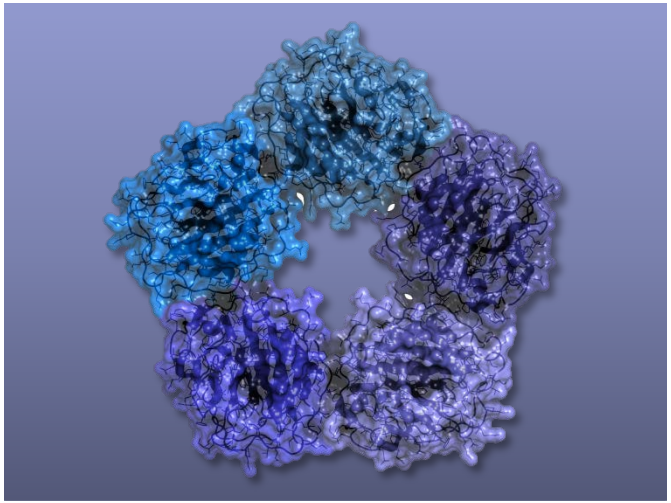
The purpose of the addendum is to complement, provide clarification on, and update the following topics discussed in ICH S6: species selection, study design, immunogenicity, reproductive and developmental toxicity, and assessment of carcinogenic potential. Scientific advances and experience gained since publication of ICH S6 call for this addendum. This harmonized addendum will help to define the current recommendations and reduce the likelihood that substantial differences will exist among regions.

This guidance should facilitate the timely conduct of clinical trials, reduce the use of animals in accordance with the 3Rs (reduce/refine/replace) principles and reduce the use of other drug development resources. Although not discussed in this guidance, consideration should be given to the use of appropriate in vitro alternative methods for safety evaluation. These methods, if accepted by all ICH regulatory authorities, can be used to replace current standard methods.

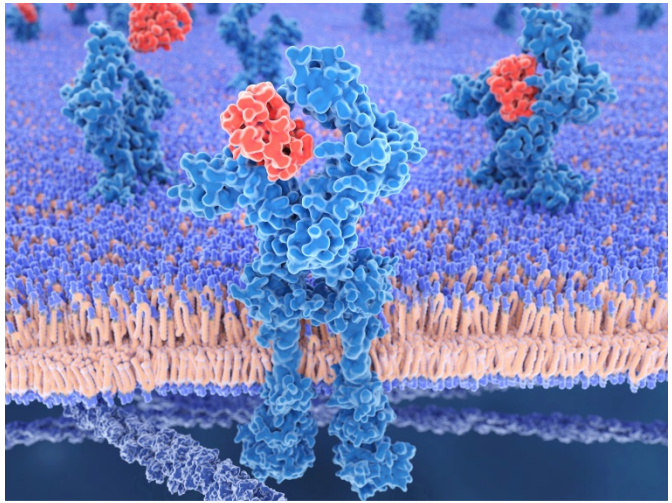
ICH S8:
Immunotoxicity Testing of Human Pharmaceuticals

- **Recommendations on nonclinical testing to identify compounds with immunotox potential**
- **'Weight of Evidence'** approach for immunotox testing decision
- **When to consider additional immunotoxicity studies?**
  - Findings from standard tox studies (Hematology, clin chem, organ weights, infection/tumors, histopathology)
  - Pharmacological properties of the drug
  - Intended patient population (immunocompromised, concurrent disease and/or treatment)
  - Structural similarity to known immunostimulators
  - Disposition of the drug (higher retention in immune system cells)
  - Clinical findings (observations in patients/subjects exposed to the drug)

Immunotoxicity Assays



Phenotypic/Biomarkers
Immunophenotyping
Immunohistochemistry (IHC)
Immunofluorescences (IF)
Biomarkers (e.g. cytokines)
Complement
ADAs (anti-drug antibodies)



Functional (*in vitro* and *ex vivo*)
Cytokine production/release
TDAR (T cell-dependent antibody response)
Cytotoxicity assays
Host resistance assays
Hypersensitivity/Allergy/Autoimmunity
Complement
Receptor occupancy (PK assessment)

Immunotoxicity Testing

Standard Toxicity Tests	Additional Assays for Immune Assessment
• Hematology • Clinical chemistry • Gross pathology • Immune organ weights • Histopathology	• Immunotox assays • Enhanced histopathology • Ancillary diagnostics (IHC, ISH)

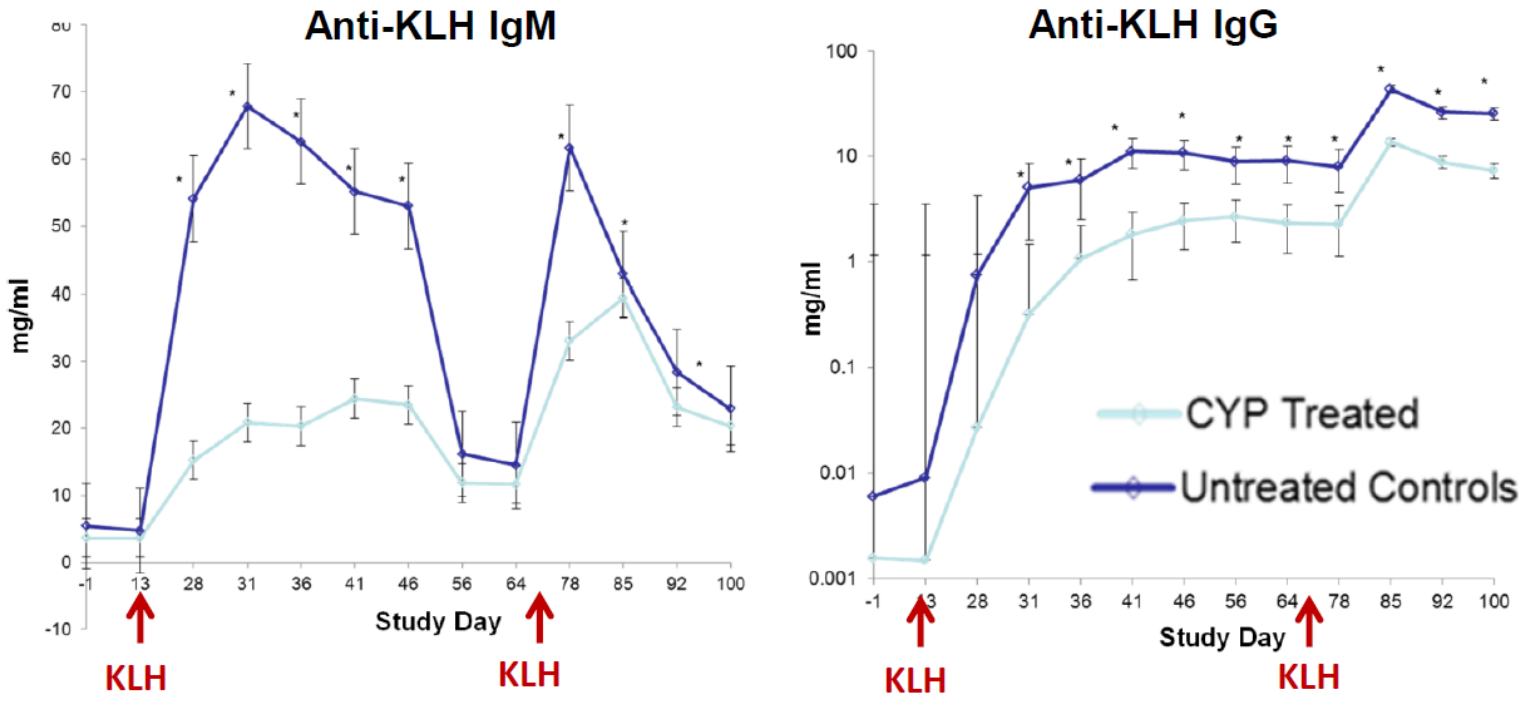
Immunotox assays

- TDAR
- Immunophenotyping
- Natural Killer Cell Activity Assays
- Host Resistance Studies
- Macrophage/Neutrophil Function
- Assays to Measure Cell-Mediated Immunity

Expanded Toolkit for Immunotox Evaluation

Immunotox manifestation	Assessment Methods		
	In-vitro	***In-vivo***	***Ex-vivo***
Immunosuppression	Human lymphocyte activation Acute phase protein and cytokine release	TDAR, TIAR **Host resistance** (to infection/s, implanted tumors)	Organ weights, histopathology, hematology
Immunogenicity	Assays to measure T cell, B cell responses	Effect on exposure, PD and endogenous protein level	ADA response and titer
Hypersensitivity	Drug specific IgE and *in-vitro* assays for biological activity	Clinical signs (e.g. skin lesions)	Coagulation parameters, ADA and circulating Immune complexes Histopathology and IHC (for immune complexes)
Adverse Immune Stimulation	Assessment of humoral and T cell responses	Cytokine release assessment	CRP, fibrinogen, histopathology (leukocyte infiltration in tissues; diffuse capillary leakage)
Autoimmunity	Ab response to self antigens	Popliteal lymph node assay	Histopathology

T-lymphocyte Dependent Antibody Response (TDAR)



KLH = Keyhole limpet hemocyanin, **CYP**= cyclophosphamide

Cytokine Release Assay

- ***In vitro*** human cytokine release assays remain a priority for hazard ID for cytokine release syndrome (CRS)
- Multiplex array Luminex® system which included detection of the following: IFN-γ, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, TNF-α, IL-12p70
- **Points to consider:**
  - Importance of using right control (LPS, known CRS-causing Abs)
  - Donor variability
  - Non-GLP
  - In-vitro is not physiologic
  - Concordance: NHP > Rat (less sensitive?)

The effect of assay platform

	mAb tested		
	Strong CRS association	Weak or rare CRS association	No CRS association
Assay Platform	• OKT3 • Alemtuzumab • CD28 super-agonist	• Rituximab • Trastuzumab	• Infliximab
Solid phase	Cytokines detected	Cytokines detected	Cytokines detected
High density PBMC Pre-culture	Cytokines detected	No cytokines detected	Not done
PBL-HUVEC co-culture	Cytokines detected	No cytokines detected	No cytokines detected

Grimaldi et al (2016) Cytokine 85:101-108

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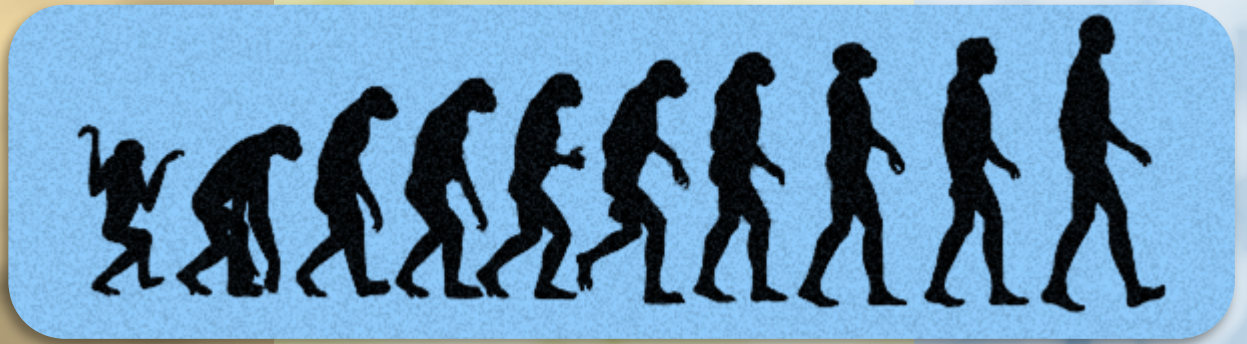
4. Translational Implications of Immunotox Findings in Nonclinical Studies



Species Relevance

Concordance

Challenges

The Value of Nonclinical Immunotox Studies?

- Toxicity of biologics is mainly driven by their pharmacologic activity
- Many biotherapeutics are highly target-/species-specific
  - Relevant animal species may not be available
  - Even in cross-reactive species, there may be PK/PD differences
  - Type III hypersensitivity rxns in nonclinical studies are poorly translated to humans
- Adverse events *may* be predicted from knowledge of the target, data from knockout (KO) mice (or target deficient humans), mouse studies done with surrogate molecules
- However, KO mice can either *over- or under* predict the human risk

Translational Challenge: Species Differences

- **NHPs and human have differences in the immune system-**
  - Expression and function of immunologic co-stimulatory molecules and factors
  - CD28 expression on memory T cells : TeGenero (TGN 1412) experience in 2006
  - Complement pathway components
  - IgG–FcγR interactions
    - Identical IgG1-FcγR interaction and effector function profile in human and cynos → effector-function-related effects of human IgG1 Abs in cynos are expected to be predictable for humans
    - Cynomolgus inhibitory FcγRIIb receptor shows strongly increased affinity for IgG2 than human → in vitro and in vivo results for human IgG2 and IgG4 obtained in the cynos have to be cautiously interpreted
    - Release of cytokine and inflammation markers, such as c-reactive protein, generally occur at lower levels in rats than in monkeys→ Rat not a sensitive species for CRA

Drug Discov Today. 2013 Dec;18(23-24):1138-43.
Cray C et al. (2009), Acute phase response in animals: a review. Comp Med 59(6):517-526
Haley, P (2003) Toxicology 188:49-71

Species Differences (contd…)

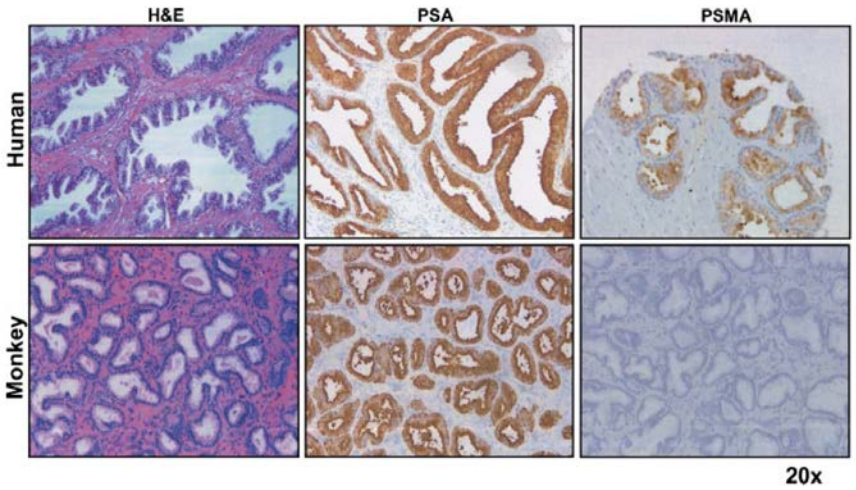
- **Anatomical differences**
  - Variation: sex, age, strain
  - Intra-animal variation in lymph nodes (central versus peripheral)
  - Hematopoietic activity in rodent (mice >> rat) spleen

- **Functional differences:**
  - Significant differences in NK cell activity between mouse and man
  - Mouse IgG1 is reaginic (not rats and guinea pigs)
  - Shock organs of mice (vasculature and gut) different than those in human (lung and vasculature)
  - Mice do not have IgG3 and IgG4 subclasses
  - Mice require 1000 times the dose of humans to elicit comparable DTH responses

Species Differences (contd….)

Bispecific CD3 Ab well tolerated in NHPs than in human

- **Bispecific Ab targeting CD3 and prostate antigen PSMA**
  - ~16000 fold differences in animal-to-human doses, comparing HNSTDs to human MTDs/ HHDs
- ***PSMA***
  - Expressed in high levels by human prostate
  - Is not expressed in any significant amount in the prostates of mouse, beagle dog, or macaque monkeys
  - These non-human species, therefore, are not suitable toxicologic models to assess prostate damage from PSMA-activated intraprostatic prodrug/protoxin therapies
    - **Pharmacologic relevance?**



Aggarwal S et al. (2006) Comparative study of PSMA expression in the prostate of mouse, dog, monkey, and human. Prostate 66, 903e910.

Nonclinical and Human Adverse Effects of Biologics

Concordance

- **29 approved drugs** evaluated
  - **15 cell surface targets and 14 soluble targets**
- Good concordance with human PD between NHPs or surrogate treated mice
- Poor PD concordance in genetically deficient mice
- Poor concordance for human adverse events

	Accurate reflection of the major effects of the biopharmaceutical in humans
	Some of the major effects in humans are not reflected in the preclinical data
	Major effects in humans are not reflected in the preclinical data

Target	Surrogate in Rodent	Surrogate in NHP	Concordance of Human AE and Rodent Genetic Deficiency	Concordance of Human AE and Rodent AE	Concordance of Human AE and NHP AE
Cell Surface					
CD3	+	+	-	+	?
CD11a (LFA-1)	+		-	-	-
CD20	+		-	-	-
CD25	+		-	-	-
CD49d (VLA-4)	+		-	-	-
CD52	+		-	-	-
CD126 (IL-6R)	+		-	-	-
CD152 (CTLA4)	+		+	±	?
EGFR	+		-	?	+
GPIIb/IIIa	+		+	±	+
Her2 (ErbB2)			+	+	-
CD58-Ig	+		-	-	-
CD152-Ig			-	-	-
Soluble					
TNFα	+		±	±	±
IL-1β	+		±	±	-
IL-12/23	+		±	±	-
IgE	+		±	-	±
Complement C5	+		±	-	?
RANK-L	+		±	±	+
VEGF	+		-	-	±

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Bugelski and Martin (2012) 166:823-846

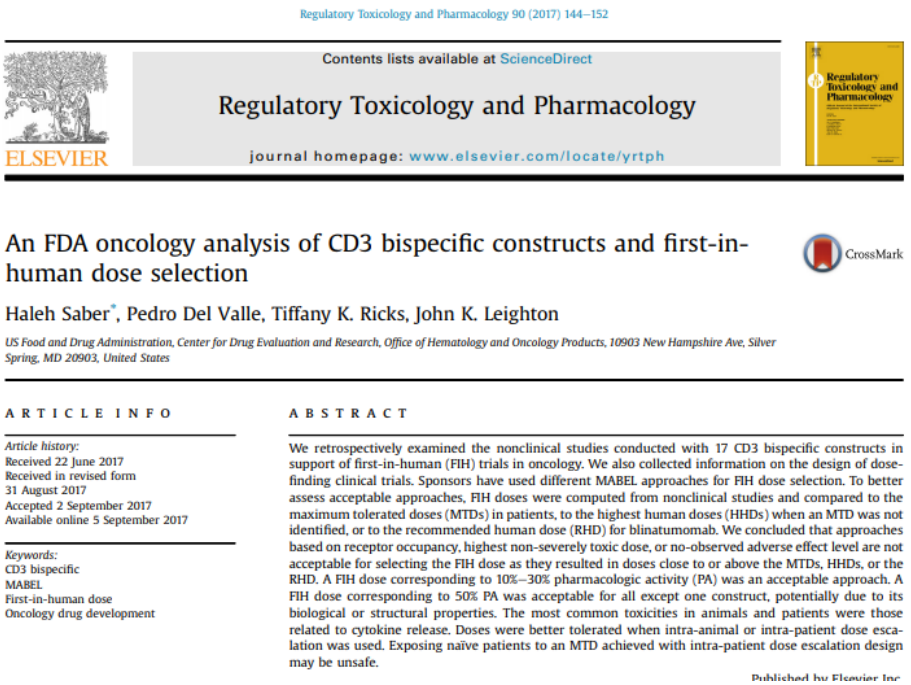
Martin and Bugelski (2012) 166:806-822

Sometime There is Concordance

- 17 INDs for CD3 bispecific constructs (Oncology drugs) were analyzed
- Drug-related toxicities in animal studies
- Cytokine release syndrome (CRS) and inflammatory responses were common
- **High concordance in animals and patients for the occurrence of CRS**
- CNS toxicity was observed in both patients and animals;

***HOWEVER*, no correlation in side-by-side product wise comparison.**

- Neurotox in patients assessed via clinical observations: dizziness, confusion and speech disorder (not done in animals)
- Neurotox in nonclinical studies via histopath (not done in patients)



Regulatory Toxicology and Pharmacology 90 (2017) 144–152

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journal homepage: www.elsevier.com/locate/yrtph

An FDA oncology analysis of CD3 bispecific constructs and first-in-human dose selection

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US Food and Drug Administration, Center for Drug Evaluation and Research, Office of Hematology and Oncology Products, 10903 New Hampshire Ave, Silver Spring, MD 20903, United States

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ABSTRACT

We retrospectively examined the nonclinical studies conducted with 17 CD3 bispecific constructs in support of first-in-human (FIH) trials in oncology. We also collected information on the design of dose-finding clinical trials. Sponsors have used different MABEL approaches for FIH dose selection. To better assess acceptable approaches, FIH doses were computed from nonclinical studies and compared to the maximum tolerated doses (MTDs) in patients, to the highest human doses (HHDs) when an MTD was not identified, or to the recommended human dose (RHD) for blinatumomab. We concluded that approaches based on receptor occupancy, highest non-severely toxic dose, or no-observed adverse effect level are not acceptable for selecting the FIH dose as they resulted in doses close to or above the MTDs, HHDs, or the RHD. A FIH dose corresponding to 10%–30% pharmacologic activity (PA) was an acceptable approach. A FIH dose corresponding to 50% PA was acceptable for all except one construct, potentially due to its biological or structural properties. The most common toxicities in animals and patients were those related to cytokine release. Doses were better tolerated when intra-animal or intra-patient dose escalation was used. Exposing naïve patients to an MTD achieved with intra-patient dose escalation design may be unsafe.

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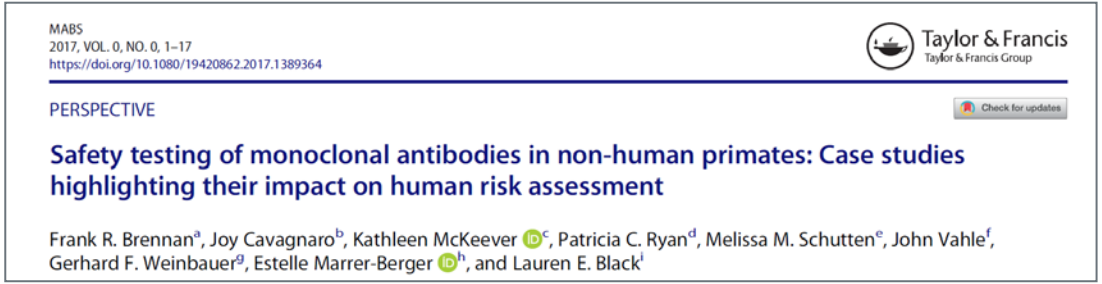
Saber H et al (2017) Regul Tox Pharmacol 90:144-152

Value of NHP Studies for Human Risk Assessment



MABS
2017, VOL. 0, NO. 0, 1–17
https://doi.org/10.1080/19420862.2017.1389364

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PERSPECTIVE

Check for updates

Safety testing of monoclonal antibodies in non-human primates: Case studies highlighting their impact on human risk assessment

Frank R. Brennan[a], Joy Cavagnaro[b], Kathleen McKeever[c], Patricia C. Ryan[d], Melissa M. Schutten[e], John Vahle[f], Gerhard F. Weinbauer[g], Estelle Marrer-Berger[h], and Lauren E. Black[i]

- **18 case studies** of mAbs
- Knowledge of the target, data from transgenic rodents, and genetically deficient human is not adequate for human risk assessment
- Pharm/Tox properties of mAbs with novel scaffolds, novel targets and multi-targeting domains are less predictable
- Human translatability of NHP findings was demonstrated → utilized to enable program progression/ termination
- NHP studies also indicated that some target/pathways with predicted liabilities based on target biology, in vitro and KO mouse data could still be administered safely to advance into clinical trials →would not have been otherwise pursued

Brennan et al (2018) MAbs 10:1-17

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NHP Studies for Human Risk Assessment

Molecule/ Target	Potential human risk	Relevant Tox species	Tox finding	Value of NHP study	Fate
Anti-GM-CSF IgG4	Inhibition of macrophage function→ PAP, Lung failure	NHP	Foamy macrophage, human PAP like disease at high multiples of clinical doses	Allowed human dose modeling and safety margin prediction	Progression to clinic
Anti-DLL-4F(ab)'2	Inhibn of normal blood vessels, liver and heart tox, vascular tumors	NHP, rodent	Dose dependent endothelial proliferation and fibrosis in heart and pulmonary artery (PAH?)	Identified new toxicity, likely translatable to human	Candidate and target terminated
Anti-TGFb IgG4	CV tox, skin acanthomas, immune activation	NHP, rodent	Tox only in NHP: epithelial hyperplasia, enteropathy, renal tubular lesions	Identified safe starting dose, dosing frequency and monitoring scheme	Progression to clinic
Anti-cytokine mAb IgG4	None (based on KO mice and adult NHP tox studies)	NHP	Dystocia and hemorrhage in ePPND study (Target involved in labor-associated inflammation→ cervical ripening)	Identified life-threatening hazard for women and infants during advanced gestation stage (effect not seen in KO mice or pregnant mice with surrogate molecule)	In development for autoimmune dz; warning label
Anti-human cell memb protein IgG2a	None based on pharmacology	NHP	Severe thrombocytopenia purpura after single dose (immune mediated platelet phagocytosis); Negative in human and cyno platelet activation assay	Identified new human-relevant toxicity not detected in vitro, allowed continuation of target program with safer lead mol. Importance of *in vivo* testing	Continuation of the target

Brennan et al (2018) MAbs 10:1-17

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Examples and Case Studies

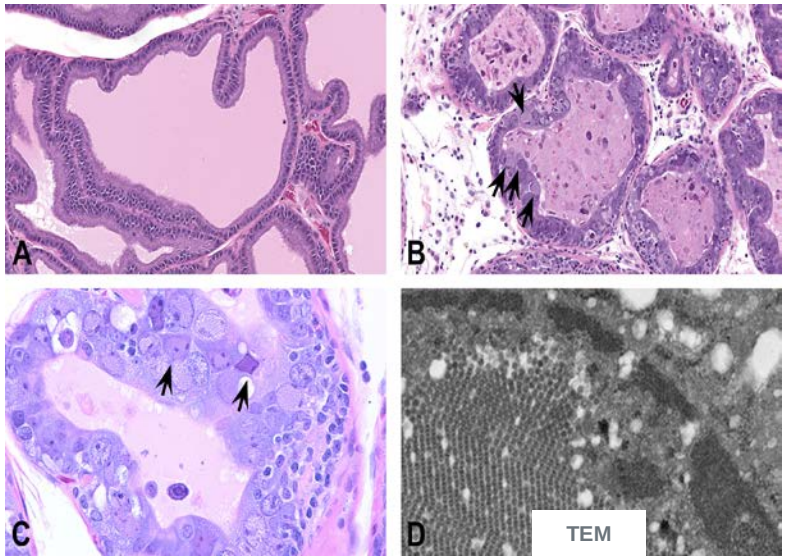
Immunosuppression

Prostatitis in Wistar Han Rats in a Chronic Toxicity Study

- 2 of the 15 male rats in the high dose group in a 6-month tox study of an immunomodulatory test article
- No functional deficits (urine output/fertility etc.)
- **Histopathology:** Moderate, multifocal, predominantly lymphocytic inflammation in the prostate gland that was associated with prominent atypia of epithelial cells lining involved glands → Chronic prostatitis, lymphocytic with **intranuclear and cytoplasmic inclusion-like material**
- Lower dose animals were fine
- No serological or PCR confirmation
- TEM was strongly suggestive of **polyoma virus**
- Similar to a RatPyV2 described in immunodeficient rats (**next slide**)

- Impact on NOAEL assessment? Translational relevance?

Prostate gland histopathology



A: Control rat; **B-D:** High dose rat, arrows: intranuclear inclusions

K. Masek-Hammerman et al. (2017); *Toxicol Pathol* 45, 589-592.

Immunosuppression

Rat Polyoma virus (RatPyV2) in immunodeficient rats

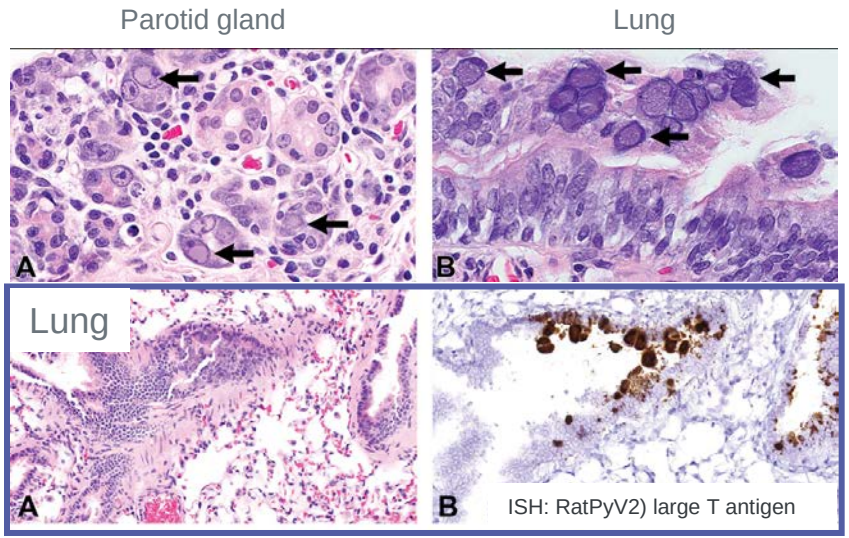
Severe combined immunodeficient F344 rats with null Prkdc and IL-2rg genes

Clinical:

- Weight loss, decreased fecundity, mortality

Histopath:

- Severe atrophy of the salivary glands, the exorbital lacrimal glands and Harderian glands
- Large basophilic intranuclear inclusions were observed in epithelium of the respiratory tract, salivary and lacrimal glands, uterus, and prostate gland
- Confirmed by viral metagenomic sequencing, ISH, qPCR, exptl infection in Foxn1rnu nude rats
- Serologic survey revealed anti-RatPyV2 antibodies in 480/1500 serum samples; PCR + in 7 of 1000 fecal samples
- Population surveys found antibodies to this novel virus in **32% of immunocompetent rats** used in biomedical research in North America. https://www.idexxbioresearch.eu/updates/2016/11/7/new-virus-discovered)



Arrows: Intanuclear inclusions

C. Besch-Williford et al. *Toxicol Pathol* 45, 593-603.

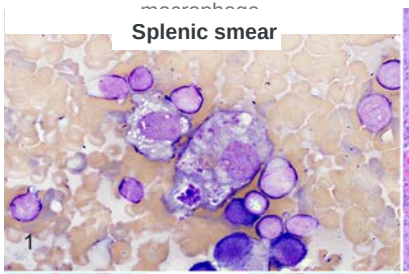
Immunosuppression

Talaromycosis (Penicillosis) in a cyno

- A sexually mature Chinese-origin female cyno
- High-dose group in a 26-week tox study with an experimental immunomodulatory therapeutic antibody (a CD40L antagonist fusion protein)
- The animal was clinically normal throughout the study
- **Study day 141:** Abnormal clinical pathology changes
- **Study day 149:** Splenomegaly (dx supported by ultrasound) Study day 192: Scheduled necropsy→ marked splenomegaly with a nodular and discolored appearance.
- **Cytology (splenic smear):** yeast-like organisms within macrophages.
- **Histopathology:** disseminated systemic granulomatous inflammation with 2- to 3-µm oval, intracytoplasmic yeast-like organisms in multiple organs identified as *Talaromyces (Penicillium) marneffei*
- This organism, not previously reported as a pathogen in macaques, causes ***an important opportunistic infection in immunosuppressed humans*** in specific global geographic locations

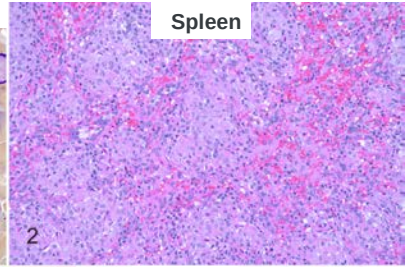
Impact on NOAEL assessment? Translational relevance?

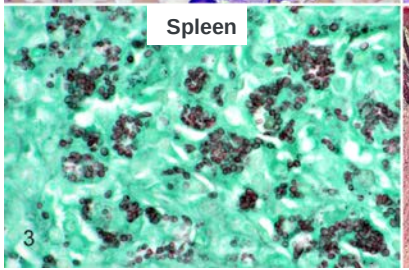
Yeast forms of *Talaromyces marneffei* in the cytoplasm of a

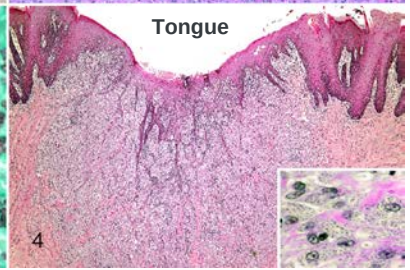


Yeast forms of *T. marneffei* in red pulp macrophages. GMS.

Foci of granulomatous inflammation in splenic red pulp.







Erosion and granulomatous inflammation. Inset: Macrophages containing yeast forms of *T. marneffei*.

W. O. Iverson et al. (2018); Vet Pathol 55, 591-594.

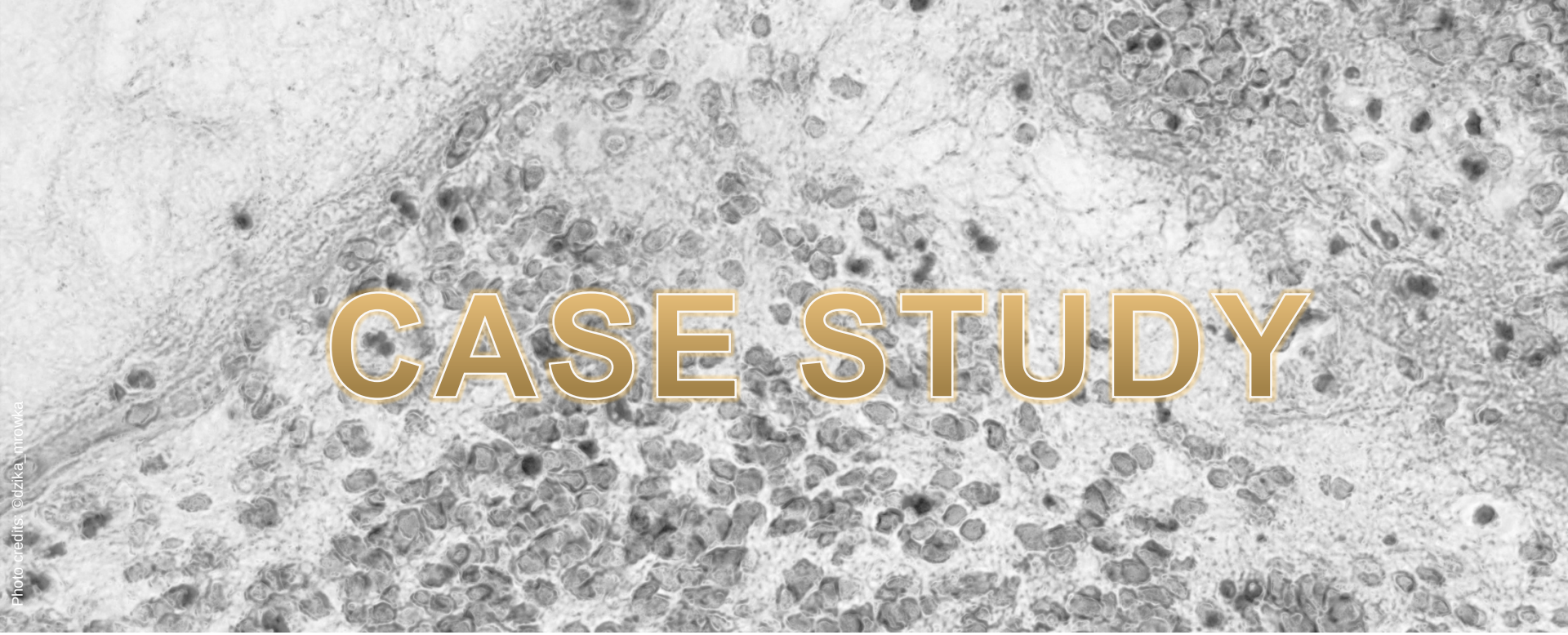


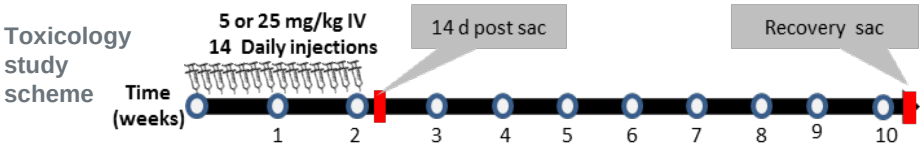
Photo credits: ©dzika_mrowka

CNS Lesions in Mice Following Intravascular Administration of a T Cell-targeting Antibody Surrogate

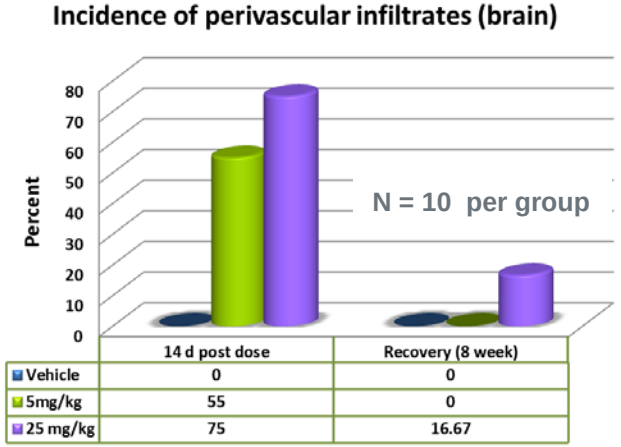
SANOFI

Tox Study with Surrogate Murine Antibody in CD-1 Mice

- A T cell-targeting humanized antibody was developed for treatment of autoimmune diseases.
- Human-specific clinical candidate was **non-cross reactive to any preclinical tox species** (except Gorilla)
- A surrogate monoclonal antibody (Ab) was developed for nonclinical study in mice



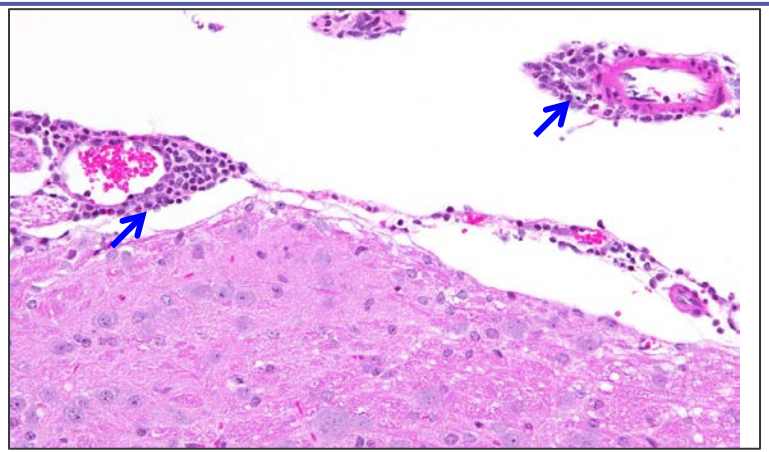
- In the toxicology study, **minimal to mild perivascular inflammatory cell infiltrates were observed in brain and spinal cord f**ollowing 14 daily IV doses of Ab @ 5 or 25 mg/kg in outbred CD-1 mice.
- Following 8 week recovery period: **complete recovery in the low dose group** and partial recovery in the high dose group.

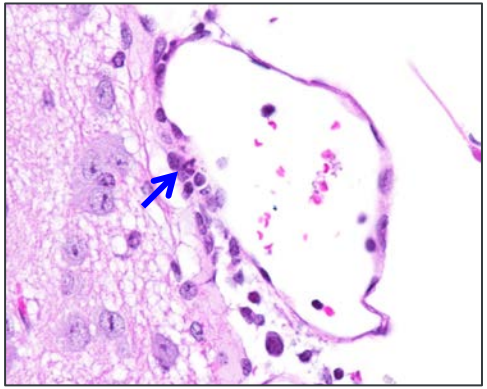


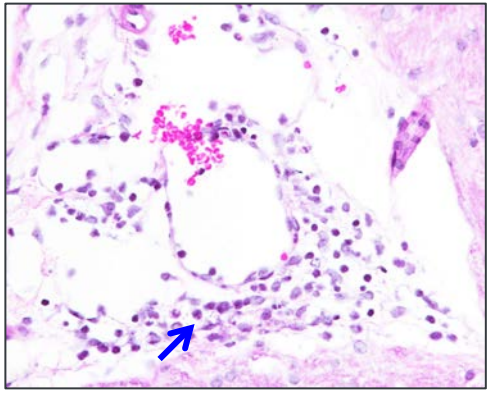
	14 d post dose	Recovery (8 week)
Vehicle	0	0
5mg/kg	55	0
25 mg/kg	75	16.67

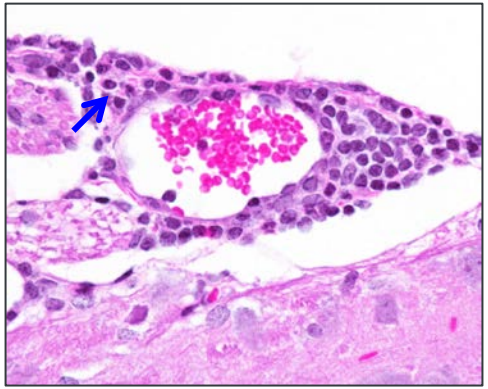
SANOFI

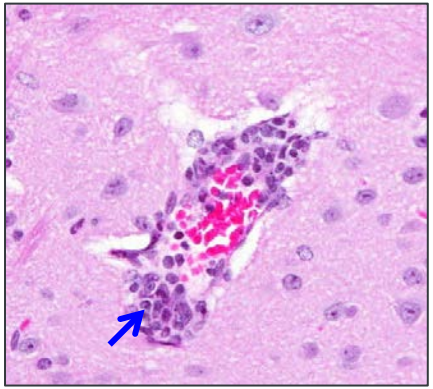
Perivascular Infiltrates in Brain and Spinal Cord











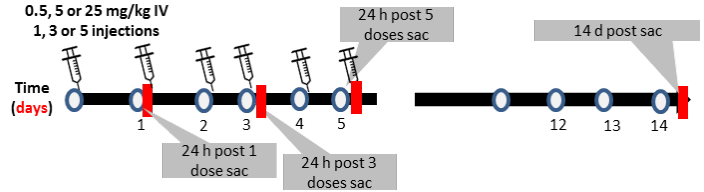
Follow-up Study #1

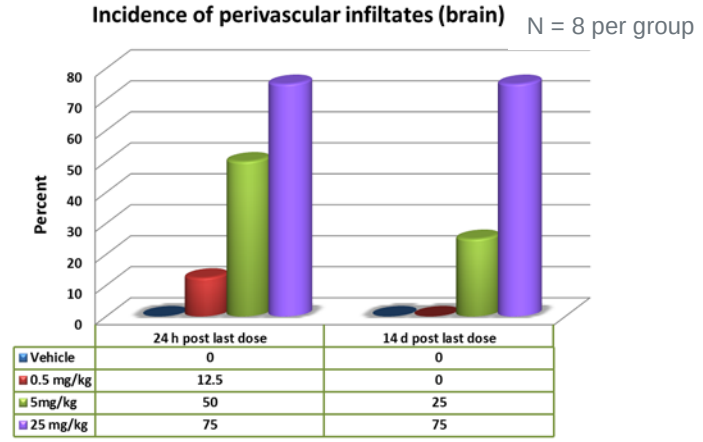
Multiple Doses and Frequencies of Surrogate Ab in CD-1 Mice

- Multiple doses (0.5, 5 or 25 mg/kg) and dosing frequencies (1, 3 or 5 daily IV doses) were tested.
- Brain and spinal cord were evaluated at 24 h or 14 d post last dose.

- CNS lesions **(multifocal minimal to mild perivascular infiltrates)** were observed in all Ab treated groups including the single 0.5 mg/kg IV dose group (examined at 24 h post dose).
  - Blood vessels (veins and venules) of meninges (pia mater) of the brain and spinal cord were affected (next slide)
  - Parenchymal blood vessels also affected occasionally
  - Infiltrates comprised of mononuclear cells (macrophages and lymphocytes) and rarely granulocytes (neutrophils and eosinophils) (next slide)

Study scheme





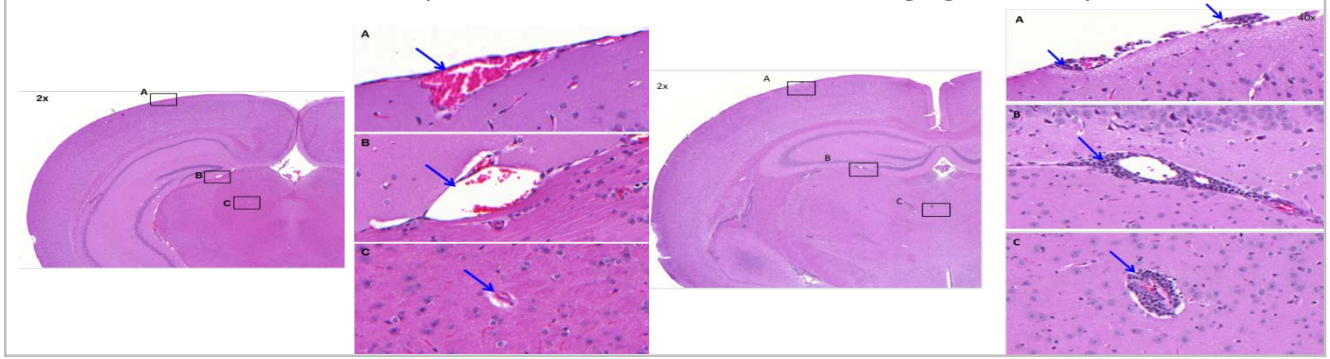
CNS Lesions Following Ab administration in CD-1 Mice

A

Brain

Vehicle, 14 d post dose

Ab, 0.5 mg/kg x 5, 14 d post dose

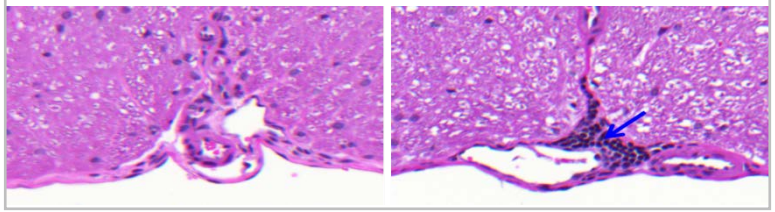


B

Spinal Cord

Vehicle

Ab; 5mg/kg x 5, 14d



Photomicrographs showing histologic changes in **A**) brain and **B**) spinal cord. Blue arrows indicate sites of perivascular inflammatory cell infiltrates in the brain and spinal cord of Ab-treated mice.

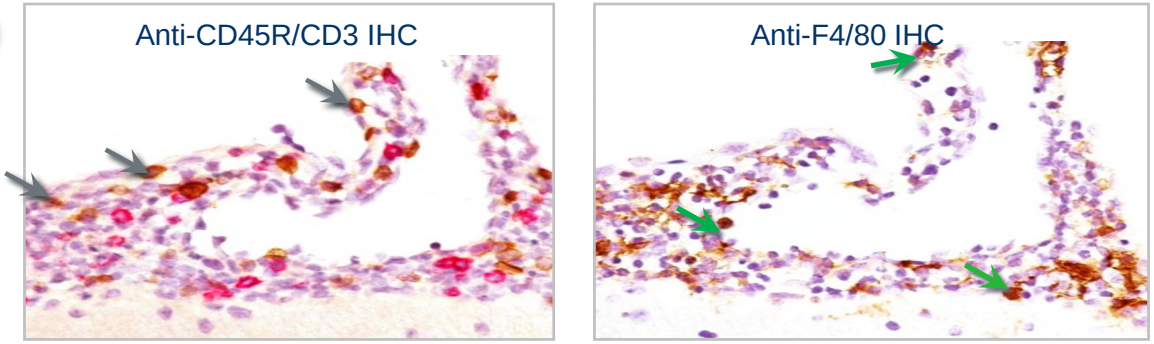
Boxed areas A, B and C in brain images are shown at higher magnification on right side panels.

SANOFI

Perivascular Infiltrates in CNS

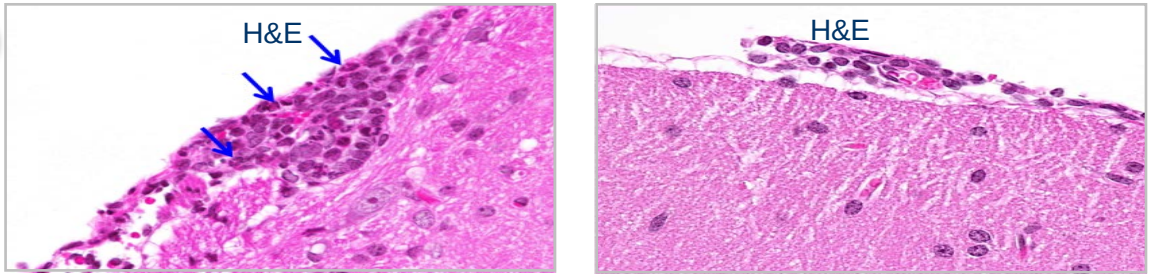
IHC Characterization

A



Ab at 5 mg/kg x 5 24h post last dose,
brain stem (60x)

B



Ab at 5 mg/kg x 5 24h post dose,
brain stem (60x)

Ab at 5 mg/kg x 5 14d post dose,
brain stem (60x)

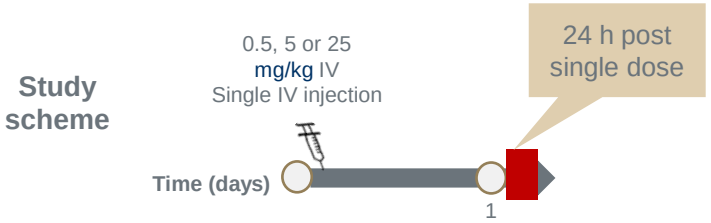
Legends:
- Black arrows: T cells
- Pink-red stained cells: B cells
- Green arrows: Macrophages
- Blue arrows: Neutrophils

Photomicrographs showing perivascular inflammatory cell infiltrates in **CD-1 mice** treated with surrogate Ab. **a)** IHC reveals **CD3+** (T cells), **CD45R+** (B cells) and **F4/80+** (macrophages) cells within these infiltrates; **b)** H&E photomicrographs also show a few granulocytes within inflammatory infiltrates.

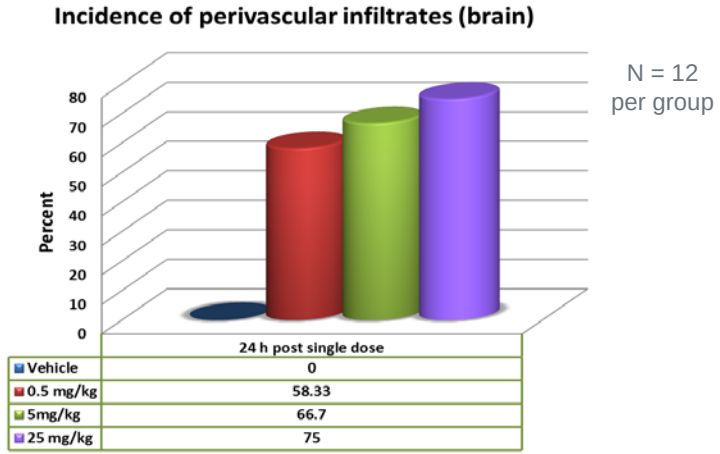
Follow-up Study #2:

Single Injection, Multi-dose 24 h Study in CD-1 Mice

Since lesions appeared within 24 hours of Ab dosing, the goal of this study was to determine if serum cytokine levels correlated with the histopathological findings.



- Minimal to mild perivascular infiltrates were observed in the brain and spinal cord of CD-1 mice at 24 h post Ab dose.
- There was an apparent dose-dependent trend for higher incidence of these infiltrates in CD-1 mice.
- There was no correlation between serum cytokine levels and the incidence of CNS lesions.

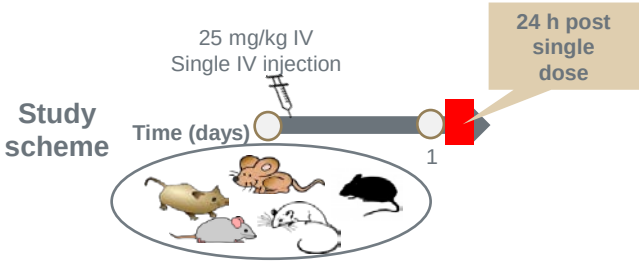


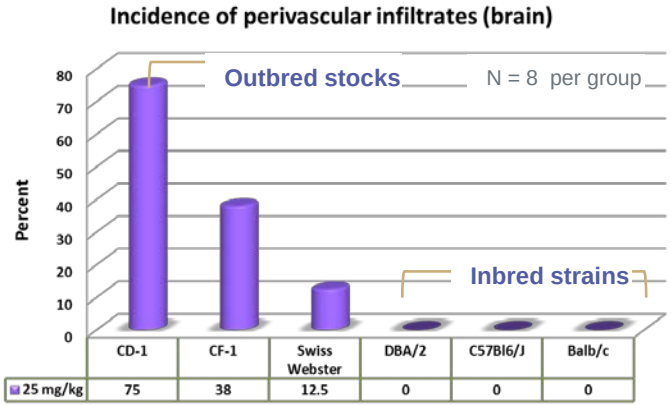
Follow-up Study 3:

Single Dose 24 h Study in Inbred Strains and Outbred Stocks of Mice

- Are the CNS findings CD-1 mouse specific?
- Two additional outbred (CF-1 and Swiss Webster) and three inbred strains (C57BL/6, BALB/c and DBA/2) of mice were dosed with a single IV dose of 25 mg/kg Ab
- Brain and spinal cord evaluated via histopathology

- CNS lesions were **observed in all three outbred stocks** indicating that these were not CD-1 mouse specific.
  - Susceptibility of the three stocks was: ***CD-1 > CF-1 >>Swiss Webster***
- CNS lesions were **not noted in the inbred strains of mice.**





In-vitro Proliferation Assay Using Splenocytes

From Inbred and Outbred Mice

- Mouse splenocytes were tested *in vitro* for proliferation activity following treatment with the surrogate Ab.
- Splenoyctes from the three outbred stock mice showed variable incidence of proliferation in response to Ab, similar to the incidence of CNS findings *in vivo.*
- Inbred mouse splenocytes did not show cell proliferation in response to Ab treatment.

	Strain or Stock	Test Article	Cell Type	Cell Proliferation (incidence)	CNS Lesions
Outbred	CD-1	Ab (Surrogate)	Splenocytes	**Yes** (7/16)	**Yes**
Outbred	CF-1	Ab (Surrogate)	Splenocytes	**Yes** (2/8)	**Yes**
Outbred	Swiss Webster	Ab (Surrogate)	Splenocytes	**Yes** (1/8)	**Yes**
Inbred	**C57BL/6J**	Ab (Surrogate)	Splenocytes	No	No
Inbred	**BALB/c**	Ab (Surrogate)	Splenocytes	No	No
Inbred	**DBA/2**	Ab (Surrogate)	Splenocytes	No	No
	Human	Clinical candidate	PBMC	No	Unknown

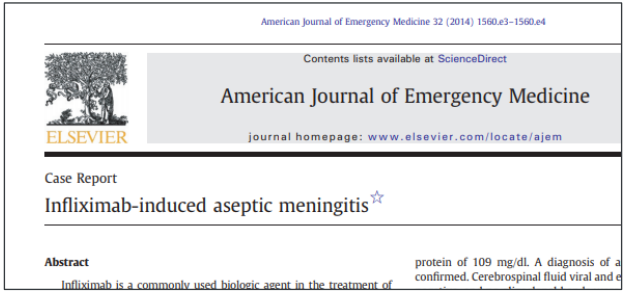
The clinical candidate also did not elicit a proliferative response in human peripheral blood mononuclear cells (PBMCs) similar to the results obtained in the inbred mouse strains.

Surrogate Study in Mice: Summary

- CNS lesions were also observed in outbred stocks but not in any of the inbred strains
- CSF analysis was not performed in our studies
- Significant hematologic differences were not noted
- Inbred versus outbred stock studies underscore **the importance of mouse genetics and background** → contribution of **host factors** → **implications in preclinical safety assessment**
- Clear effect of strain/species background on drug effects → **consideration for nonclinical species selection**

Translational Relevance

- Relevance to the clinical candidate is unclear.
- Reminiscent of **drug-induced aseptic meningitis (DIAM)** in people.
- DIAM as a **rare and generally self-limiting adverse event** has been associated with NSAIDs, antibiotics, **intravenous immunoglobulins, and pan-T cell antibody** administration.
  - The cause of DIAM is unknown but **allergic hypersensitivity** may be implicated.
  - Headache, stiff neck and fever are the main clinical signs. Elevated CSF protein and pleocytosis (increased cell count) aid in clinical diagnosis.



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Case Report

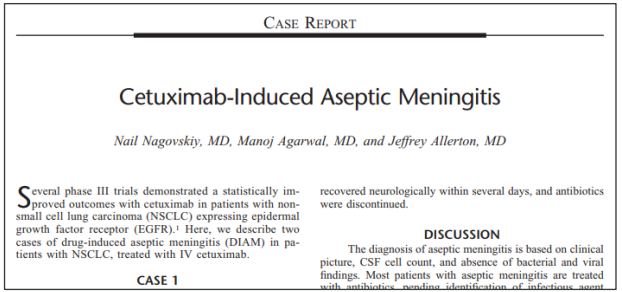
Infliximab-induced aseptic meningitis☆

Abstract

Infliximab is a commonly used biologic agent in the treatment of

protein of 109 mg/dl. A diagnosis of a

confirmed. Cerebrospinal fluid viral and e



CASE REPORT

Cetuximab-Induced Aseptic Meningitis

Nail Nagovskiy, MD, Manoj Agarwal, MD, and Jeffrey Allerton, MD

Several phase III trials demonstrated a statistically improved outcomes with cetuximab in patients with non-small cell lung carcinoma (NSCLC) expressing epidermal growth factor receptor (EGFR).[1] Here, we describe two cases of drug-induced aseptic meningitis (DIAM) in patients with NSCLC, treated with IV cetuximab.

CASE 1

recovered neurologically within several days, and antibiotics were discontinued.

DISCUSSION

The diagnosis of aseptic meningitis is based on clinical picture, CSF cell count, and absence of bacterial and viral findings. Most patients with aseptic meningitis are treated

Am J Em Med 32:1560-e3-1560e4
Ulrich A et al. (2015) J Clin Neurosci 22:1061-1063

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Take Home Points

1. Biopharrmaceutical drug development is complex and entails many challenges including immunotoxicity; novel biologic targets and innovative (e.g. multi-specific) formats warrant ***tailor-made*** **nonclinical development strategies**
2. Immunotoxicity of biopharmaceuticals may be either related to exaggerated pharmacology or is an off target effect
3. Immunotox assessment of biologics is undertaken on a '**case-by-case' basis using weight of evidence** from standard tox studies and as per regulatory guidance
4. Nonclinical in-vivo/ex-vivo immunotox studies are helpful for identifying the human safety risk of the biopharmaceuticals, but require critical understanding of the target, species relevance for both pharmacology and toxicology, and **multi-disciplinary approach to data analysis and integration**
5. Toxicologic pathologists have a critical role in discussion of translatability of nonclinical immunotox findings / Concordance of nonclinical studies and clinical studies

Thank you

STP-India

Sanofi R&D

- Translational In-vivo Models Research Platform
- Global Discovery Pathology
- Sanofi Preclinical Safety
- Biomedical Media Services

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