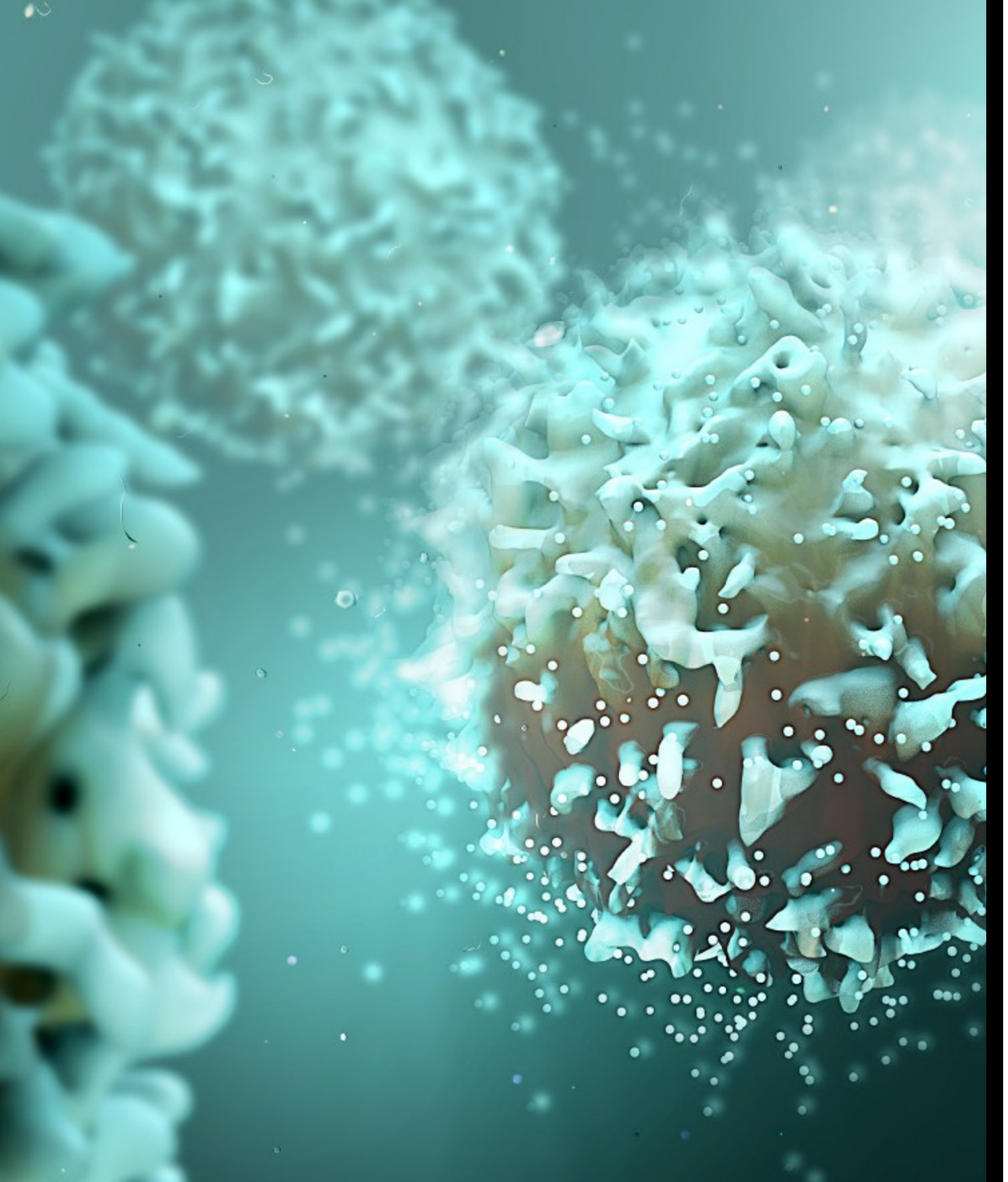
HESI IMMUNO-SAFETY TECHNICAL COMMITTEE

On-demand Training Course

Cytokine Release Syndrome (CRS)

Jennifer Hall (Boehringer-Ingelheim), M.S.



Learning Objectives

- **Define Cytokine Release Syndrome (CRS)**

  Triggers, Clinical Symptoms, Risk Factors, Grading & Management

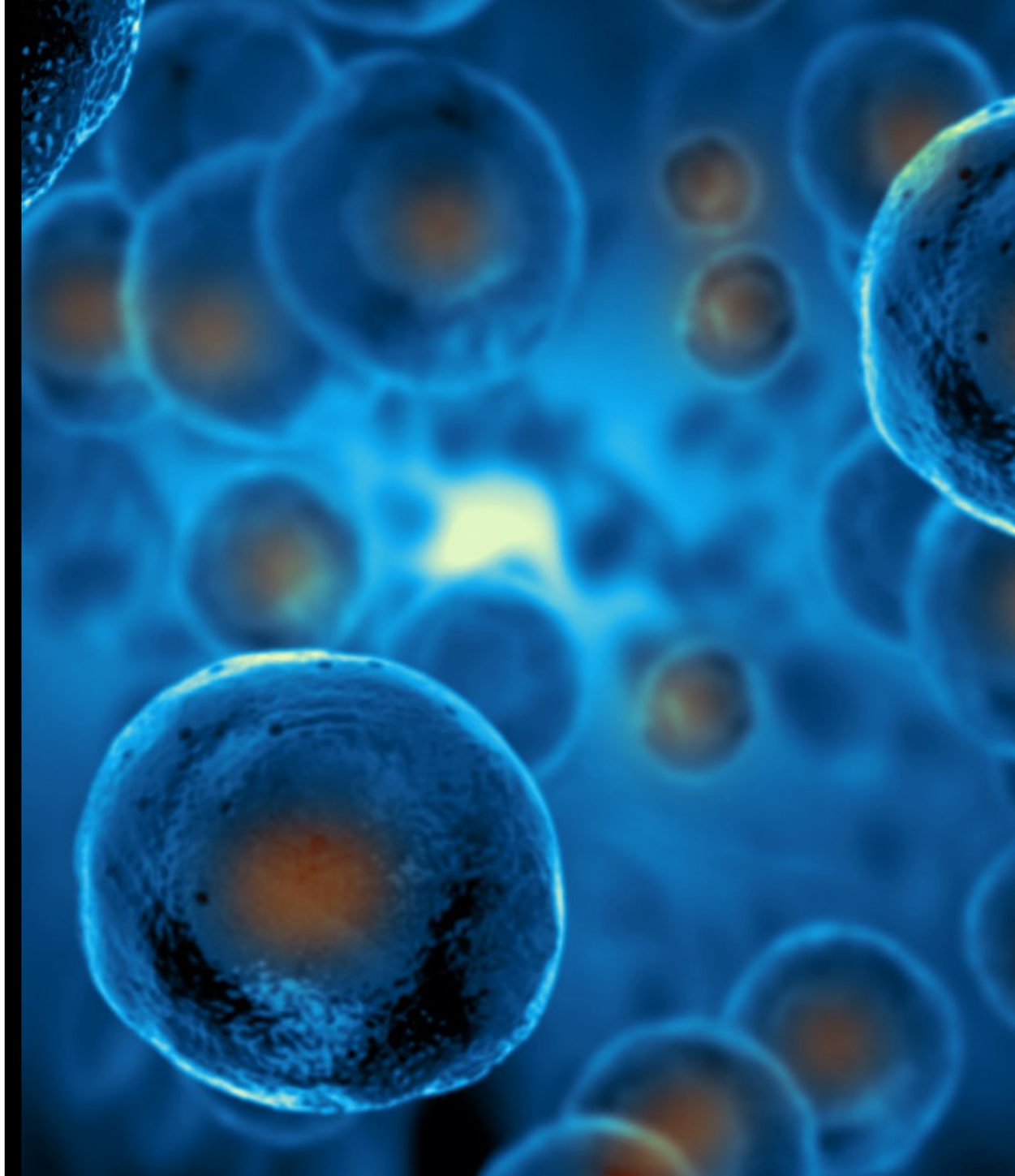
- **Identify Notable Case Examples**

  OKT3, Campath-1H, Rituxan & TGN1412, Kymriah, BLINCYTO & Kimmtrak

- **Describe the use of *in vitro cytokine release assays (CRAs) to help predict in vivo CRS***

  Common formats, Interpretation, Challenges & Current Status

Cytokine Release Syndrome (CRS)



Cytokine Release Syndrome (CRS)

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- **Excessive & rapid release of pro-inflammatory cytokines** from immune cells into the bloodstream
- An **acute systemic inflammatory response**, usually associated with first infusion reaction
- Severity can range from mild-to-moderate-to severe & life-threatening; symptoms can be progressive

Potential Triggers

- Treatments that enhance an immune response
  - Monoclonal antibodies
  - Immunostimulatory therapies for cancer
    -CAR T-cell therapy*
    -Checkpoint inhibitors
    -Bispecific T cell-engagers (BiTEs)
  - Infections (COVID-19)

Clinical Symptoms

FEVER +

Mild

- Chills
- Fatigue
- Nausea
- Vomiting
- Diarrhea
- Headache
- Rash

Moderate

- Tachycardia
- Hypotension
- Respiratory Distress
- Hypoxia

Severe

- Systemic Capillary Leak Syndrome
- Cardiac Shock
- Pulmonary Edema
- Disseminated Intravascular Coagulation
- Multi-organ Failure
- Neurotoxicity

DEATH

*chimeric antigen receptor (CAR)

Cytokine Release Syndrome (CRS)

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Potential Risk Factors

- Type of disease
  -Autoimmune diseases (rheumatoid arthritis or lupus)
- High disease/tumor burden
- CAR-T cell dose
- Graft-versus-host disease

Cytokine Release Syndrome (CRS)

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Clinical Grading Scales for CRS

Penn
University of Pennsylvania

ASTCT
The American Society for Transplant and Cellular Therapy

MSKCC
Memorial Sloan Kettering Cancer Center

Typically, grading is based on cross-classification of CRS according to several grading scales

Lee

Neelapu

CTCAE
National Cancer Institute Common Terminology Criteria for Adverse Events

CARTOX
CAR T-cell therapy-Associated Toxicity

Cytokine Release Syndrome (CRS)

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Clinical Grading Scales for CAR T-cell Therapy

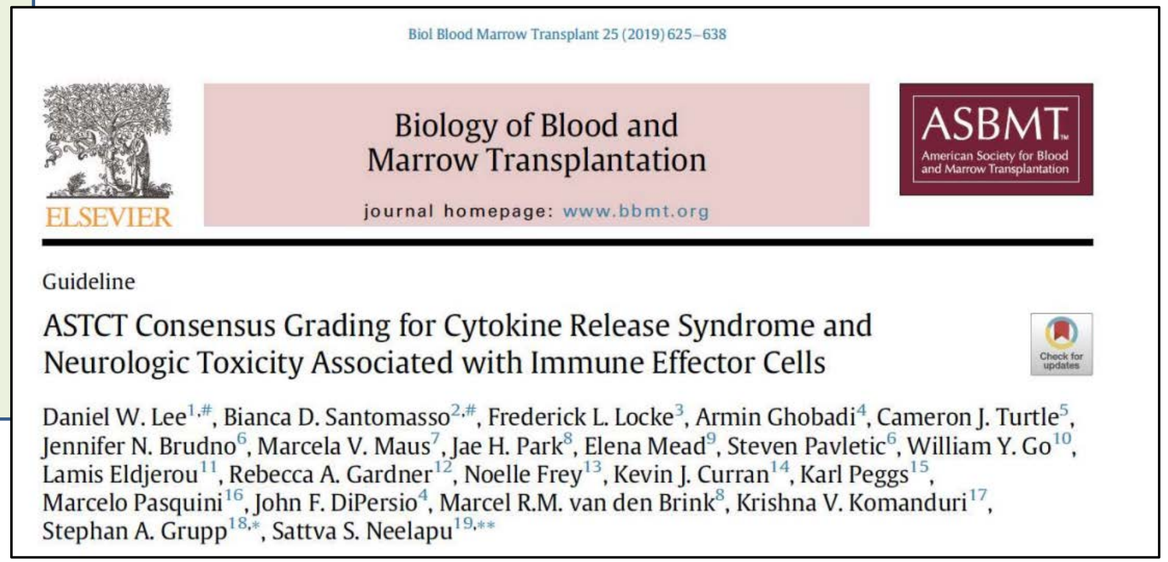
- ASTCT Consensus Grading Guideline

  The American Society for Transplant and Cellular Therapy

  -Created by a multidisciplinary panel of medical doctors, nurses, clinical trialists & advocacy experts

  -Recommendations to help aid in the recognition, workup, evaluation & management in 7 easy-to-navigate tables

  -Considered the optimal grading scale for assessing CRS & neurologic toxicity associated with immune effector cells (CAR-T cells)



Biol Blood Marrow Transplant 25 (2019) 625–638

ELSEVIER

Biology of Blood and Marrow Transplantation

journal homepage: www.bbmt.org

ASBMT American Society for Blood and Marrow Transplantation

Guideline

ASTCT Consensus Grading for Cytokine Release Syndrome and Neurologic Toxicity Associated with Immune Effector Cells

Daniel W. Lee[1,#], Bianca D. Santomasso[2,#], Frederick L. Locke[3], Armin Ghobadi[4], Cameron J. Turtle[5], Jennifer N. Brudno[6], Marcela V. Maus[7], Jae H. Park[8], Elena Mead[9], Steven Pavletic[6], William Y. Go[10], Lamis Eldjerou[11], Rebecca A. Gardner[12], Noelle Frey[13], Kevin J. Curran[14], Karl Peggs[15], Marcelo Pasquini[16], John F. DiPersio[4], Marcel R.M. van den Brink[8], Krishna V. Komanduri[17], Stephan A. Grupp[18,*], Sattva S. Neelapu[19,**]

Cytokine Release Syndrome (CRS)

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Clinical Management

- Close monitoring for CRS is recommended to allow for early recognition & intervention
  - Onset of CRS can occur ~30-120 mins with monoclonal/bispecific antibodies & checkpoint inhibitors
  - Onset can last longer with CAR T cells
- Prompt treatment is key to reducing exaggerated immune responses
- Depends on the grading score
- Symptomatic management with supportive care (e.g., oxygen, antihistamines, antipyretics, transfusions & intravenous fluids)
- Systemic high dose Corticosteroids (Dexamethasone)
- Vasopressors
- Step-up dosing strategy: particularly effective for dosing with T-cell engagers
  - an approach that involves administering a lower dose of a drug initially & then gradually increasing the dose over time until a target dose is reached
  - Used to maintain the efficacy of the drug while reducing the incidence & severity of CRS

Cytokine Release Syndrome (CRS)

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Clinical Management

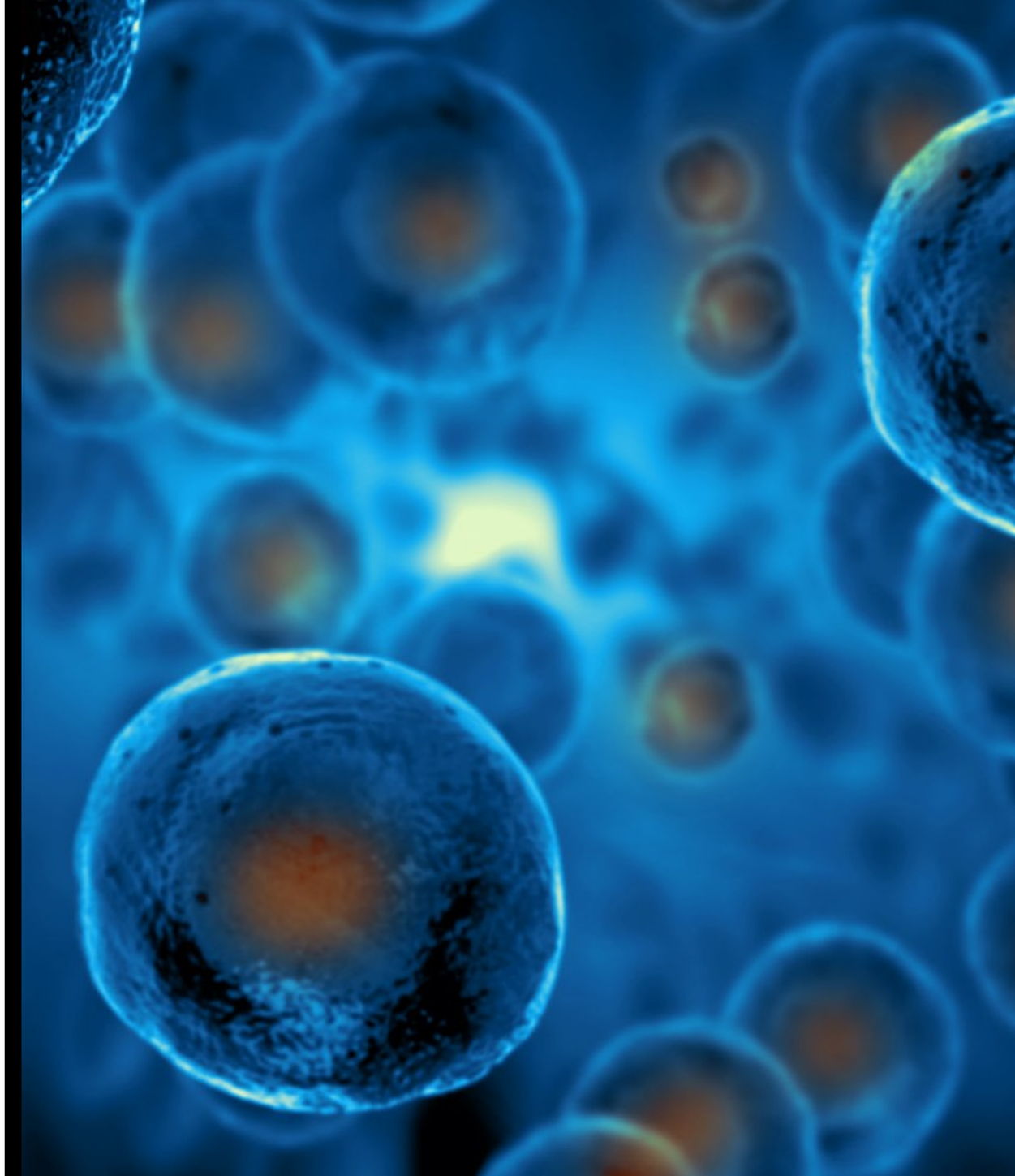
Monoclonals & T-cell Engagers
1st line treatment
Systemic Corticosteroids
Typically, effective

CAR T-cells, T-cell Engagers and Monoclonals
Anti-IL-6 blockade
Roche's Tocilizumab-Actemra®
BeiGene & EUSA Pharma
Siltuximab-Sylvant

Anti-TNFα blockade
Amgen's Etanercept-Enbrel
Janssen's Infliximab-Remicade

Consider risk verse benefit approach when choosing treatments for CRS

Notable Examples of CRS in the Clinic



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Monoclonal Antibodies

Product	Type of Molecule	Mechanism of Cytokine Release	Clinical Severity
1st **Muromonab-CD3 Orthoclone OKT3®** Marketed by Janssen-Cilag Approved: 1986 Withdrawn: 2010	• T-cell mitogenic murine anti-human CD3 • Immunosuppressant drug given to prevent renal, heart & liver allograft rejection	• Activation of T-cells	• Moderate/ Severe • CRS in ~50% patients
Rituximab **Rituxan®** Marketed by Biogen + Genentech Approved: 1997	• B lymphocyte-depleting chimeric (mouse/human) anti-CD20 • Drug used to treat autoimmune diseases (RA) & certain cancers	• Complement-mediated & antibody-dependent cellular cytotoxicity (CDC & ADCC)	• Mild/ Moderate
Alemtuzumab **Campath-1H®** Marketed by Genzyme Approved: 2001 Withdrawn: 2012 Relaunched: Lemtrada	• Lymphocyte and monocyte-depleting humanized anti-CD52 • Drug used to treat B-cell chronic lymphocytic leukemia (CLL) & multiple sclerosis	• FcγR engagement of effector cells such as natural killer (NK) cells	• Moderate/ Severe

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Theralizumab AKA TGN1412

Incident

- Super-agonist humanized anti-CD28 monoclonal antibody (IgG4κ) that directly stimulated T-cells developed by TeGenero
- Originally indicated for B-cell chronic lymphocytic leukemia & Rheumatoid arthritis
- Phase I trial was conducted on March 13, 2006, at the Northwick Park & St. Mark's Hospital in London, UK, under the supervision of PRAEXEL International (CRO)
- 8 healthy male volunteers (6 received TGN1412 & 2 received saline placebo)
- Single *i.v.* infusion at 0.1 mg/kg (1/500th of the highest dose tested in monkeys); infusion given in succession, ~10 mins apart
- All 6 males receiving TGN1412 experienced a sudden and severe systemic inflammatory response

Theralizumab AKA TGN1412

Incident Cont'd

- Onset of a massive "cytokine storm" occurred within ~ 90 mins of infusion (excessive induction of pro-inflammatory cytokines, headache, myalgia, nausea, diarrhea, vasodilation & hypotension)
- Life-threatening symptoms followed within the next 12-24 hours: respiratory stress, multiple organ failure (pulmonary & kidney), disseminated intravascular coagulation & severe depletion of lymphocytes & monocytes
- All 6 males survived; however, not without significant long-term physical and mental consequences

Theralizumab AKA TGN1412

Investigations

MHRA

stands for

Medicines and Healthcare products Regulatory Agency (UK)

- Thorough investigation confirmed no discrepancies with PAREXEL facility, equipment, quality systems & documentation or batch release criteria

Expert Scientific Group: Phase 1 clinical trials

THE ROYAL SOCIETY

The Royal Society is a Fellowship of many of the world's most eminent scientists and is the oldest scientific academy in continuous existence.

Chair:
Professor
Gordan Duff
"Duff's Report"

- Made 22 recommendations of factors to be considered before FIH clinical trials of agents with novel mechanism of action
  -study design & initial dose selection
  -appropriate observation period between dosing
  -need for a structured set of principles for evaluating high-risk agents in humans

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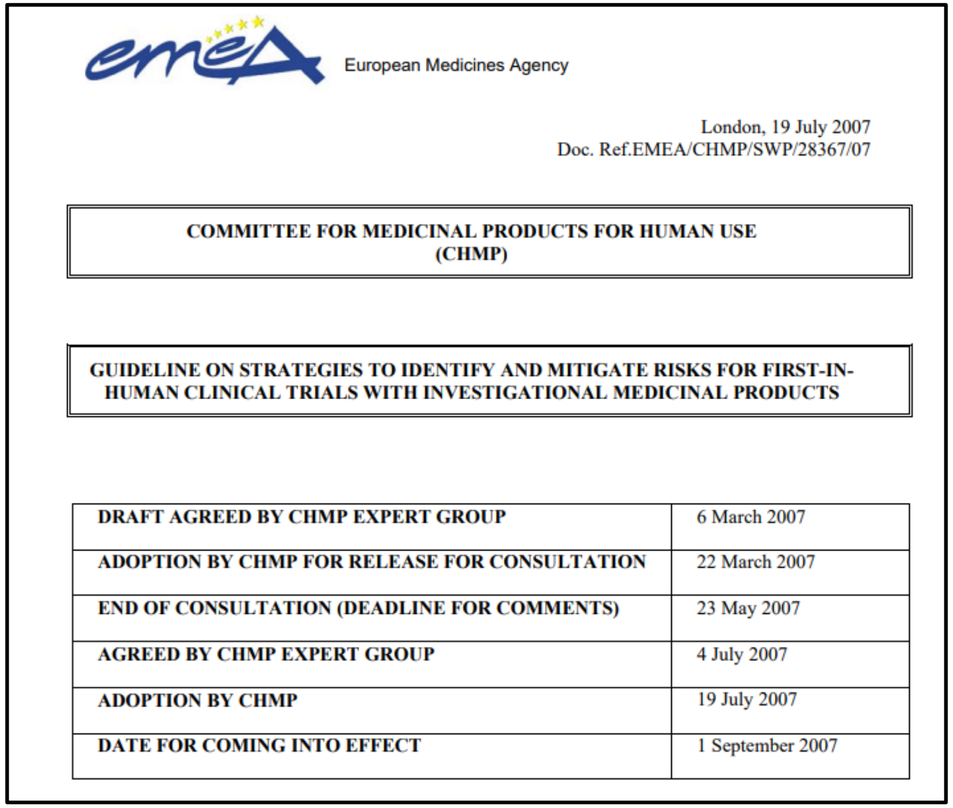
Theralizumab AKA TGN1412

Regulatory Action



European Medicines Agency

London, 19 July 2007
Doc. Ref.EMEA/CHMP/SWP/28367/07

COMMITTEE FOR MEDICINAL PRODUCTS FOR HUMAN USE (CHMP)

GUIDELINE ON STRATEGIES TO IDENTIFY AND MITIGATE RISKS FOR FIRST-IN-HUMAN CLINICAL TRIALS WITH INVESTIGATIONAL MEDICINAL PRODUCTS

DRAFT AGREED BY CHMP EXPERT GROUP	6 March 2007
ADOPTION BY CHMP FOR RELEASE FOR CONSULTATION	22 March 2007
END OF CONSULTATION (DEADLINE FOR COMMENTS)	23 May 2007
AGREED BY CHMP EXPERT GROUP	4 July 2007
ADOPTION BY CHMP	19 July 2007
DATE FOR COMING INTO EFFECT	1 September 2007

Main Highlights

- Emphasized the importance of non-clinical safety studies to identify potential risks **before** FIH trial
- Recommended a **stepwise approach**, with dose escalation only after careful evaluation of all safety & PK data
- Required the inclusion of healthy volunteers in the initial trial stages only after sufficient safety data has been obtained
- Recommended the use of adaptive trial designs to allow for modifications to the trial based on emerging data
- Required the **establishment of a safety monitoring committee** to oversee the conduct of the trial & evaluate safety data

- **Identify**
- **Mitigate**
- **Monitor**

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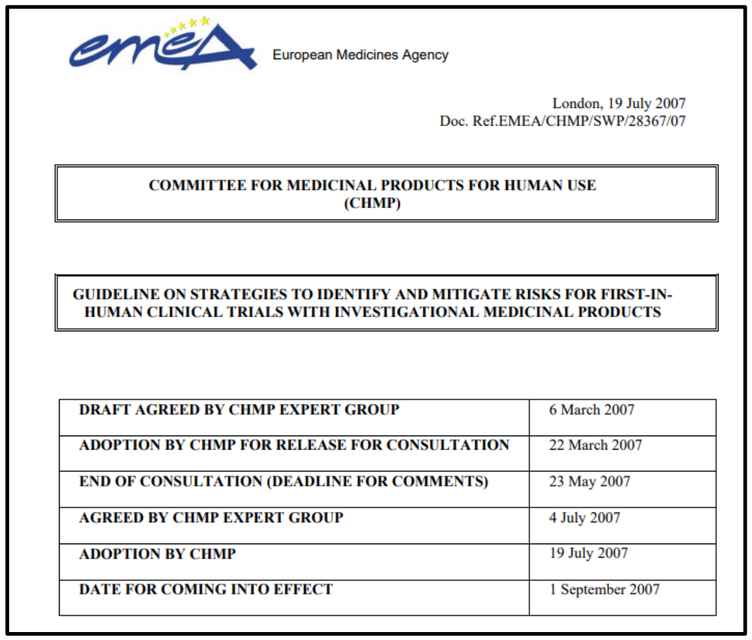
Theralizumab AKA TGN1412

MABEL Approach: Minimal Anticipated Biological Effect Level

Estimation of the first dose in humans



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- MABEL dose calculation should use ALL preclinical *in vitro* & *in vivo* information available from PK/PD data

1. target binding and receptor occupancy studies *in vitro* in target cells from human and the relevant animal species
2. concentration-response curves *in vitro* in target cells from human and the relevant animal species and dose/exposure-response *in vivo* in the relevant animal species
3. exposures at pharmacological doses in the relevant animal species

A conservative method for estimating the starting dose intended to maximize participant safety in clinical trials by identifying the lowest dose that is likely to produce a specific biological effect

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J Immunol Methods. 2015 Sep;424:43-52. doi: 10.1016/j.jim.2015.04.020. Epub 2015 May 7.

Cytokine release assays for the prediction of therapeutic mAb safety in first-in man trials--Whole blood cytokine release assays are poorly predictive for TGN1412 cytokine storm

S Vessillier [1], D Eastwood [2], B Fox [2], J Sathish [3], S Sethu [3], T Dougall [2], S J Thorpe [2], R Thorpe [2], R Stebbings [4]

- Preclinical testing failed to predict the severity of the cytokine storm experienced by 6 healthy male donors dosed with TGN1412 during the Phase 1 clinical trial

  -classical (most widely used) whole blood CRA format was not predictive of TGN1412 response in humans; however, can be successful for identification of cytokine release by molecules with other mechanisms of action

- This prompted the development of new *in vitro* cytokine release assays (CRAs) for mimicking *in vivo* cytokine release and lymphoproliferation

Although not mandatory, there is a regulatory expectation to perform CRA testing after TGN1412 incident

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Developments in Cytokine Release Assays

Cytokine. 2011 Jul;55(1):141-51. doi: 10.1016/j.cyto.2011.03.019. Epub 2011 Apr 13.

Endothelial cells co-stimulate peripheral blood mononuclear cell responses to monoclonal antibody TGN1412 in culture

Lucy Findlay [1], Giles Sharp, Bernard Fox, Christina Ball, C Jane Robinson, Christopher Bird, Richard Stebbings, David Eastwood, Meenu Wadhwa, Stephen Poole, Robin Thorpe, Susan J Thorpe

After TGN1412: Recent developments in cytokine release assays

R. Stebbings, D. Eastwood, S. Poole & R. Thorpe

Pages 75-82 | Received 29 May 2012, Accepted 10 Jul 2012, Published online: 11 Sep 2012

Summary:

- TGN1412 presented *in vitro* to peripheral blood mononuclear cells (PBMCs) in the presence of primary human umbilical vein endothelial cells (HUVEC), retaining classic endothelial markers mediated specific cytokine release

PBMC+HUVEC was predictive of cytokine release with TGN1412

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Theralizumab AKA TGN1412

Developments in Cytokine Release Assays

IMMUNOBIOLOGY

Preculture of PBMCs at high cell density increases sensitivity of T-cell responses, revealing cytokine release by CD28 superagonist TGN1412

Paula S. Römer,[1] Susanne Berr,[1] Elita Avota,[1] Shin-Young Na,[1] Manuela Battaglia,[2] Ineke ten Berge,[3] Hermann Einsele,[4] and Thomas Hünig[1]

[1]Institute for Virology and Immunobiology, University of Würzburg, Würzburg, Germany; [2]San Raffaele Diabetes Research Institute, Milan, Italy; [3]Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands; and [4]Medical Clinic II, University of Würzburg, Würzburg, Germany

Summary:

- Preculture of PBMC's at high density results in cytokine release during subsequent stimulation with soluble TGN1412
- Resetting PBMCs to lymph node-like activity improves the predictive value

High density PBMC preculture was predictive of cytokine release with TGN1412

Theralizumab AKA TGN1412

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Workshop Held

Workshop on Cytokine Release: State-of-the-Science, Current Challenges and Future Directions
Organized by the HESI Immunotoxicology Technical Committee

22 October 2013
Sheraton Silver Spring
Silver Spring, MD

Cytokine 85 (2016) 101–108

Contents lists available at ScienceDirect

Cytokine

journal homepage: www.journals.elsevier.com/cytokine

Cytokine release: A workshop proceedings on the state-of-the-science, current challenges and future directions

Christine Grimaldi [a], Deborah Finco [b], Madeline M. Fort [c], Daniel Gliddon [d,1], Kirsty Harper [d], Whitney S. Helms [e], Jane A. Mitchell [f], Raegan O'Lone [g], Stanley T. Parish [g,*], Marie-Soleil Piche [h], Daniel M. Reed [f], Gabriele Reichmann [i], Patricia C. Ryan [j], Richard Stebbings [k,2], Mindi Walker [l]

[a] Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT 06877, USA
[b] Pfizer Inc., 1 Eastern Point Road, Groton, CT 06340, USA
[c] Amgen, Inc., Comparative Biology and Safety Sciences, USA
[d] Envigo, Woolley Road, Alconbury, Huntingdon, Cambridgeshire PE28 4HS, United Kingdom
[e] US Food and Drug Administration, 10903 New Hampshire Ave, Rockville, MD, USA
[f] Imperial College London, London SW3 6LY, United Kingdom
[g] ILSI Health and Environmental Sciences Institute, 1156 15th St NW, Suite 200, Washington, DC 20005, USA
[h] Charles River Laboratories, 20222 Transcanadien, Senneville, QC H9X 3R3, Canada
[i] Paul-Ehrlich-Institute, Federal Institute for Vaccines and Biomedicines, Paul-Ehrlich Str. 51-59, 63225 Langen, Germany
[j] Medimmune, Inc., One MedImmune Way, Gaithersburg, MD 20878, USA
[k] The National Institute of Biological Standards and Controls, Blanche Ln, South Mimms, Potters Bar EN6 3QG, United Kingdom
[l] Johnson and Johnson, Inc., 1 Johnson and Johnson Plaza, New Brunswick, NJ 08933, USA

Objective:

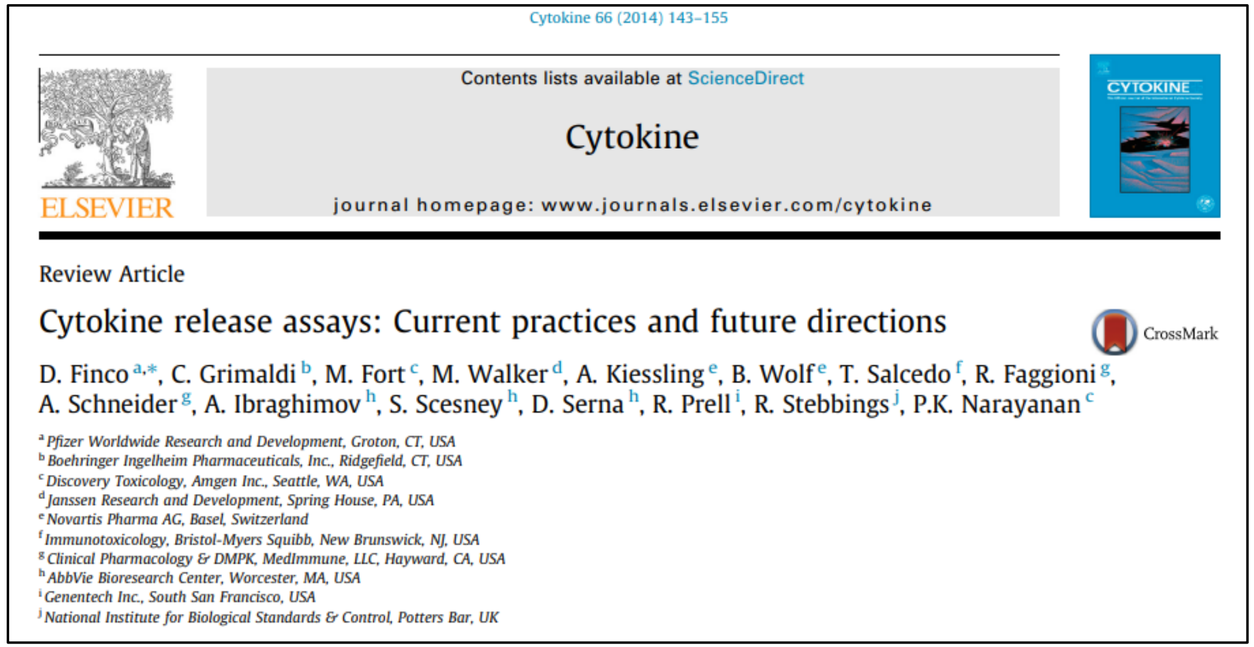
Bring together academic, pharmaceutical, CRO & health authority scientists to discuss novel approaches & current challenges for the use of cytokine release assays (CRAs) for the identification of CRS potential of novel monoclonal antibodies

Topics:

- Assessments of novel CRA formats
- Translatability/impact on dosing in the clinic
- Challenges with data interpretation
- Use of preclinical animal species to predict cytokine release
- Emerging/novel technologies for cytokine measurements
- Regulatory perspective

Theralizumab AKA TGN1412

Survey Conducted



Cytokine 66 (2014) 143–155

Contents lists available at ScienceDirect

Cytokine

journal homepage: www.journals.elsevier.com/cytokine

Review Article

Cytokine release assays: Current practices and future directions

D. Finco [a,*], C. Grimaldi [b], M. Fort [c], M. Walker [d], A. Kiessling [e], B. Wolf [e], T. Salcedo [f], R. Faggioni [g], A. Schneider [g], A. Ibraghimov [h], S. Scesney [h], D. Serna [h], R. Prell [i], R. Stebbings [j], P.K. Narayanan [c]

[a] Pfizer Worldwide Research and Development, Groton, CT, USA
[b] Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, USA
[c] Discovery Toxicology, Amgen Inc., Seattle, WA, USA
[d] Janssen Research and Development, Spring House, PA, USA
[e] Novartis Pharma AG, Basel, Switzerland
[f] Immunotoxicology, Bristol-Myers Squibb, New Brunswick, NJ, USA
[g] Clinical Pharmacology & DMPK, MedImmune, LLC, Hayward, CA, USA
[h] AbbVie Bioresearch Center, Worcester, MA, USA
[i] Genentech Inc., South San Francisco, USA
[j] National Institute for Biological Standards & Control, Potters Bar, UK

A single most appropriate system to predict CRS does not exist

A case-by case approach is recommended

Highlights

- Provided an overview of different approaches used by the pharmaceutical industry & CROs, for the use & application of CRAs based upon a survey & post survey follow-up conducted by HESI's Immunotoxicology Committee CRA Working Group

- Identified gaps/future directions

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Types of Survey Questions

- What CRA assays formats are you currently using?
- What cytokines do you measure?
- What assay format is used to measure cytokine levels?
- Have you evaluated any other approaches to assess for potential cytokine release?
- If using whole blood, is it undiluted or diluted?
- How many donors do you use?
- Do you report data relative to known positive controls?
- Do you report data as pg/mL?
- Would you stop a program based upon *in vitro* CRA results?

Total of 22 Questions

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Cytokine Release Assays (CRAs)

Challenges

- Lack of standardized assay formats & strategies for use
- Lack of standardized [+] and [-] controls
- Lack of consensus on interpretation of data

  -high Inter- & intra- donor variability

  -Target distribution or density in healthy donors may not be representative of patients
- Cannot be used to determine a threshold where the levels of cytokines release may be associated with serious CRS in the clinic
- Clinical translatability and predictability of CRS in the clinic
- Uncertainty regarding exactly how data should be used
- Lack of regulatory expectations

Only useful for hazard identification

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CAR T-cells

- Carry a particularly high risk of CRS & neurotoxicity

1st

KYMRIAH® (tisagenlecleucel) Suspension for IV infusion

NOVARTIS

- **Drug Class**: CD19-directed CAR T-cell immunotherapy
- **Indication**: B-cell acute lymphoblastic leukemia (ALL) & r/r ALL
- **First FDA approved**: August 30, 2017
- **CRS**: 61/79 patients (77%) with r/r ALL, including ≥ grade 3 in 48% patients; median onset of 3 days

The Food and Drug Administration (FDA) has approved six CAR T-cell therapies:

- **Abecma** (idecabtagene vicleucel)
- **Breyanzi** (lisocabtagene maraleucel)
- **Kymriah** (tisagenlecleucel)
- **Tecartus** (brexucabtagene autoleucel)
- **Yescarta** (axicabtagene ciloleucel)
- **Carvykti** (ciltacabtagene autoleucel)

Treatment of different blood cancers (either)

- Acute lymphoblastic leukemia [ALL]
- B-cell lymphoma, follicular lymphoma [FL]
- Mantle cell lymphoma
- Multiple myeloma

* Over 700 clinical trials of CAR T therapy have been registered at clinicaltrials.gov and many of these trials focus on solid tumors. However, **no CAR therapy has been approved for solid tumors yet**.

Bispecific T-cell Engagers

- Carry a particularly high risk of CRS and neurotoxicity

FIC for blood tumors

- **Drug Class**: Bispecific CD19-directed CD3 T-cell engager
- **Indication**: adults with acute B-cell lymphoblastic leukemia (ALL); r/r ALL
- **First FDA approved**: December 3, 2014
- **CRS**: 15% of patients with r/r ALL experience CRS (mostly mild), typically onset within 2 days; dose-limiting CRS observed

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Bispecific T-cell Engagers

1st

FIC for solid tumors

KIMMTRAK
(tebentafusp-tebn)
Injection for Intravenous Use 100 mcg/0.5 mL
IMMUNOCORE

- **Drug Class**: Bispecific gp100 peptide-HLA-A*0.2:01 directed CD3 T-cell engager
- **Indication**: Unresectable or metastatic m uveal melanoma
- **First FDA approved**: January 25, 2022
- **CRS**: Occurred in 89% of patients (mostly mild) with 0.8% being Grade 3-4

CD3 Bispecific T-cell Engagers

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PUBLIC

FDA-HESI Public Workshop: Preclinical and Translational Safety Assessment of CD3 Bispecifics

OCTOBER 1 - 2, 2018

JOURNAL OF IMMUNOTOXICOLOGY
2020, VOL. 17, NO. 1, 67–85
https://doi.org/10.1080/1547691X.2020.1729902

Taylor & Francis
Taylor & Francis Group

RESEARCH ARTICLE

OPEN ACCESS Check for updates

Summary of a workshop on preclinical and translational safety assessment of CD3 bispecifics

Cris Kamperschroer[a*], Jacintha Shenton[b*], Hervé Lebrec[c], John K. Leighton[d], Paul A. Moore[e] and Oliver Thomas[f]

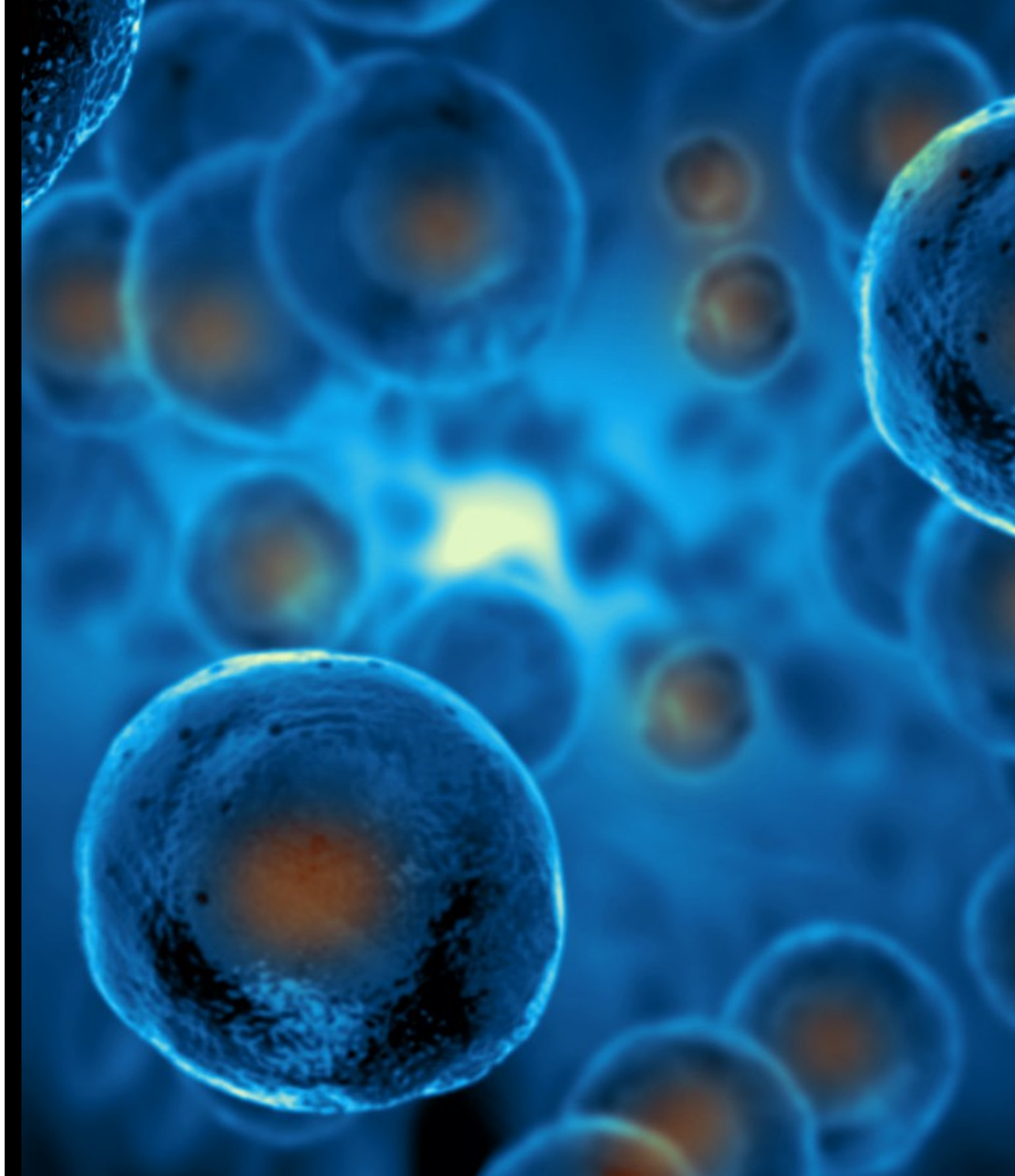
[a]Pfizer Worldwide Research and Development, Groton, CT, USA; [b]Nonclinical Safety, Janssen R&D, Spring House, PA, USA; [c]Translational Safety, Amgen Research, South San Francisco, CA, USA; [d]U. S. Food and Drug Administration, Silver Spring, MD, USA; [e]MacroGenics, Rockville, MD, USA; [f]Translational Safety, Amgen Research, Munich, Germany

Objective:

- Brought together subject matter experts from pharmaceutical, academic, health authority & CROs to discuss the current-state-of-the-science, challenges and future directions of CD3 bispecifics
- Provide insights into their preclinical and translational safety assessments

- CD3 bispecifics and their effect on T-cell biology,
- Target (tumor antigen) expression and liability assessment,
- Relevance of molecular design and bioactivity to toxicity assessment and potential,
- In-vivo pharmacology and toxicology,
- In-vitro assays to assess cytokine release,
- First-In-Humans (FIH) dose selection,
- Clinical Experience
- Translation of nonclinical findings to the clinic.

In Vitro Cytokine Release Assays (CRAs)



Cytokine Release Assays (CRAs)

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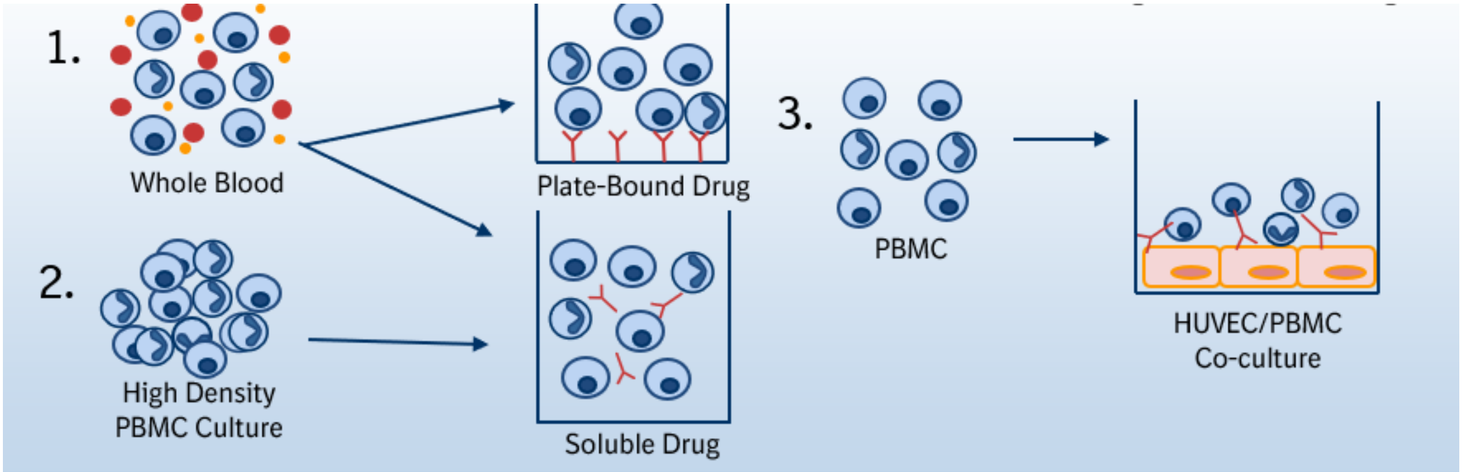
Common CRA formats	Considerations
Whole Blood (soluble & plate-bound)	Most widely used, closely replicates *in vivo* conditions, especially for targets expressed on monocytes & neutrophils; cells in circulation; sensitive for molecules that stimulate FcγRI-mediated cytokine release
High Density PBMC pre-culture*	PBMCs (T cells, B cells, NK cells & monocytes); extracted from whole blood so less physiologically relevant, more sensitive to T-cell activation; cells are in a "primed" lymph node-like state; predictive of TGN1412 unmatched donor samples
PBMC/HUVEC co-culture*	Targets expressed on endothelial cells; cell contact dependent, produces target cross-linking & Fc binding; predictive of TGN1412
PBMC/BOEC Co-culture^	Produces target cross-linking & Fc binding matched donor samples Patented assay; only at CRO

PBMC-peripheral blood mononuclear cells
HUVEC-human umbilical vein endothelial cells
BOEC- blood outgrowth endothelial cells

*2011 Romer et al
^2015 Mitchelle, et al



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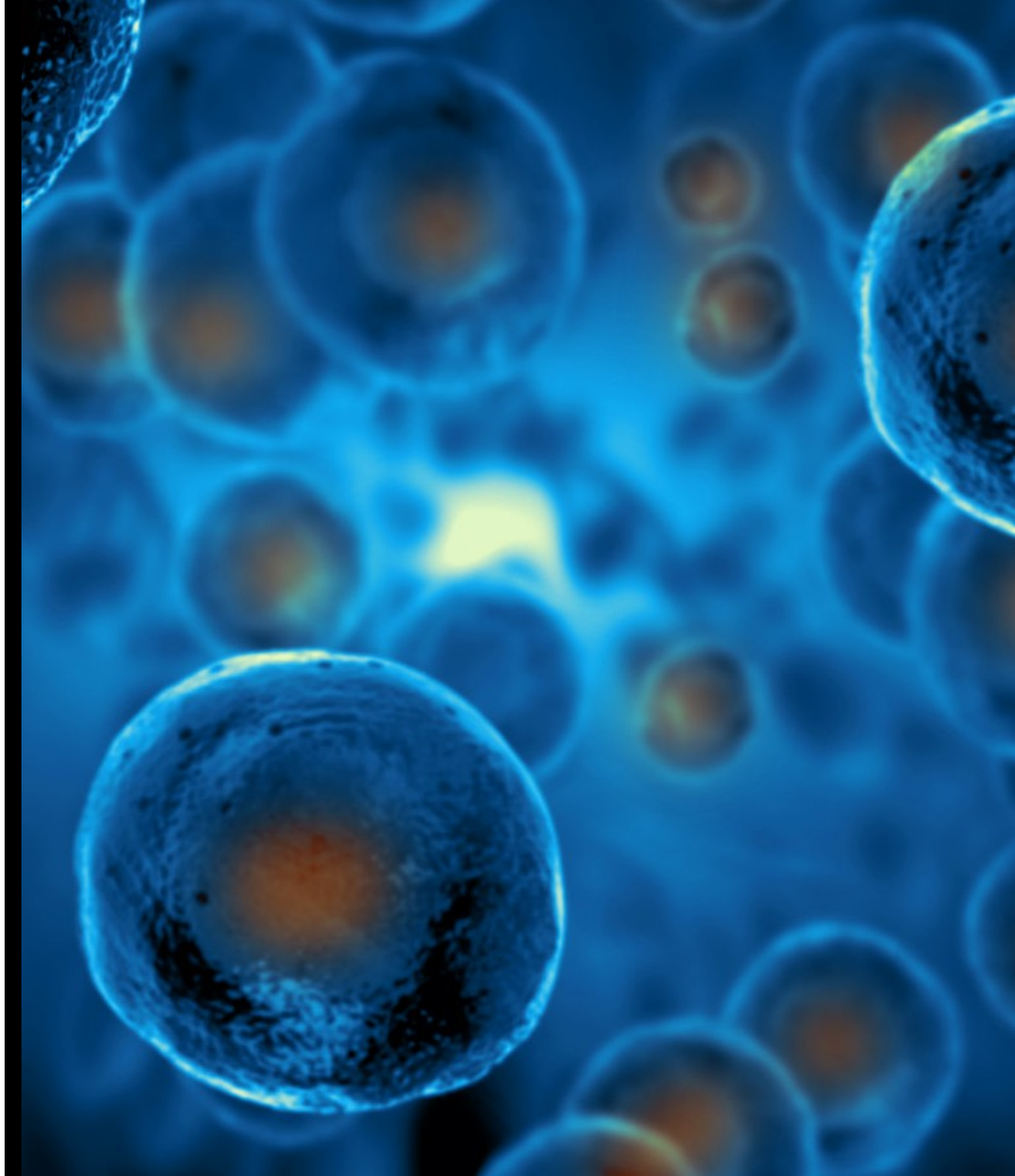
Cytokine Release Assays (CRAs)

- **Multiple healthy human donors:**
  Minimum of 10 (more the better)
- **Pro-inflammatory cytokine standard panel:**
  IL-2, IL-6, TNFα & IFNγ
- **Relevant positive controls:**
  Depends on the mechanism of action of drug product being evaluated
  OKT3®, Campath-1H®, Rituxan®, BLINCYTO & PMA + IONO
  Phorbol Myristate Acetate + Ionomycin
- **Relevant negative controls: Erbitux (anti-EGFR), media only, PBS only**
- **Drug product titrations**
  ~ 100 – 0.1 ug/mL (titrate 3-fold)
- **Platform:**
  Meso Scale Discovery (MSD) Electrochemiluminescence
  Luminex

Data Interpretation

- ❑ **Readout: absolute cytokine concentrations (pg/mL)**
- ❑ **Individual cytokine responses to test article is compared to "all" [-] & [+] controls**
- ❑ **Individual cytokine responses to each [+] control is compared to each [-] control**
- ❑ **Supported by statistical analysis**

Current Status



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General Strategy

Parameter	Consideration
Target	• Secreted/soluble monomeric proteins • Membrane/surface protein; soluble oligomer
Target Expression	• Target is not expressed on peripheral whole blood cells or endothelium • Target is expressed on peripheral whole blood or endothelium
Antibody Format	• Novel format (oligonucleotides, recombinant proteins, oncolytic viruses, adeno-associated virus)
Antibody Fc	• Antibody-dependent cellular cytotoxicity and/or complement-dependent cytotoxicity (CDC) activity
Mechanism of Action	• Robust pharmacology data exists & the hazard for cytokine release has already been identified • Immunostimulatory & agonistic antibodies which may stimulate (indirectly or directly) & have exaggerated pharmacological activity

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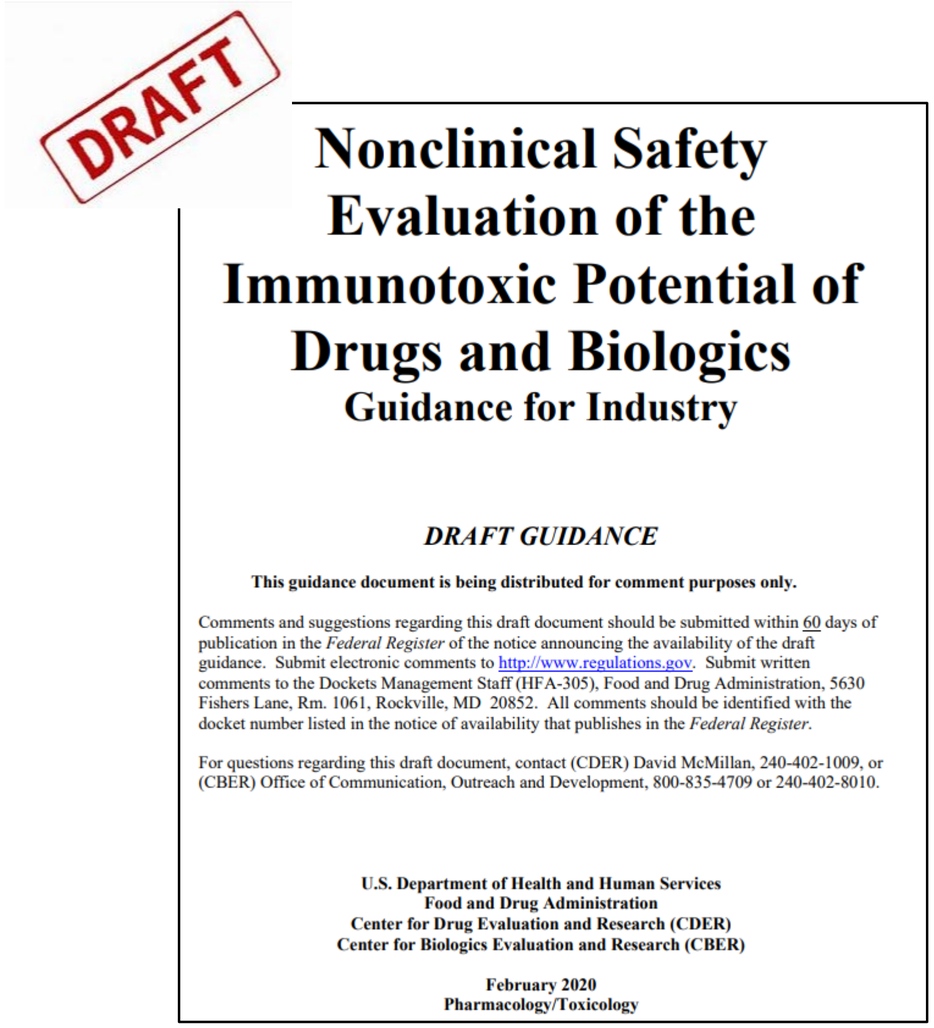
Current Status



Excessive release of cytokines can cause severe adverse reactions as shown by the near-fatal clinical responses to the monoclonal antibody TGN 1412.[8] There are now commonly used in vitro models available to evaluate the potential for this risk.[9] As alternative models are developed and refined, additional assays may become available.

An assessment of the potential for cytokine release syndrome caused by therapeutic proteins using unstimulated human cells in both plate-bound (or other assays that can assess the contribution of crosslinking of receptors) and soluble formats with appropriate positive and negative controls.[10] These assays are considered critical for hazard identification. If the assays used to characterize the primary pharmacology of the product have already demonstrated that the product has a clear potential to directly cause cytokine release (e.g., a CD3 bispecific T cell redirector), these assays are usually not necessary, as the hazard has already been identified. Similarly, if one assay is positive, then an assay in the other format may not be needed.

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Cytokine: X 2 (2020) 100042

Development of the first reference antibody panel for qualification and validation of cytokine release assay platforms – Report of an international collaborative study

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11 Laboratories

Challenge:

- Lack of availability of standard positive and negative control monoclonal antibodies for use in cytokine assay qualification

Objective

Develop a positive & negative control reference panel to increase confidence in the robustness of a CRA platform to identify a potential CRS for novel immunomodulatory drugs

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Study design:

The **National Institute for Biological Standards and Control** (NIBSC) developed a reference panel of lyophilized monoclonal antibodies known to induce CRS in the clinic, manufactured according to the respective published sequences of **Campath-1H® , OKT-3® & TGN1412**, as well as 3 isotype matched negative controls

The relative capacity of these control monoclonal antibodies known to stimulate the release of **IFN-γ, IL-2, TNF-α and IL-6** *in vitro* was evaluated

Assay type	IncubationTime	Number of participants	Number of donors (all participants)
PBMC-SP	18–24 h	4	35
	48 h	3	26
PBMC-AQ	24 h	1	8
PBL/HUVEC	24 h	1	8
WB-AQ	24 h	6	54
	48 h	1	8
dWB-AQ	48 h	1	12
dWB-SP*	48 h	1	15

PBMC: Peripheral Blood Mononuclear Cells; PBL: peripheral blood leukocytes; HUVEC: human umbilical vein endothelial cells; WB: Whole Blood; dWB: diluted Whole Blood; SP: solid phase; AQ: Aqueous Phase; dWB-SP* corresponds to the bead coated method.

Samples & cytokine measurements:
(1)NIBSC: Meso Scale Discovery (MSD)
(2)Participants: MSD or Luminex

Interpretation of results:
Fold increase in cytokine release between positive controls and their respective isotypes

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Summary:

- The performance of the positive controls in the various CRA formats support the hypothesis that **no single CRA platform is optimal for every drug with CRS potential**

- However, the relative ability of each positive control to induce either **IL-2, TNF-α, IL-6, or IFN-γ varied with each assay platform**. Therefore, **careful understanding of the mechanism of action** of a test antibody is critical for appropriate choice of a CRA platform to identify potential CRA risk

- These results emphasize the **value of the use of multiple positive and negative controls** for the appropriate qualification of a CRA.

- The results confirm that the positive control **monoclonal antibodies produced by NIBSC induced the release of IFN-γ, TNFα, IL-2 and IL-6 in a variety of CRA platforms**, replicating previously published data generated by the corresponding clinical therapeutics

- This panel of positive control antibodies and the negative isotype controls are **suitable for use for the qualification and validation of CRAs, comparison of different CRAs** (eg solid vs aqueous phase), and intra- and inter-laboratory comparison of CRA performance

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Innovative Medicines Initiative

The Innovative Medicines Initiative (IMI) is the world's biggest public-private partnership (PPP) in the life sciences. It is a partnership between the European Union (represented by the European Commission) and the European pharmaceutical industry (represented by EFPIA, the European Federation of Pharmaceutical Industries and Associations). For the period 2014-2020 the IMI2 programme budget amounts to €3.3 billion.

imi innovative medicines initiative

The imSAVAR is an Innovative Medicines Initiative funded project that aims to develop a standard for integrated nonclinical safety overviews for immune-modulatory investigational new drugs (IND) and clinical trial applications (CTA).

imSAVAR

28 partners from 11 countries with a multitude of expertise

CRA working group focused on standardizing CRA formats, data interpretation & *in vitro*-to-*in vivo* translatability & prediction of cytokine responses

Summary

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- CRS is a potentially life-threatening adverse event characterized by an excessive release of pro-inflammatory cytokines
- In the clinic, signs of CRS should be closely monitored & appropriate mitigation steps taken to reduce symptoms
- Although there is not full alignment on a single grading system for CRS, cross-classification according to multiple grading scales offers guidance to clinicians in determining severity
- Immunotherapies for cancer, like CAR T cell & T-cell engagers pose a particularly high risk of CRS
- Significant progress has been made in designing & developing improved *in vitro* CRAs to predict potential CRS in the clinic since the “cytokine Storm” experience with TGN1412 in 2006
- Currently, there is still a lack of consistency across biopharmaceutical companies performing the assays “ONLY USEFUL FOR HAZARD ID”
- Efforts are ongoing to address these challenges (e.g., regulatory guidance & collaborative consortiums) “MORE TO COME, SO STAY TUNED”

HESI IMMUNO-SAFETY TECHNICAL COMMITTEE

Thank you!