Bispecific T-cell engagers From receptor to systems' level modeling to rationalize dose-effect relationships

No need to take pictures: get the slides

Alexander Kulesza[1,2], Wilbert de Witte[1], Luis David Jimenez Franco[1], Venetia Karamitsou[1],Stephan Schaller[1]

[1] ESQlabs, Saterland, Germany

[2] University Namur, Belgium

Phoenix, AZ

November 10 - 13, 2024

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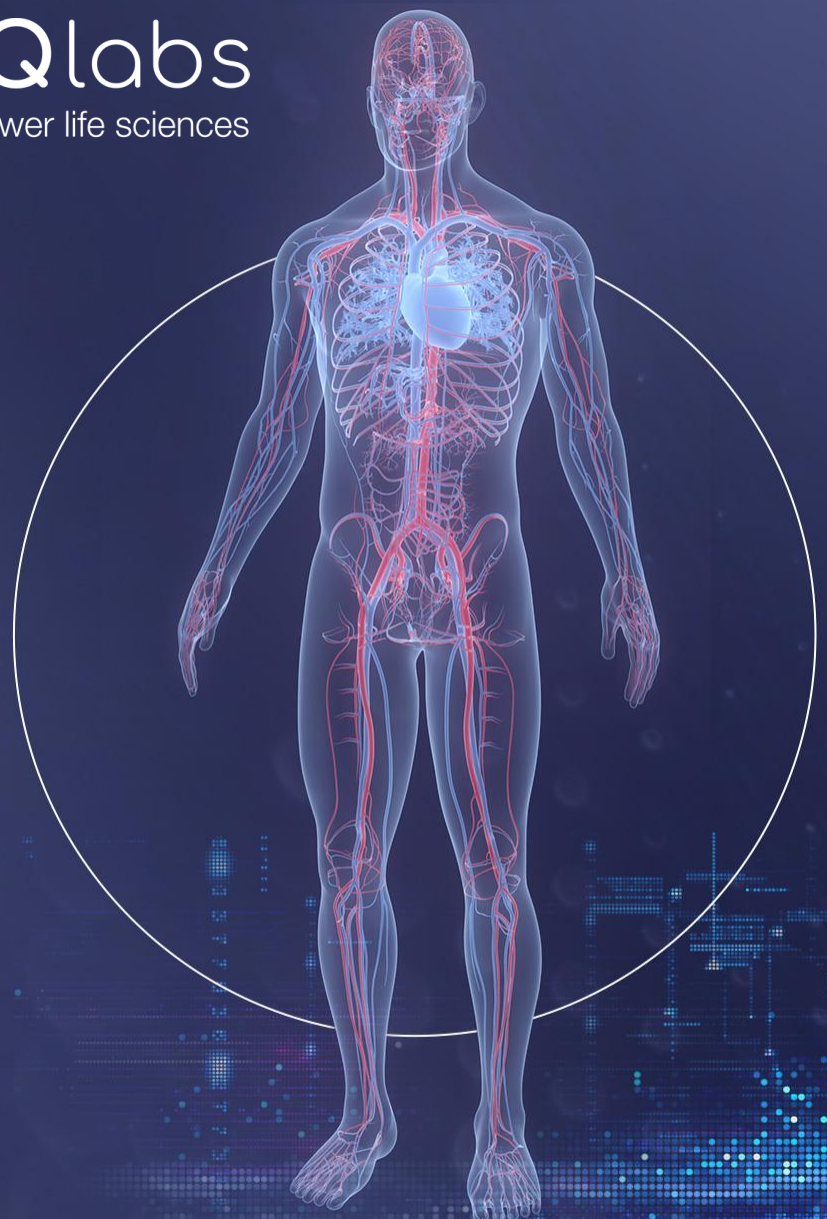

Bispecific T-cell engagers

From receptor to systems' level modeling to rationalize dose-effect relationships

Alexander Kulesza[1,2], Wilbert de Witte[1],
Luis David Jimenez Franco[1], Venetia Karamitsou[1],Stephan Schaller[1]
[1] ESQlabs, Saterland, Germany
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2024/11/12 – ACoP15 – Phoenix

Agenda

SLIDES

Disclosures:
AK is employee of ESQlabs GmbH, holds shares of CreativeQuantum GmbH and Ablatom SAS

01 BISPECIFIC T-CELL ENGAGERS?
Introduction, challenges and available models

02 OPPORTUNITES FOR MECHANISTC MODELING
Literature, published models, current work

a Cellular and systems level
A whole-body T-cell PBPK approach

b Receptor level and cellular level
Immune synapse modeling

c Cellular and systems level
Towards modeling of CRS incidence, severity and mitigation

WHAT ARE BISPECIFIC T-CELL ENGAGERS?

A very short introduction

Recombination of a Mixture of Univalent Antibody Fragments of Different Specificity[1]

A. NISONOFF
M. M. RIVERS

Department of Microbiology
University of Illinois
Urbana, Illinois

Received March 27, 1961

oxidized, the recombined product contains a considerable amount of bivalent 5S "hybrid antibody" (6, 7).[1] The recombination of fragments of two different specificities appears to be essentially random, as shown by quantitative analysis of the

sults indicate that a reconstituted 5S antibody can induce passive hemagglutination, and that reconstituted 5S hybrid antibody preparations produce passive mixed agglutination of two visually distinguishable red cell types, each coated with a different antigen. With an artificial mixture of 5S antibodies (not hybridized), mixed agglutination does not occur; the two cell types are aggluti-

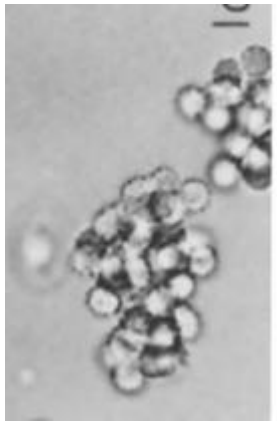
SEROLOGIC DEMONSTRATION OF DUAL SPECIFICITY OF RABBIT BIVALENT HYBRID ANTIBODY*

BY H. H. FUDENBERG, M.D., GENEVIEVE DREWS, M.D., AND A. NISONOFF, PH.D.

(From the Department of Medicine, University of California School of Medicine, San Francisco, and the Department of Microbiology, University of Illinois, Urbana)

PLATE 5

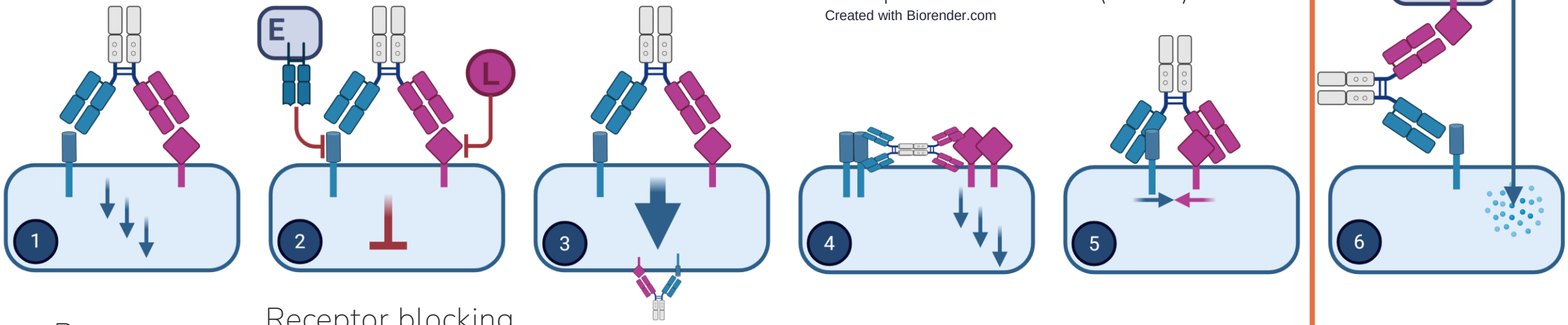
(Received for publication, September 4, 1963)



Obtained by peptic digestion (pepsin plus mild reduction of purified antibody) and then recombined by oxidation

WHAT ARE BISPECIFIC T-CELL ENGAGERS?

A very short introduction



Adopted from Vafa (2020)

Created with Biorender.com

Receptor activation

Receptor blocking and inhibition cell-bound or soluble ligand binding

Receptor internalization

Receptor clustering

Receptor association

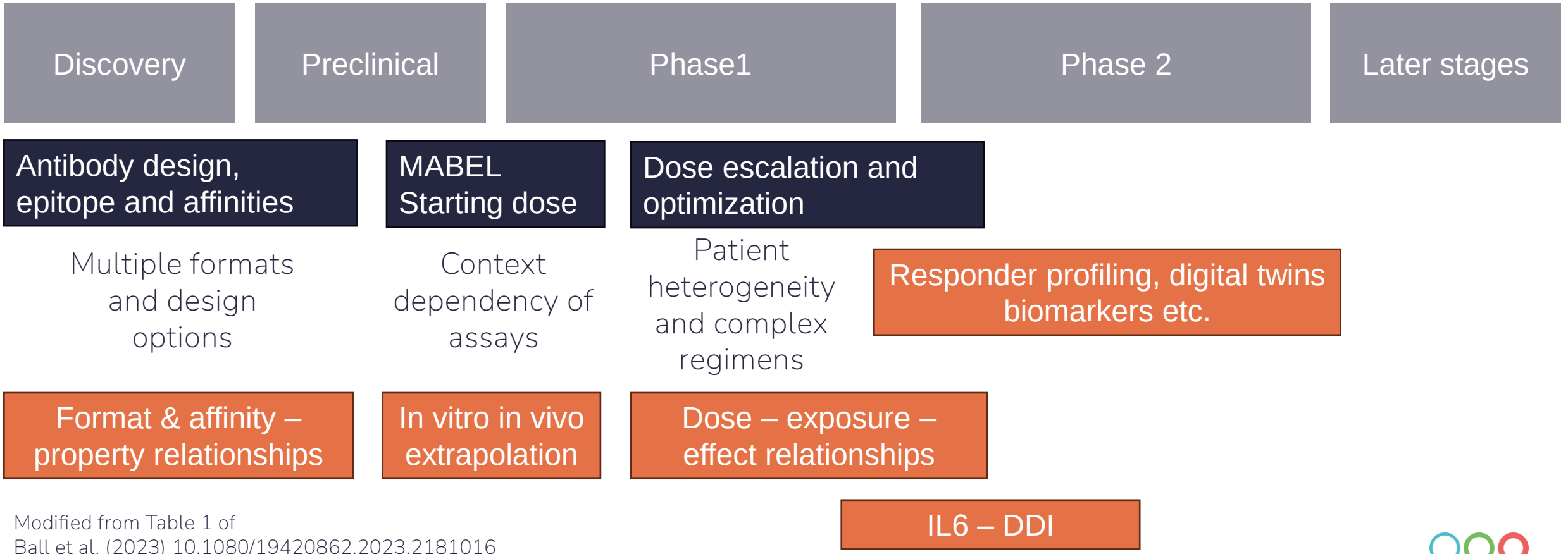
Bicellular binding and retargeting of effector cells

A bispecific T-cell engager is a protein that simultaneously binds through a target antigen on a tumor cell and CD3 on a T-cell to form a TCR-independent artificial immune synapse and circumvent HLA restriction

Vafa (2020) 10.3389/fonc.2020.00446

WHAT ARE BISPECIFIC T-CELL ENGAGERS?

A very short introduction



Modified from Table 1 of
Ball et al. (2023) 10.1080/19420862.2023.2181016
See also Qi (2023) 10.1016/j.tips.2023.09.009

The bsTCE modeling landscape

2018 Schropp et al.

2019 Betts et al.

2020 Jiang et al.

2020 Hosseini et al.

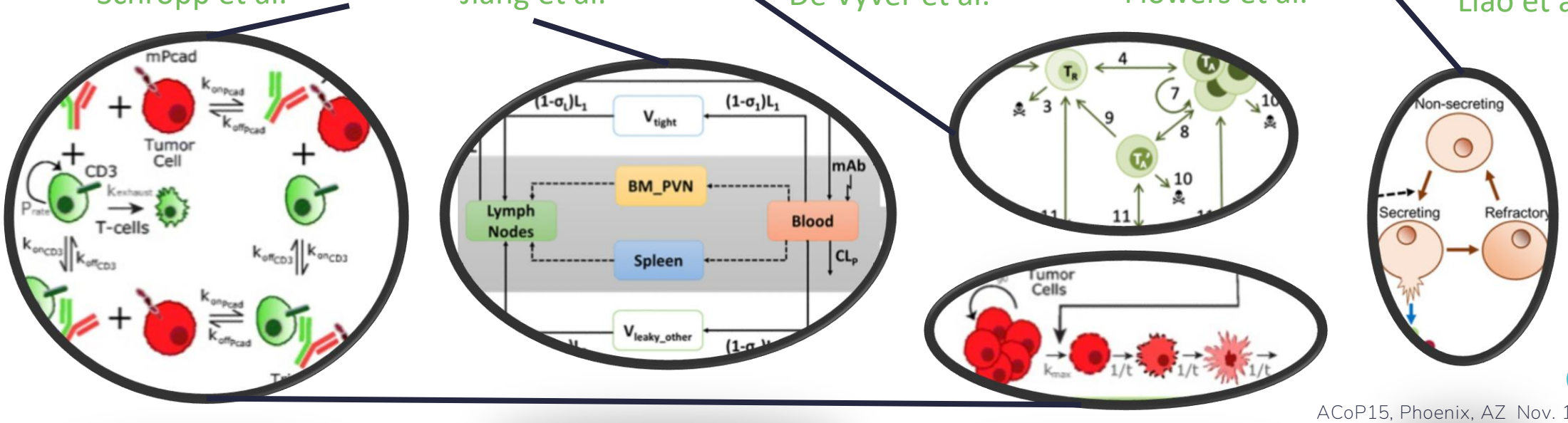
2021 De Vyver et al.

2022 Yoneyama et al.

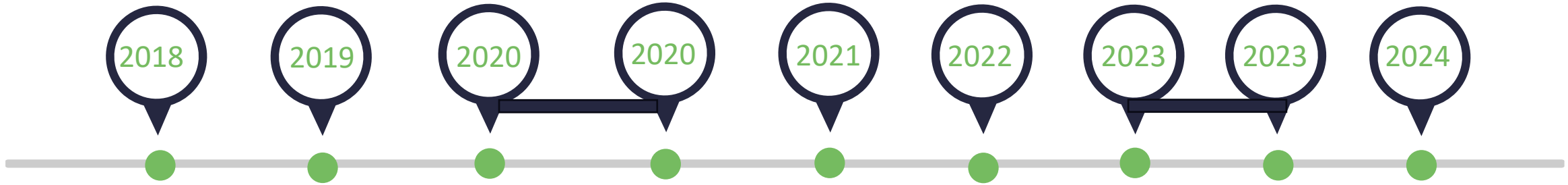
2023 Flowers et al.

2023 Wedell et al.

2024 Liao et al.



The bsTCE modeling landscape



Schropp et al.

Betts et al.

Jiang et al.

Hosseini et al.

De Vyver et al.

Yoneyama et al.

Flowers et al.

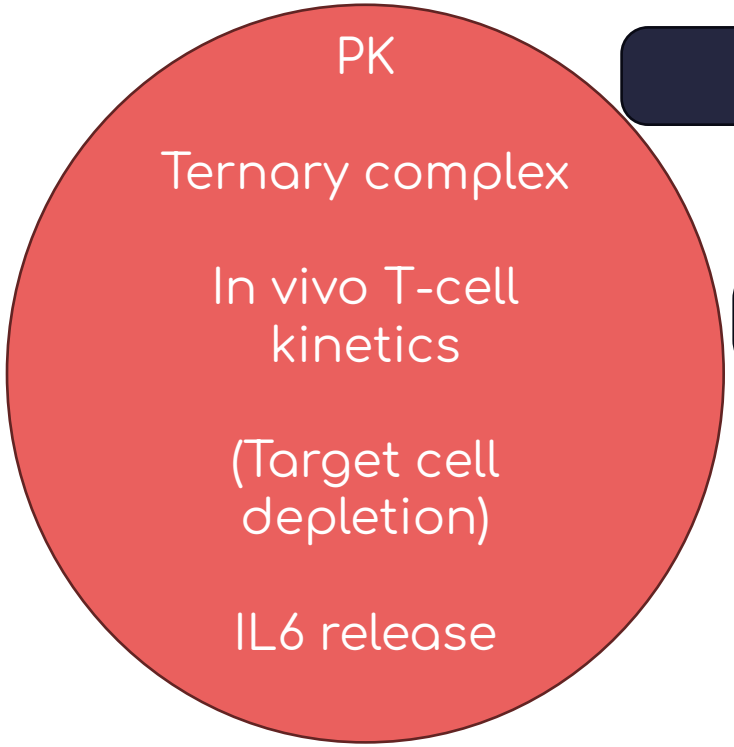
Wedell et al.

Liao et al.

COMPARTMENTS	O	O	+	+	-	++	-	O	+
RECEPTORS	++	+	O	-	O	+	++	O	+
T-CELLS	-	O	-	++	++	O	-	-	++
CYTOKINES	-	-	O	O	-	O	-	++	-

A bsTCE modeling challenge: Cytokine release syndrome

- How can we better design the therapetic window?
  - Early prediction of cytokine release profiles from affinities
- How do we proceed more efficiently from the starting dose towards the optimal doses?
  - Simulation of cytokine release under step-up dosing, escalation, CRS mitigation, with clinically relevant regimen
- How do we determine which have a low benefit/risk and how can we address that?
  - Explore between patient variability in CRS, support biomarker programs and use virtual twins



01 - Biodistribution *HER2xCD3*

Predict how components reach and penetrate tissues and tumors

02 - Immune synapse *CD19xCD3*

Better context unification for design -> T-cell function, MABEL and dose-effect

03 – Immune networks *CD19xCD3*

Better representativity of individual risk for CRS and mitigation

EMERGENT TOPICS

T-CELL BIODISTRIBUTION

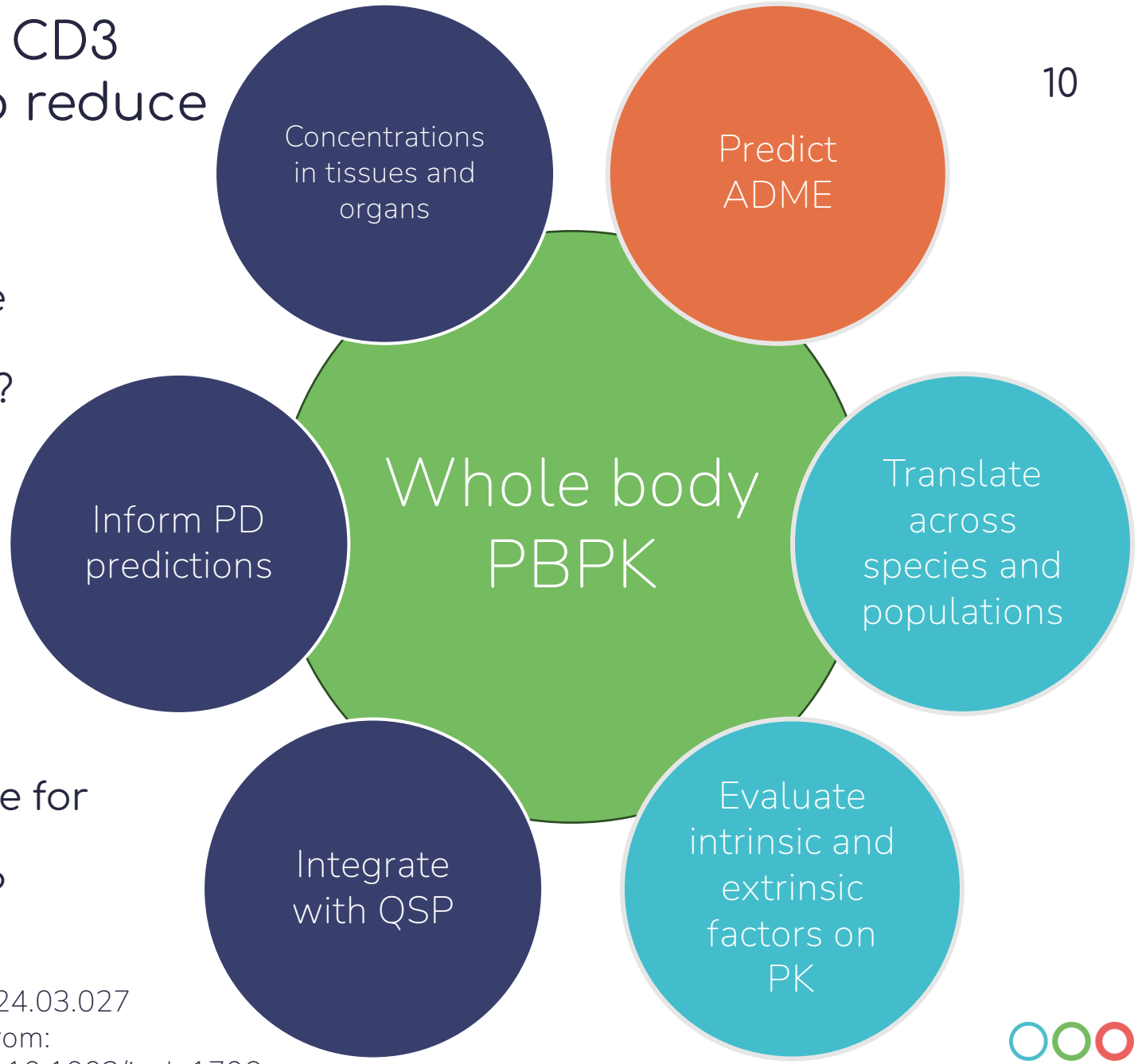
IMMUNE SYNAPSE

CRS INCIDENCE & MITIGATION

Lowering CD3 affinity to reduce CRS

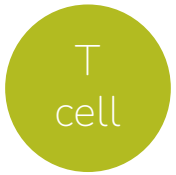
Consequece for T-cell activation ??

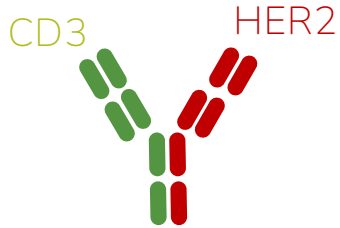
Consequence for tumor penetration?



See Qin (2024) 10.1016/j.apsb.2024.03.027
Scheme adopted from:
Gibbs et al. (2020) 10.1002/jcph.1706

A WB-PBPK T-cell / bsTCE model in PK-Sim / MoBi





1 Implement T cell in existing mouse mAb PBPK model in PKSim	2 Capture the distribution of antibody with CD3 binding only	3 Capture the distribution of TDB with CD3 and tumor target binding
Literature based T cell PBPK model from Khot *et al.* 2019	Distribution of gD/CD3 TDB	Distribution of HER2/CD3 TDB
Available data is exogenous active T cell	Distribution data from Mandikian *et al.* 2018	Distribution data from Mandikian *et al.* 2018
Optimization of T cell distribution properties in PKSim PBPK model backbone	Reproduce how CD3 affinity alters TDB distribution to T cell rich tissues	Reproduce how CD3 affinity and target binding alters TDB distribution

Measurement and Quantitative Characterization of Whole-Body Pharmacokinetics of Exogenously Administered T Cells in Mice

Antari Khot, Satoko Matsueda, Veena A. Thomas, Richard C. Koya, and Dhaval K. Shah

Journal of Pharmacology and Experimental Therapeutics March 2019, 368 (3) 503-513; DOI: https://doi.org/10.1124/jpet.118.252858

LARGE MOLECULE THERAPEUTICS | APRIL 01 2018

Relative Target Affinities of T-Cell–Dependent Bispecific Antibodies Determine Biodistribution in a Solid Tumor Mouse Model FREE

Danielle Mandikian; Nene Takahashi; Amy A. Lo; Ji Li; Jeffrey Eastham-Anderson; Dionysos Slaga; Jason Ho; Maria Hristopoulos; Robyn Clark; Klara Totpal; Kedan Lin; Sean B. Joseph; Mark S. Dennis; Saileta Prabhu; Teemu T. Junttila ✉; C. Andrew Boswell ✉

Susilo (2022) www.page-meeting.org/?abstract=10024

EMERGENT TOPICS

T-CELL BIODISTRIBUTION

IMMUNE SYNAPSE

CRS INCIDENCE & MITIGATION

A WB-PBPK T-cell model in PK-Sim / MoBi

Implemented T cell PBPK model from Khot et al. into PKSim protein PBPK model backbone

- PKSim protein PBPK model has been validated across different species and different proteins (from small peptides to mAbs)

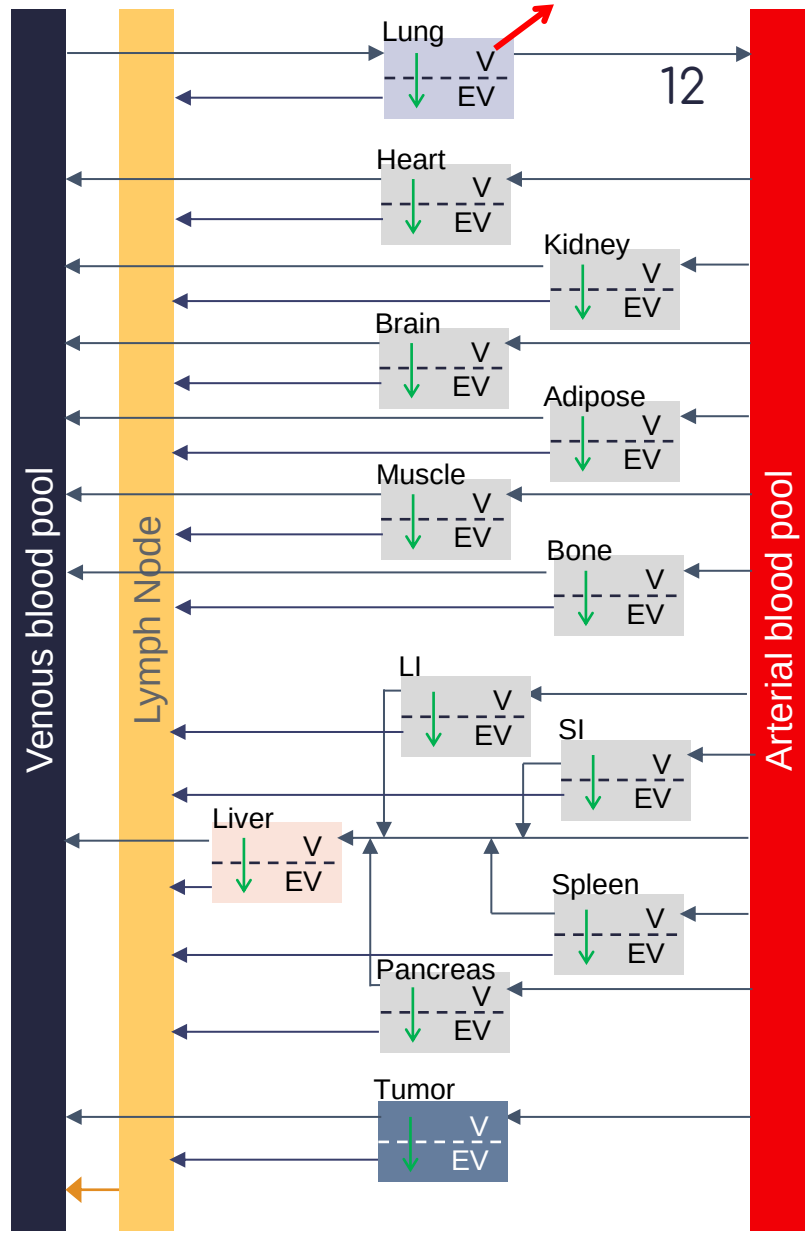
Single location-agnostic lymph node compartment

Two organ-specific parameters to calibrate:

- T cell transmigration rate: T cell migration from vascular to extravascular space
- T cell retention factor: accounting for exogenous T cell retention in the organ

Major limitations / assumptions:

- T cell elimination only in the lung vascular space
- No T cell proliferation (short time scale)



Niederalt (2018) 10.1007/s10928-017-9559-4
Khot (2019) 10.1124/jpet.118.252858
Susilo (2022) www.page-meeting.org/?abstract=10024

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T-CELL BIODISTRIBUTION

IMMUNE SYNAPSE

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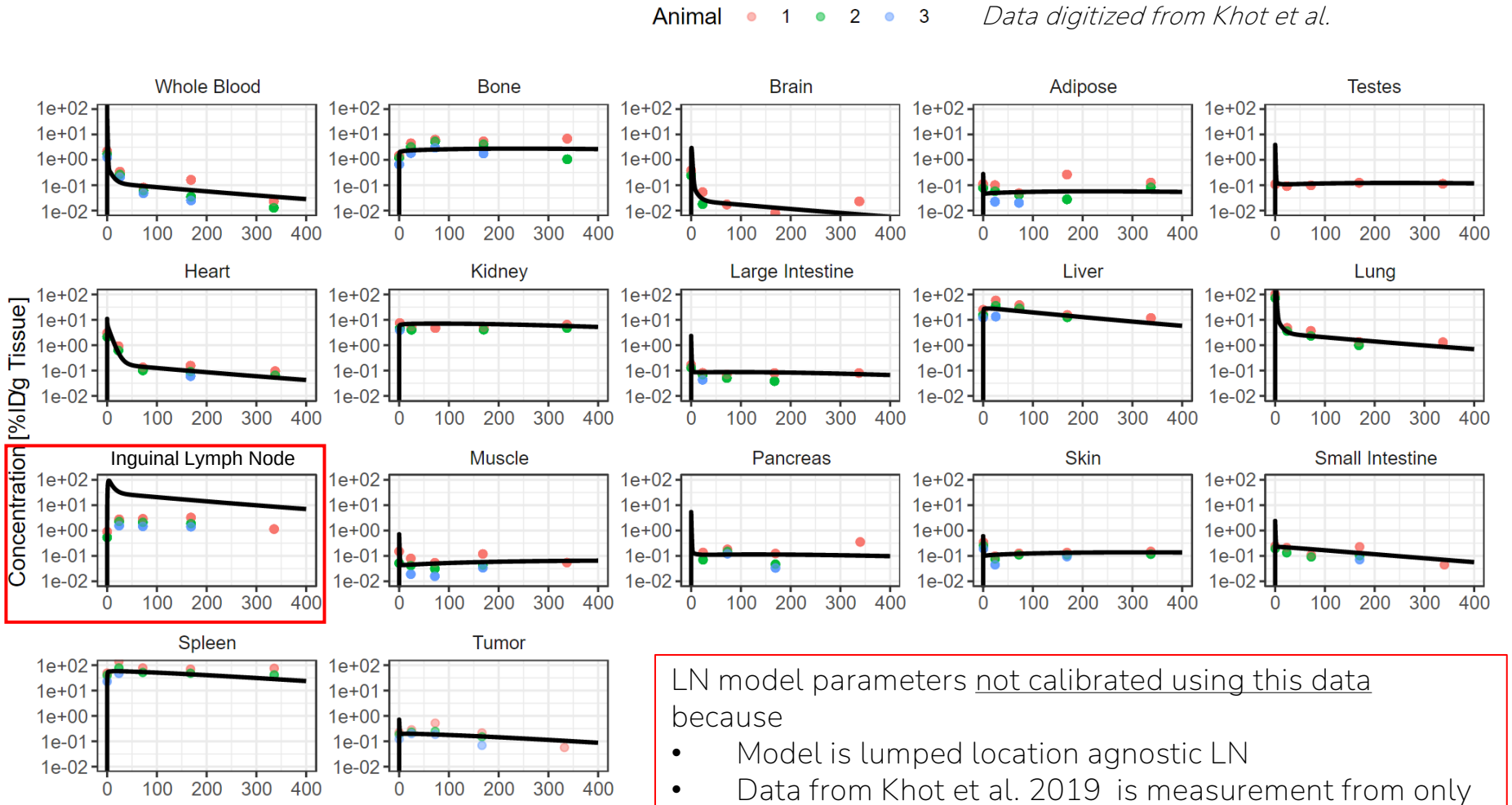
A WB-PBPK T-cell model in PK-Sim / MoBi

Model simulation captures the distribution of exogenously administered active T cells

→ Base understanding on T cell distribution



Khot (2019) 10.1124/jpet.118.252858

LN model parameters <u>not calibrated using this data</u> because

- Model is lumped location agnostic LN
- Data from Khot et al. 2019 is measurement from only the inguinal LN

EMERGENT TOPICS

T-CELL BIODISTRIBUTION

IMMUNE SYNAPSE

CRS INCIDENCE & MITIGATION

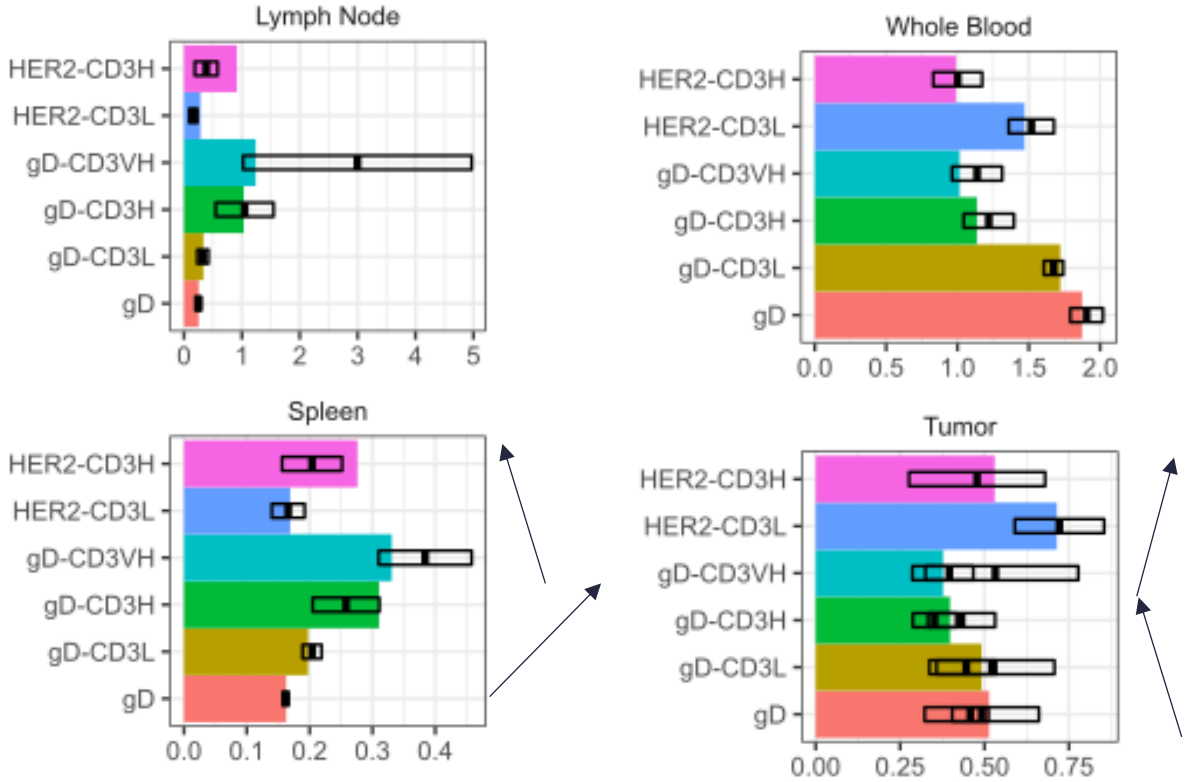
A WB-PBPK T-cell / bsTCE model in PK-Sim / MoBi

→ How affinities affect in vivo biodistribution

Higher CD3 affinity:

- Reduces plasma PK
- Increases HER2/CD3 TDB distribution to T-cell rich tissues
- Reduces HER2/CD3 TDB concentration in HER2+ tumor

Addition of HER2 target in the TDB reduces plasma and spleen concentration



Khot (2019) 10.1124/jpet.118.252858
Mandikian (2018) 10.1158/1535-7163.MCT-17-0657
Susilo (2022, 2024) www.page-meeting.org/?abstract=10024; *to be submitted for publication*

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T-CELL BIODISTRIBUTION

IMMUNE SYNAPSE

CRS INCIDENCE & MITIGATION

Summary

- It is possible to use WB-PBPK platforms for describing T-cell and biomolecular distributions with minor modifications
- Kinetics / biodistribution of exogenous T-cells and labeled bsTCE impacted by T-cell biodistribution (as a function of affinities) can be described with that framework

Ongoing work

- Investigating synapse formation and TMDD in the WB-PBPK framework extending „affinity – effects“ to „affinity – on/off tumor effects“

M. Susilo et al.

Genentech
A Member of the Roche Group

Next steps

- Fully modular bsTCE model in MoBi v12
- Integration with mechanistic and structured TGI models to describe indication-specific tumor penetration and modulation of the TME

A basic CD3-Target receptor binding model

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T-CELL BIODISTRIBUTION

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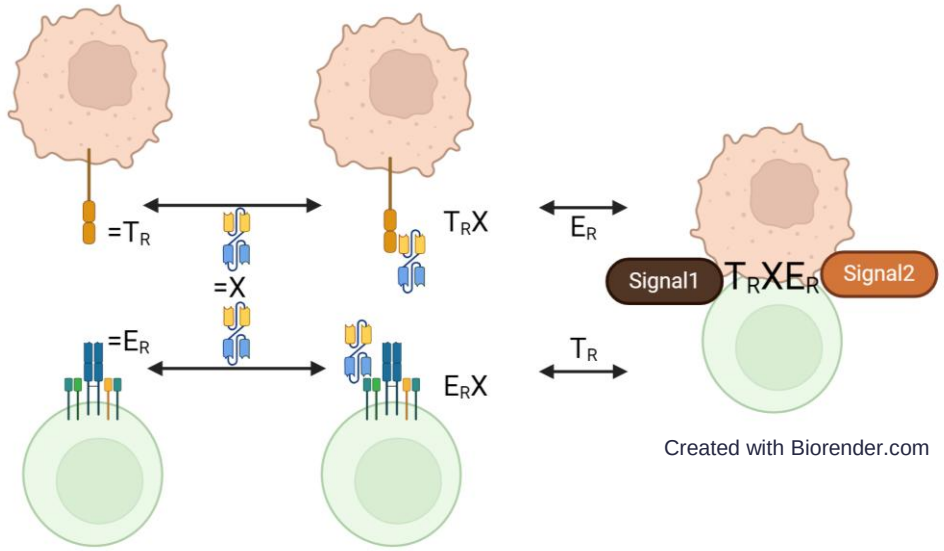
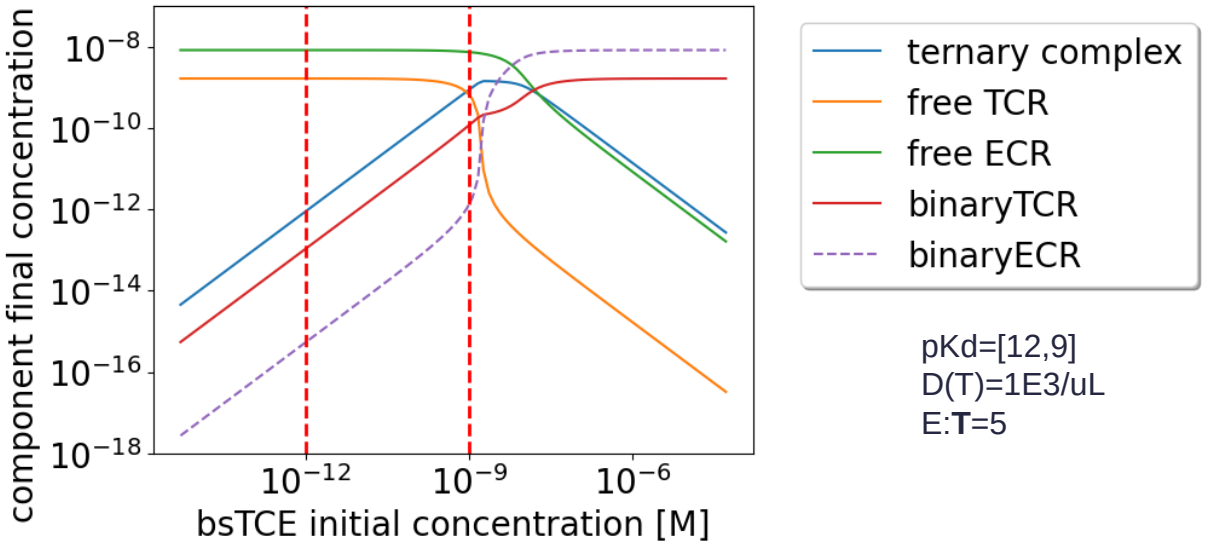
CRS INCIDENCE & MITIGATION



Created with Biorender.com

An often used model for the immune synapse is a simple version of the homogeneous, well stirred phase binding between the bispecific and freely diffusible receptors leading to ternary complex formation.



pKd=[12,9]
D(T)=1E3/uL
E:**T**=5

Models are sensitive to

CD3/target affinity

Cell density

E:T ratio

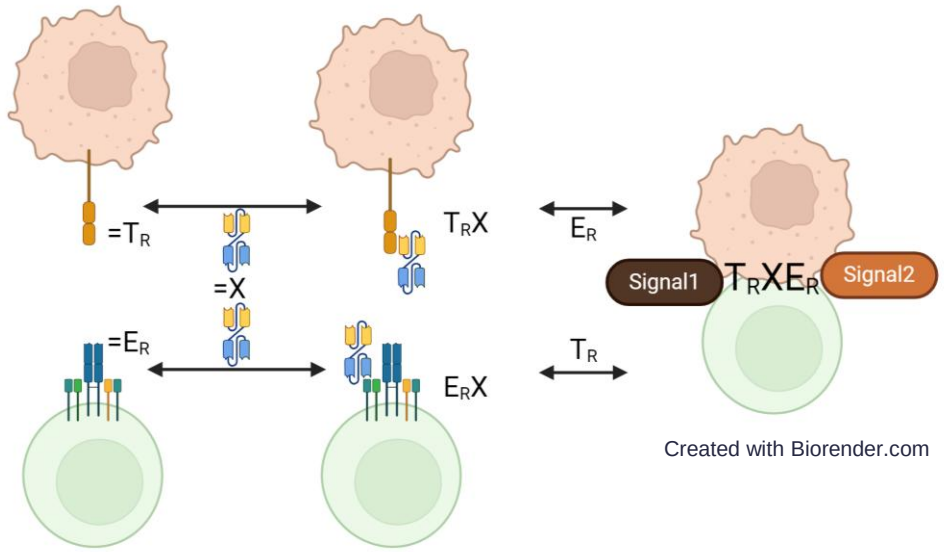
A basic CD3-Target receptor binding model

EMERGENT TOPICS

T-CELL BIODISTRIBUTION

IMMUNE SYNAPSE

CRS INCIDENCE & MITIGATION



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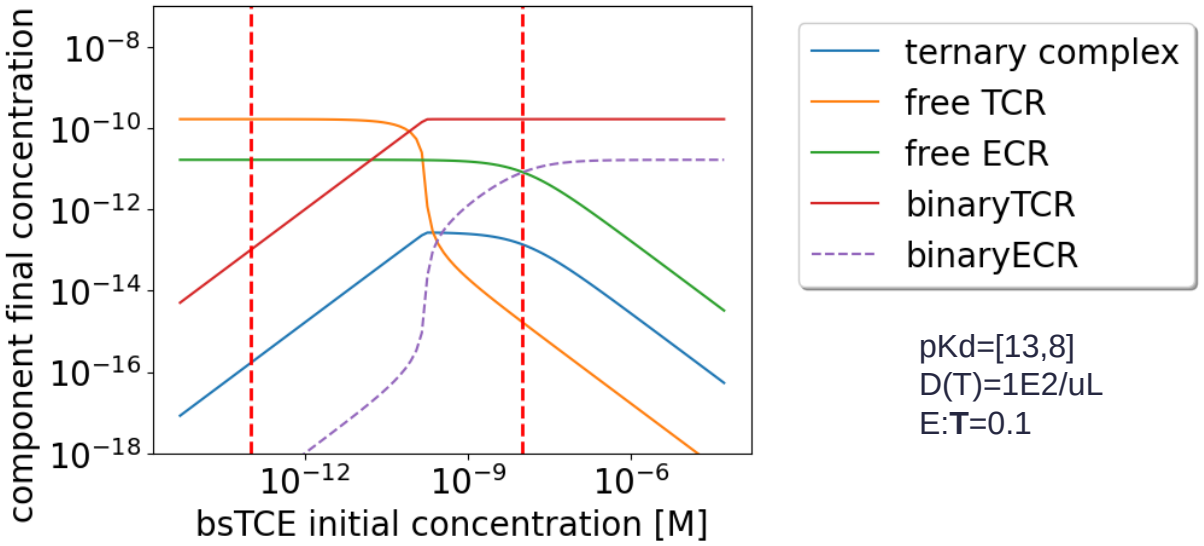


pKd=[13,8]
D(T)=1E2/uL
E:**T**=0.1

Models are sensitive to

CD3/target affinity

Cell density

E:T ratio

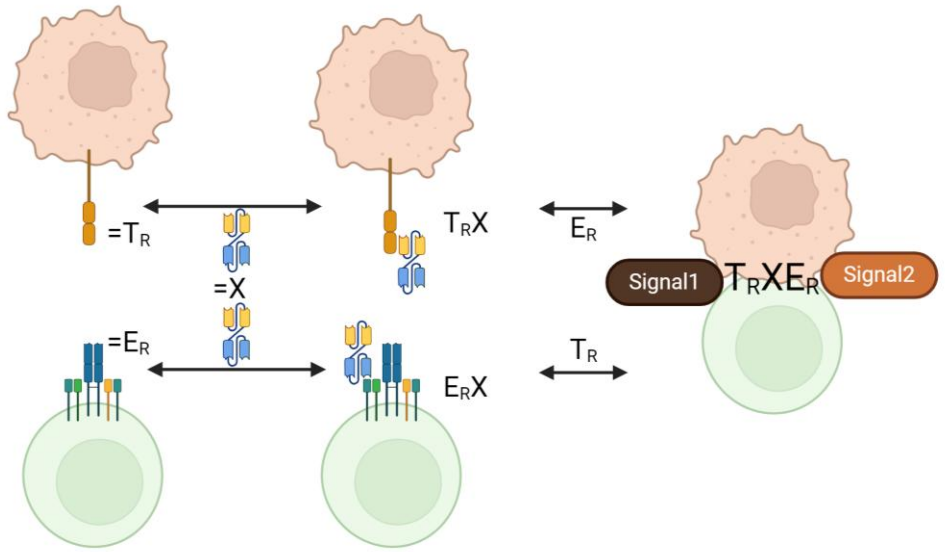
EMERGENT TOPICS

T-CELL BIODISTRIBUTION

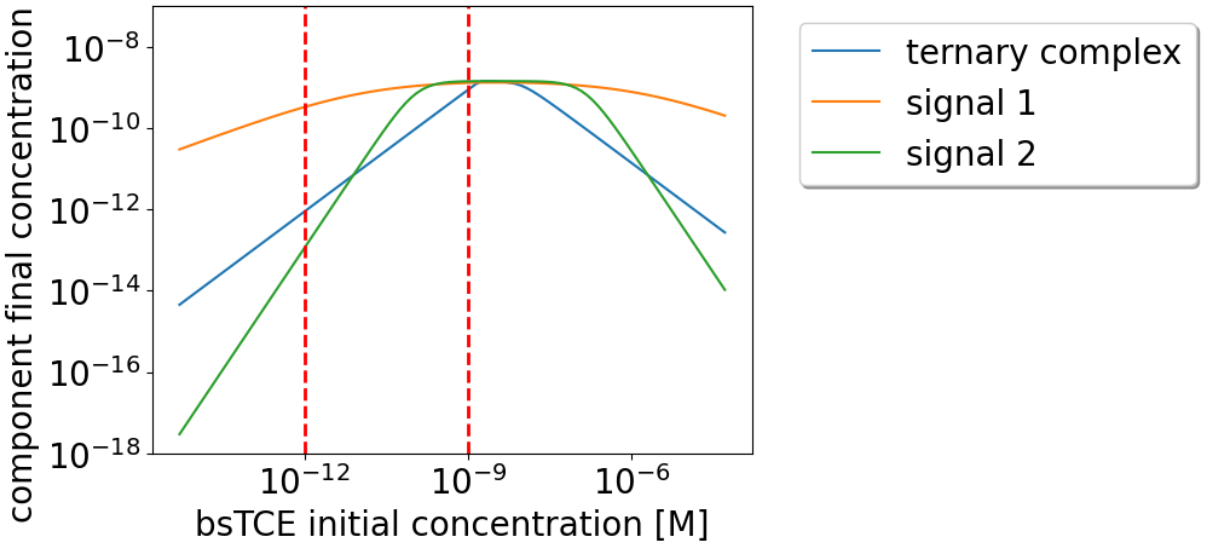
IMMUNE SYNAPSE

CRS INCIDENCE & MITIGATION

A basic CD3-Target receptor binding model



An often used model for the immune synapse is a simple version of the homogeneous, well stirred phase binding between the bispecific and freely diffusible receptors leading to ternary complex formation.



Then empirical „signals“ (often sigmoid functions) translate ternary complex into different downstream effects

EMERGENT TOPICS

T-CELL BIODISTRIBUTION

IMMUNE SYNAPSE

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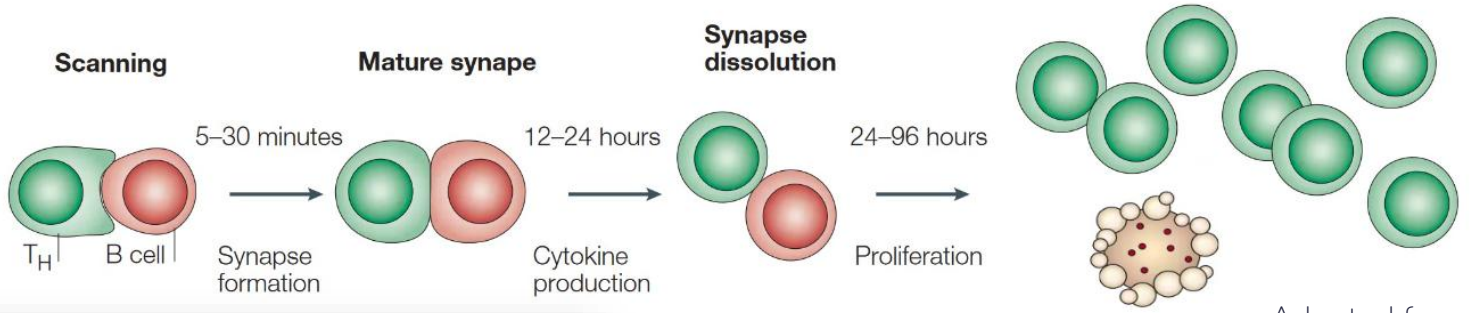
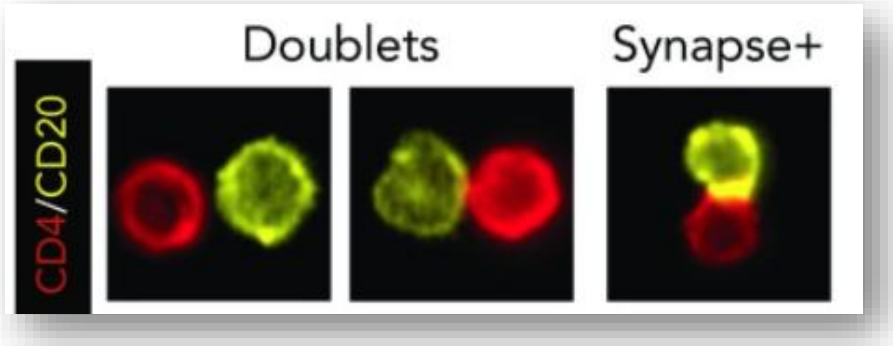
Physiology of the immune synapse

The picture of homogeneously distributed ternary complexes of freely diffusible species is not very biologically plausible as cellular rather than molecular kinetics govern the probability while complex cooperative multistep processes the strength of immune synapse formation.

There is quite some knowledge about the physiology of immune synapses



Adopted from 10.1038/nri1245, courtesy of Y. Cao



Robinson (2018) 10.1182/blood-2018-02-830992

Role of bsTCE in that process	Unclear	Established	Unclear

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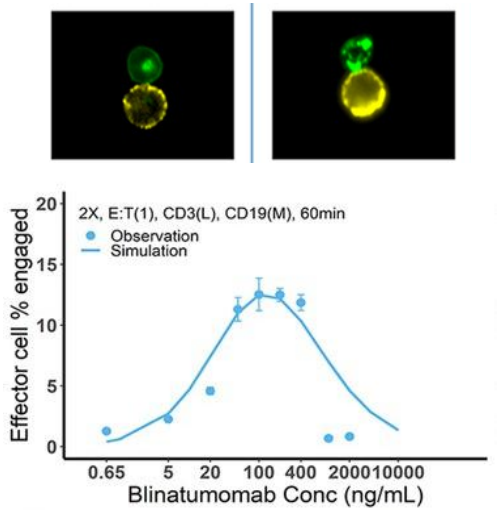
T-CELL BIODISTRIBUTION

IMMUNE SYNAPSE

CRS INCIDENCE & MITIGATION

Physiology of the immune synapse



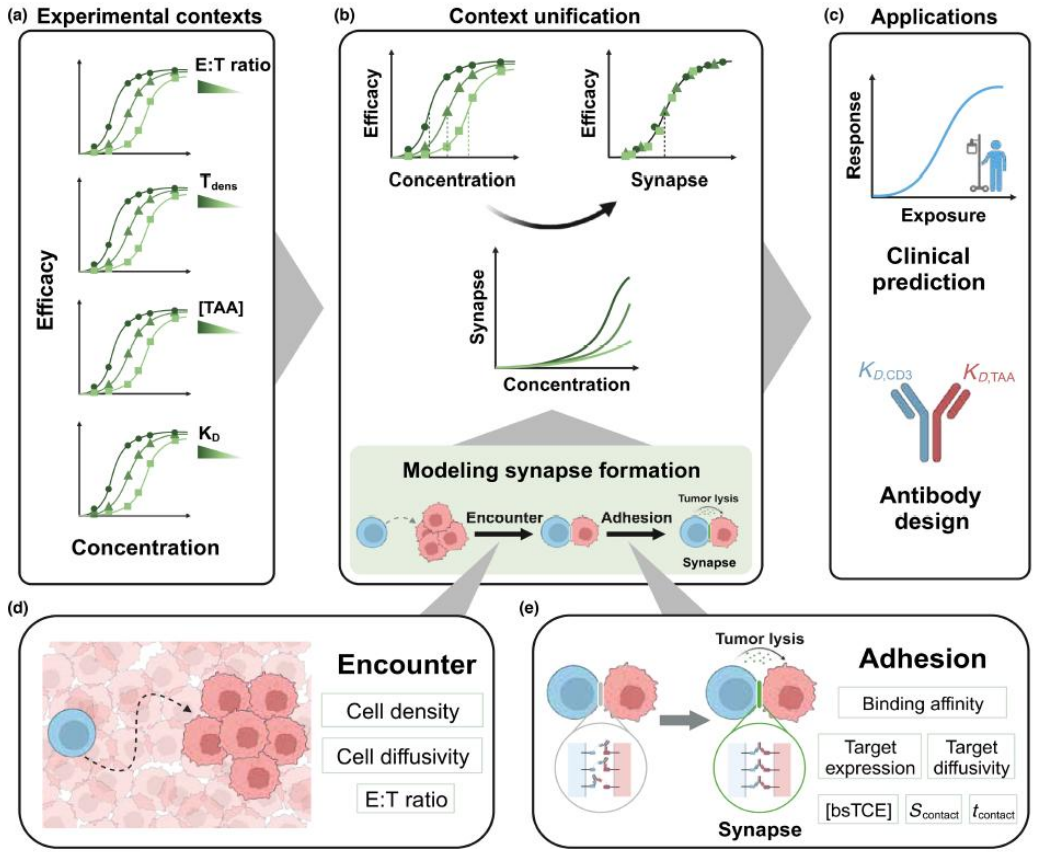
CD3/target expression

Cell density

E:T ratio

Integrated into an in vivo physiological model it could predict organ specific responses, and optimal regimen

Liu et al (2023)
10.7554/eLife.83659



Optimizing Clinical Translation of Bispecific T-cell Engagers through Context Unification with a Quantitative Systems Pharmacology Model

Xiaozhi Liao[1], Timothy Qi[1], Jiawei Zhou[1], Can Liu[1] and Yanguang Cao[1,2,*]

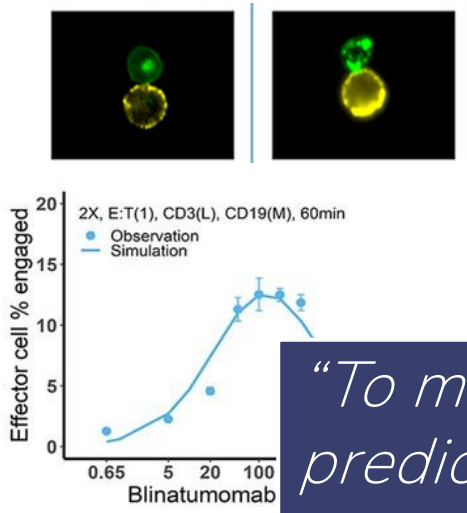
Physiology of the immune synapse

EMERGENT TOPICS

T-CELL BIODISTRIBUTION

IMMUNE SYNAPSE

CRS INCIDENCE & MITIGATION



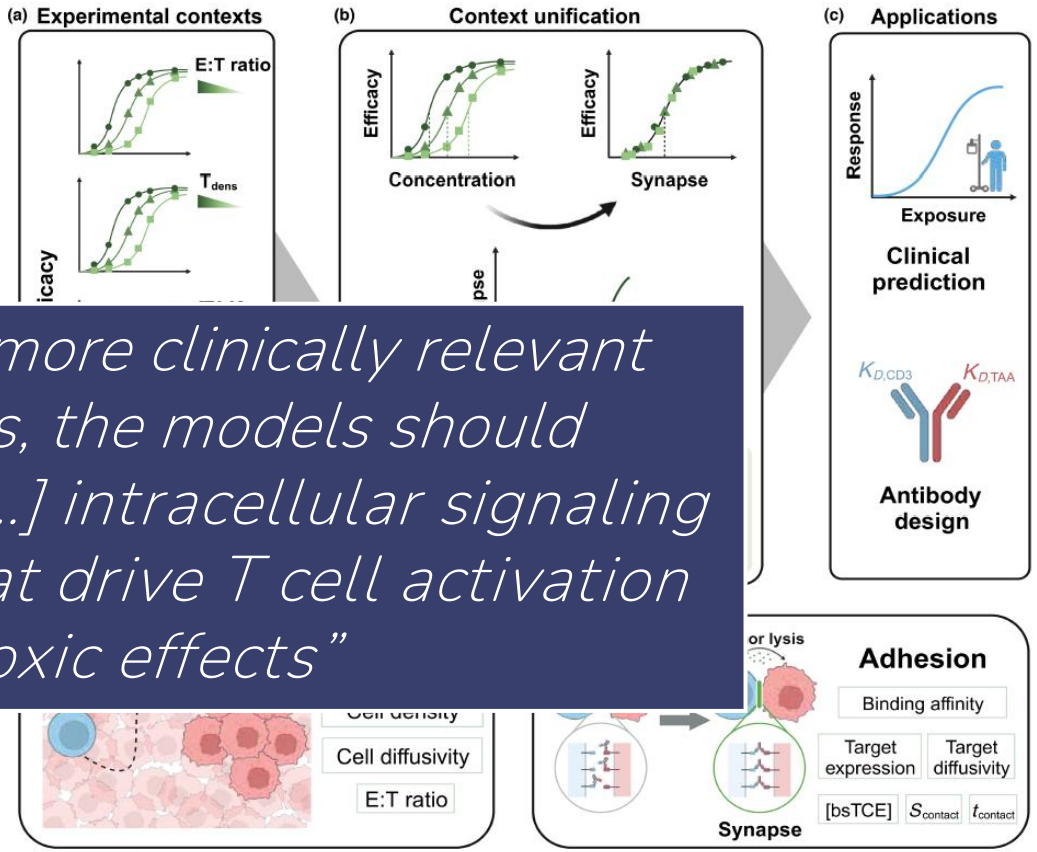
CD3/target e

Cell de

E:T ra

Integrated into an in vivo physiological model it could predict organ specific responses, and optimal regimen

"To make more clinically relevant predictions, the models should consider [...] intracellular signaling events that drive T cell activation and cytotoxic effects"



Optimizing Clinical Translation of Bispecific T-cell Engagers through Context Unification with a Quantitative Systems Pharmacology Model

Xiaozhi Liao[1], Timothy Qi[1], Jiawei Zhou[1], Can Liu[1] and Yanguang Cao[1,2,*]

Liu et al (2023) 10.7554/eLife.83659

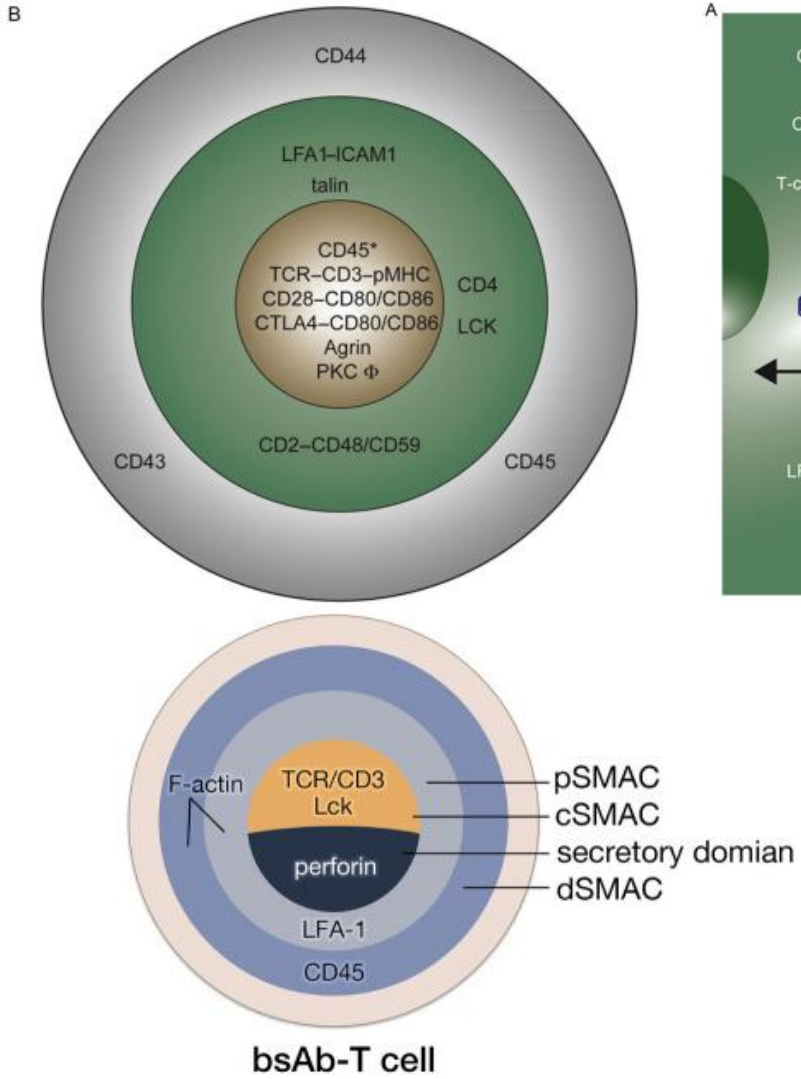
EMERGENT TOPICS

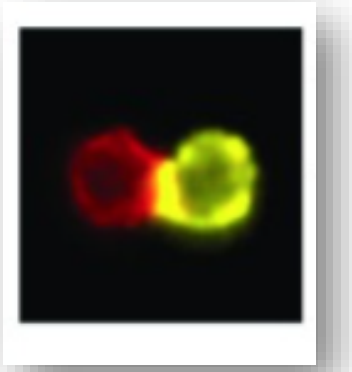
T-CELL BIODISTRIBUTION

IMMUNE SYNAPSE

CRS INCIDENCE & MITIGATION

Physiology of the immune synapse





The physiological IS has a „bulls-eye“ super structure“ with a distict signaling and adhesion – dedicated regions

And the bsTCE is believed to be structurally very similar to this structure

Huppa (2013) 10.1016/B978-0-12-407707-2.00001-1
Gao (2021) 10.3389/fimmu.2021.664329
Robinson (2018) 10.1182/blood-2018-02-830992

EMERGENT TOPICS

T-CELL BIODISTRIBUTION

IMMUNE SYNAPSE

CRS INCIDENCE & MITIGATION

Summary

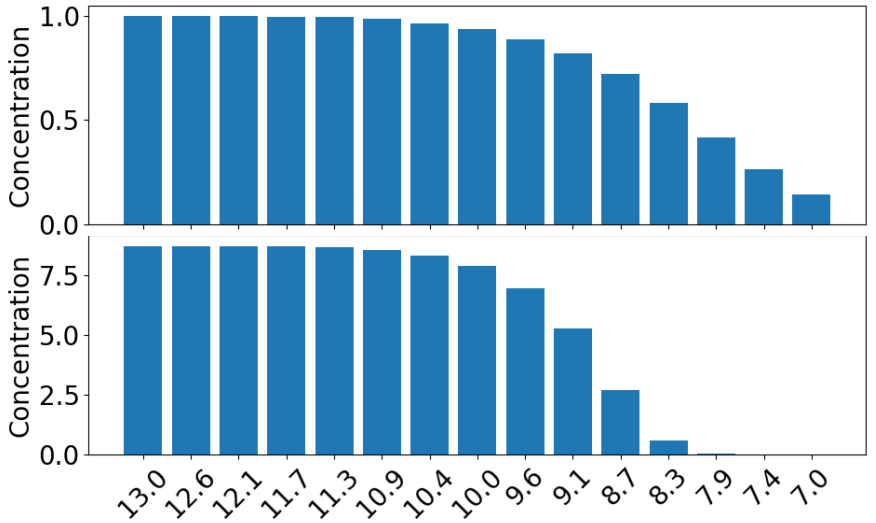
- The immune synapse needs to me modeled under consideration of receptor and cellular level mechanisms for optimal context unification
- Multi-step processes are already in place in different modeling approaches to take that into account

Y. Cao lab

UNC ESHELMAN SCHOOL OF PHARMACY

Next steps

- Kinetic proofreading explains specificity of antigen recognition that could apply to bsTCE synapses
- Testing of context unification model integrating a kinetic proofreading module for describing the adhesion kinetics once the synapse can be formed



EMERGENT TOPICS

IMMUNE SYNAPSE

T-CELL BIODISTRIBUTION

CRS INCIDENCE & MITIGATION

Cytokine release syndrome is a condition that may occur after treatment with immunotherapy. It is caused by a large, rapid and systemic release of cytokines from immune cells affected by the immunotherapy.

Adopted from NCI Dictionary of Cancer Terms

Cytokine Release Syndrome Following Blinatumomab Therapy

Pius E Ojemolon [1,✉], Sunaina Kalidindi [2], Taylor A Ahlborn [2], Osaigbokan P Aihie [3], Moyosoluwa I Awoyomi [1]

Editors: Alexander Muacevic, John R Adler

11-15% of patients who receive blinatumomab infusions develop CRS, and 2-5% have severe (grade ≥3) CRS

Blinatumomab-induced macrophage activating syndrome (MAS) in adult with B-cell acute lymphoblastic leukemia (B-ALL)

Adam Braun [1,✉,#], Salman Otoukesh [1,#], Jose Tinajero [1], Guido Marcucci [1], Ibrahim Aldoss [1]

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Other cells like (i.e. myeloid cells play a very important role)

Current concepts in the diagnosis and management of cytokine release syndrome

Clinical Trials & Observations

Daniel W. Lee, Rebecca Gardner, David L. Porter, Chrystal U. Louis, Nabil Ahmed, Michael Jensen, Stephan A. Grupp, Crystal L. Mackall

Tocilizumab (anti IL6R/sIL6R) is used front line to treat CRS

EMERGENT TOPICS

IMMUNE SYNAPSE

T-CELL BIODISTRIBUTION

CRS INCIDENCE & MITIGATION

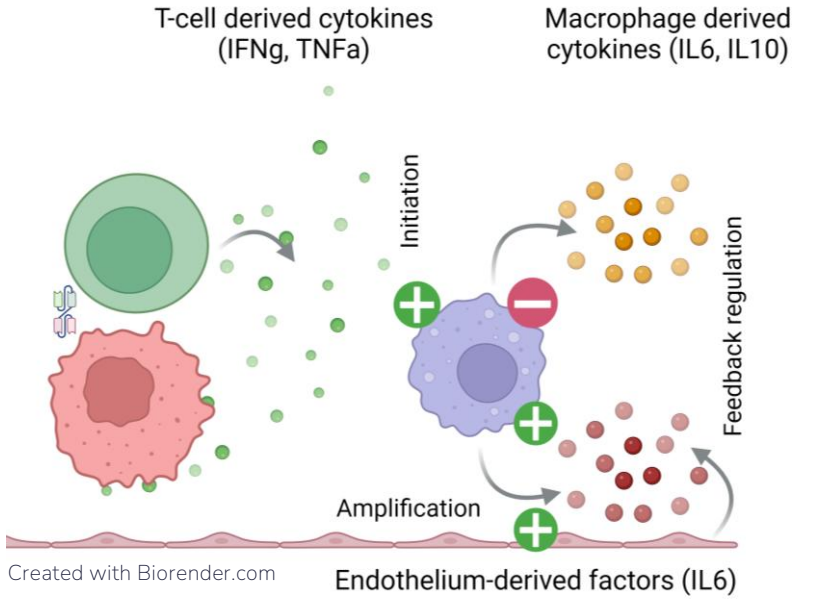
CLINICAL CANCER RESEARCH | TRANSLATIONAL CANCER MECHANISMS AND THERAPY

Dissecting the Mechanisms Underlying the Cytokine Release Syndrome (CRS) Mediated by T-Cell Bispecific Antibodies

Gabrielle Leclercq-Cohen[1], Nathalie Steinhoff[1], Llucia Albertí Servera[2], Sina Nassiri[2], Sabrina Danilin[2], Emily Piccione[3], Emilio Yángüez[1], Tamara Hüsser[1], Sylvia Herter[1], Stephan Schmeing[1], Petra Gerber[1], Petra Schwalie[2], Johannes Sam[1], Stefanie Briner[1], Sylvia Jenni[1], Roberta Bianchi[1], Marlene Biehl[1], Floriana Cremasco[1], Katerina Apostolopoulou[1], Hélène Haegel[2], Christian Klein[1], Pablo Umaña[1], and Marina Bacac[1]

IL-6 trans-signaling induces plasminogen activator inhibitor-1 from vascular endothelial cells in cytokine release syndrome

Sujin Kang [1], Toshio Tanaka [2], Hitomi Inoue [1], Chikako Ono [3], Shoji Hashimoto [4], Yoshiyuki Kioi [1], Hisatake Matsumoto [5], Hiroshi Matsuura [5], Tsunehiro Matsubara [5], Kentaro Shimizu [5], Hiroshi Ogura [5], Yoshiharu Matsuura [3], Tadamitsu Kishimoto [6]



Created with Biorender.com

Consensus

- involvement of T-cells as a major source of „activating stimulus“ (IFNg and TNFa)

- Involvement of myeloid cells (macrophages and monocytes) producing IL6, IL10 and IL8

Hypotheses

- Wiring between positive and negative cytokine feedback loops

- Importance of between IL6 cis and trans signaling

- Involvement of endothelial cells and EC derived IL6

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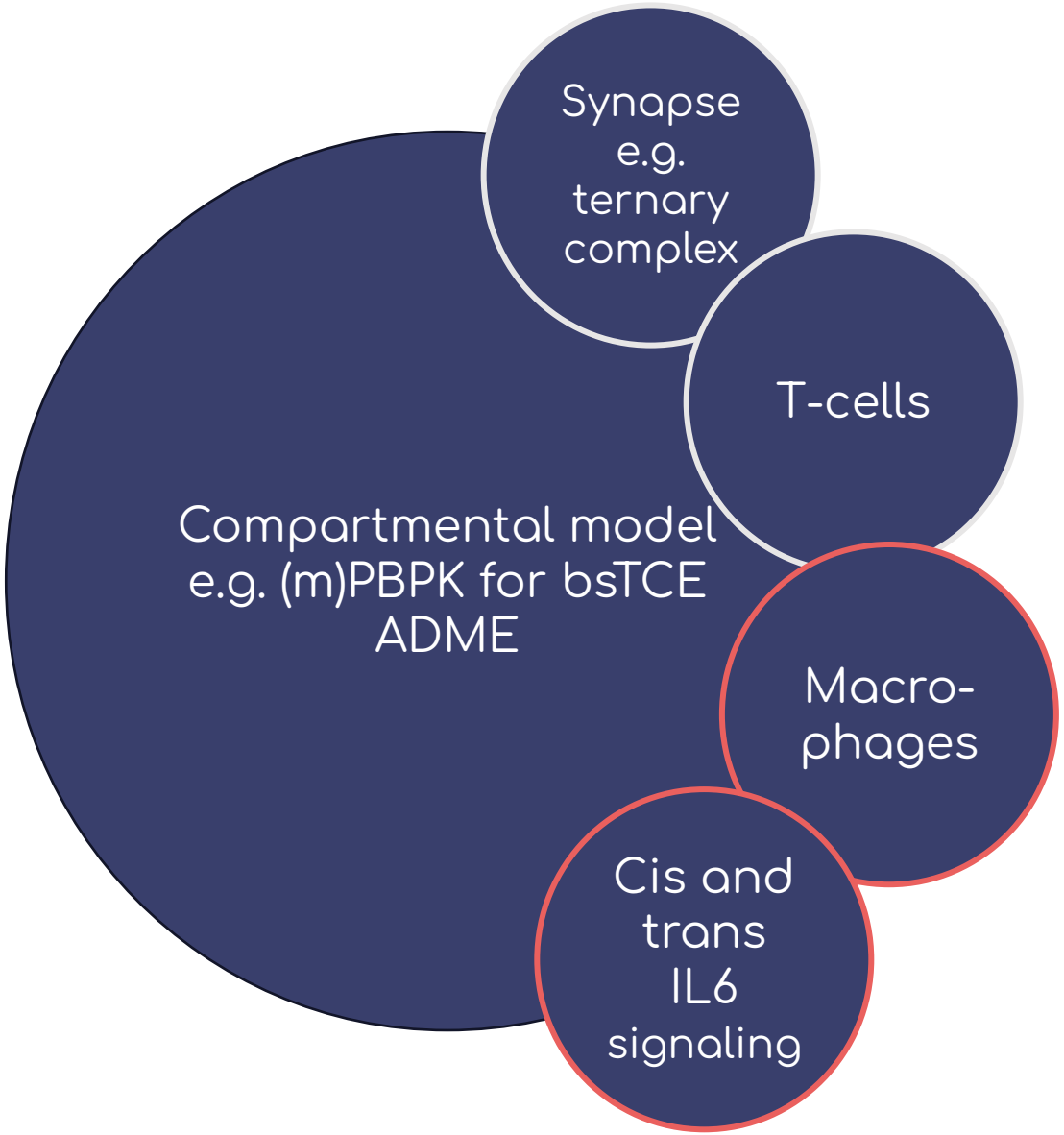
Proposed ingredients of a model to test CRS hypotheses and drive data generation



Consensus

- Context dependent immune synapse formation driving T-cell activation and effector function
- involvement of T-cells as a major source of „activating stimulus“ (IFNg and TNFa)
- Involvement of myeloid cells (macrophages and monocytes) producing IL6, IL10 and IL8

Hypotheses

- Wiring between positive and negative cytokine feedback loops
- Importance of between IL6 cis and trans signaling
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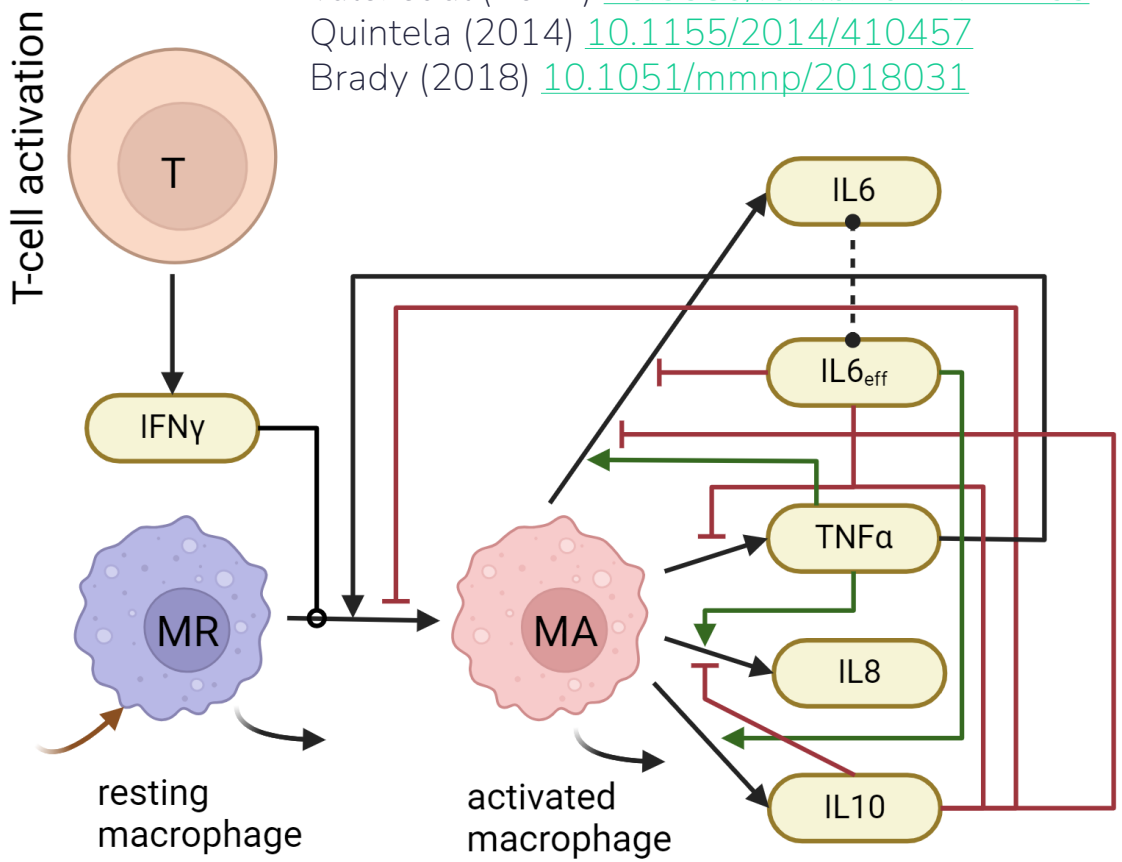
A macrophage centric cytokine secretion model

We adopted and simplified a cellular-cytokine model that has good overlap with measured cytokine concentrations and added T-cell derived interferon as master macrophage activator

Talei et al (2021) 10.3389/fcimb.2021.711153
Quintela (2014) 10.1155/2014/410457
Brady (2018) 10.1051/mmnp/2018031



Complex, non-identifiable model as an example for this talk and to highlight workflow

In preparation for more detailed IL6 modeling, IL6 signaling has been dissociated from IL6 secretion.

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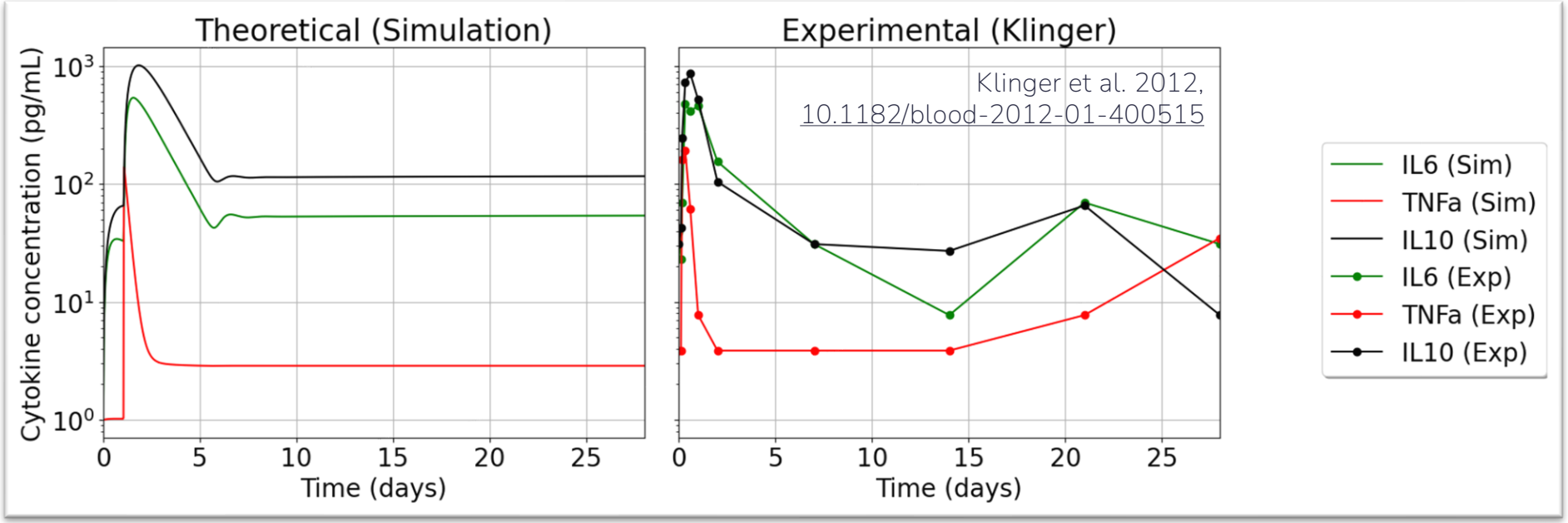
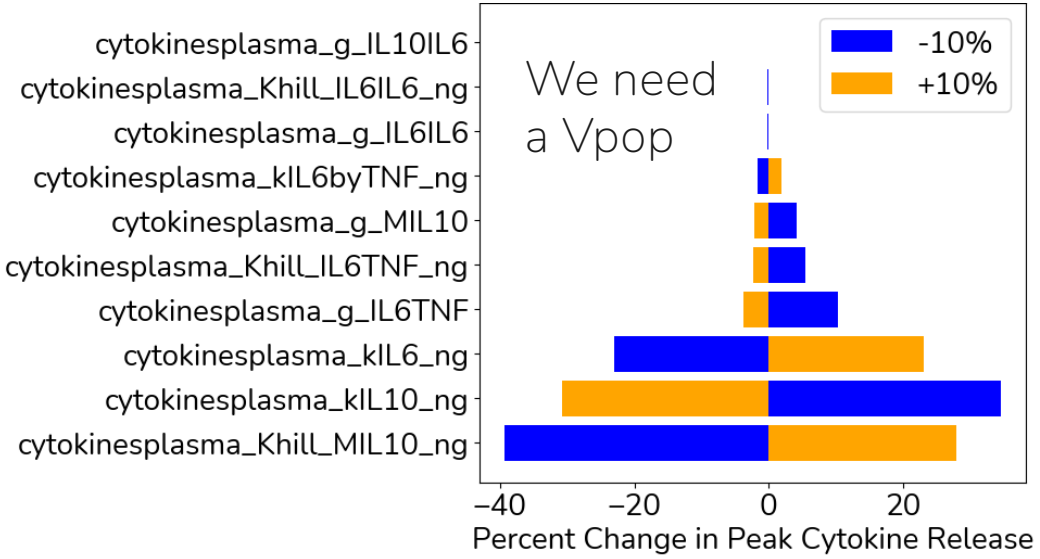


Remember?

11-15% of patients who receive blinatumomab infusions develop CRS, and 2-5% have severe (grade ≥3) CRS



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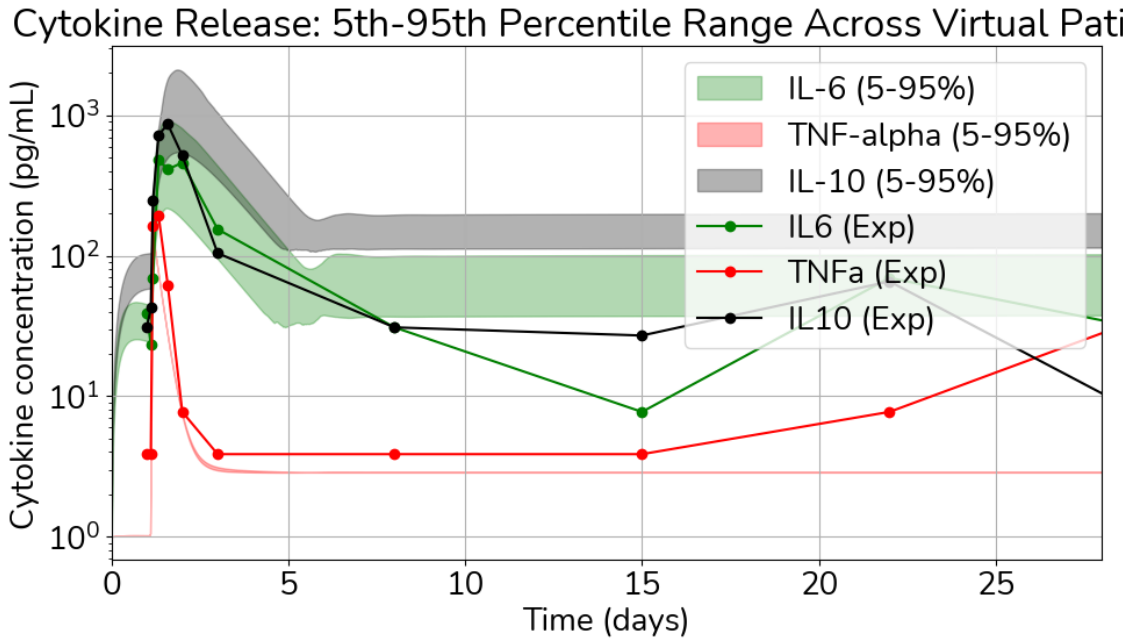

The model is too „well behaved“ to produce severe CRS, which is why we need another positive feedback loop leading to amplification in line with trans IL6 signaling

Remember?

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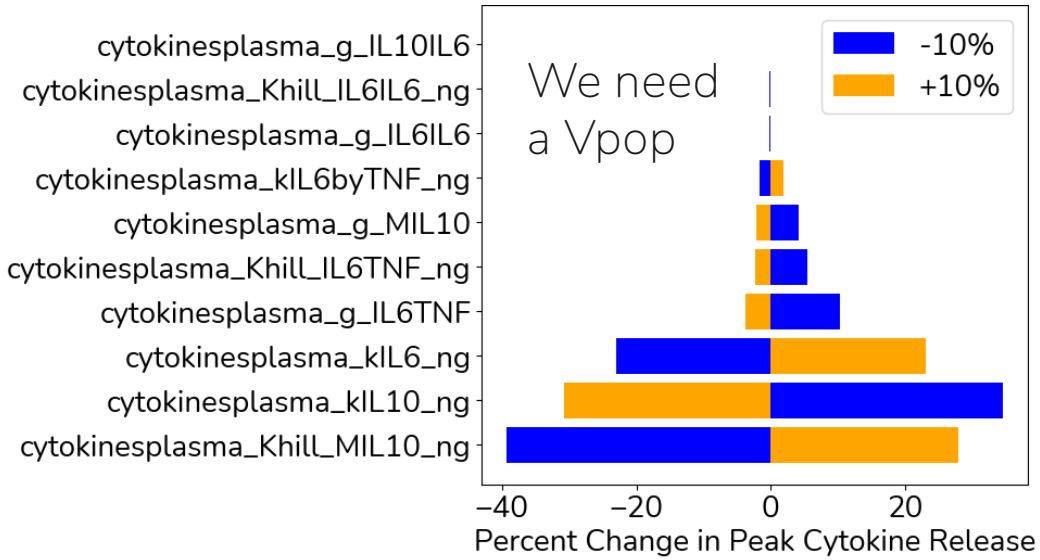

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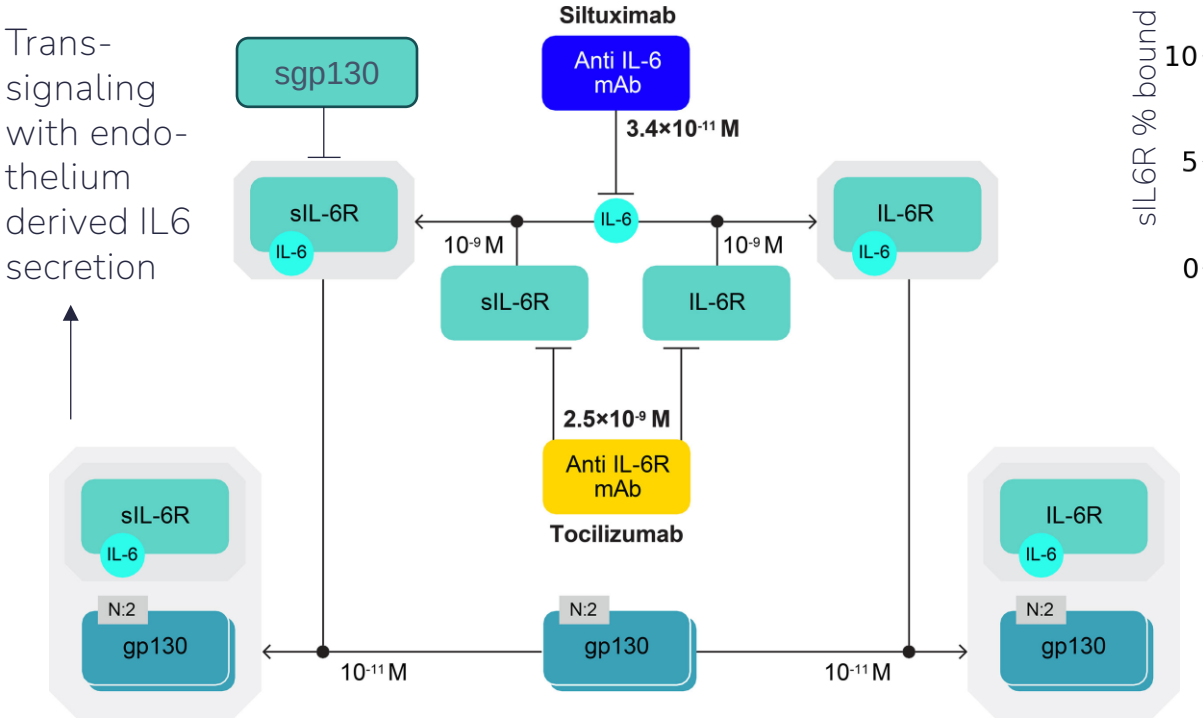
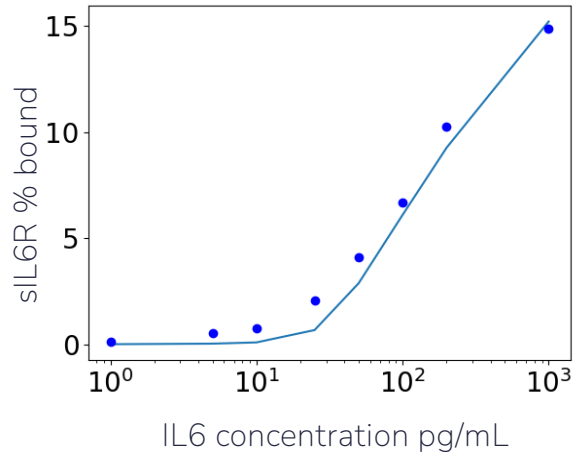
A detailed IL6 – (s)IL6R – (s)gp130 model

For proof of concept, we have integrated and extended the model by Rossi et al. (2022)
10.3389/fimmu.2022.919489

The balance of interleukin (IL)-6, IL-6·soluble IL-6 receptor (sIL-6R), and IL-6·sIL-6R·sgp130 complexes allows simultaneous classic and trans-signaling

Paul Baran [‡], Selina Hansen [‡], Georg H Waetzig [§], Mohammad Akbarzadeh [‡], Larissa Lamertz [‡], Heinrich J Huber [¶], M Reza Ahmadian [‡], Jens M Moll [‡], Jürgen Scheller [‡,1]





+ Additional between patient variability in sIL6 levels and sgp130 levels

A detailed IL6 – (s)IL6R – (s)gp130 model

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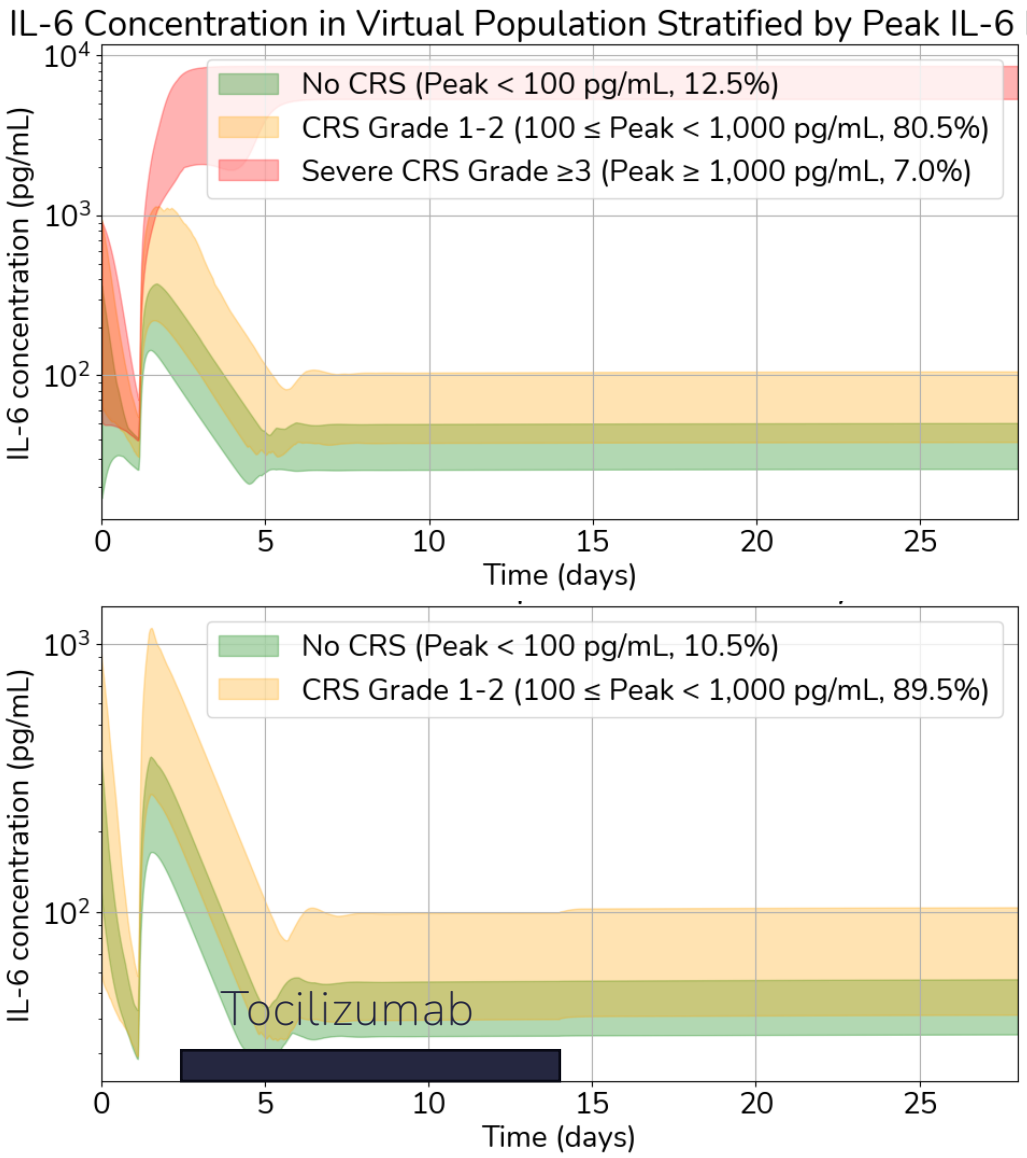

For a subset of virtual patients, IL6 crosses the threshold to activate endothelial cells which amplify IL6 secretion

Front-line tocilizumab disrupts excessive (i.e. trans) IL6 signaling

Could be also used to model risk-adapted preemptive tocilizumab

see Kadauke (2024) 10.1200/JCO.20.02477

A detailed IL6 – (s)IL6R – (s)gp130 model

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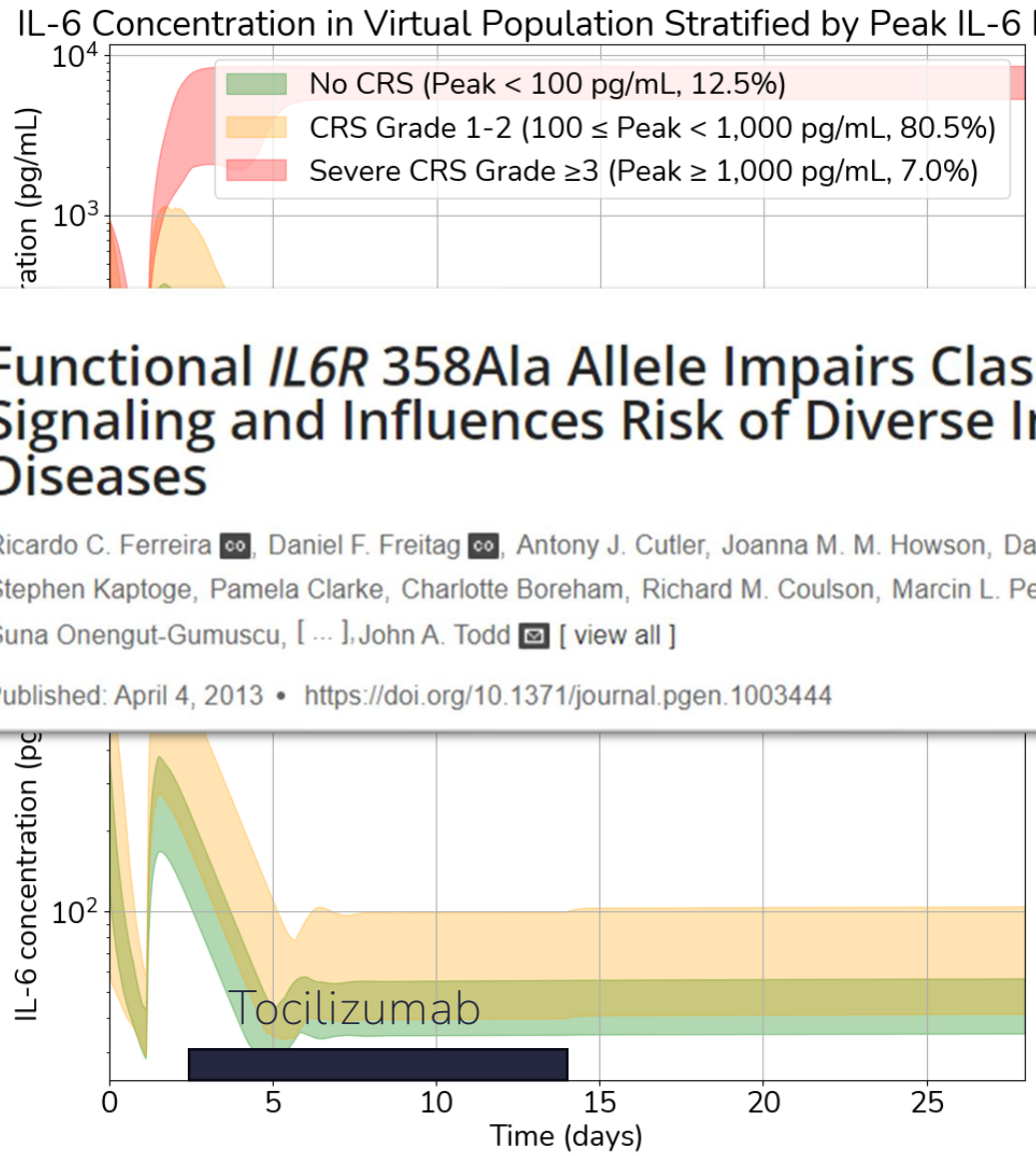

Functional *IL6R* 358Ala Allele Impairs Classical IL-6 Receptor Signaling and Influences Risk of Diverse Inflammatory Diseases

Ricardo C. Ferreira, Daniel F. Freitag, Antony J. Cutler, Joanna M. M. Howson, Daniel B. Rainbow, Deborah J. Smyth, Stephen Kaptoge, Pamela Clarke, Charlotte Boreham, Richard M. Coulson, Marcin L. Pekalski, Wei-Min Chen, Suna Onengut-Gumuscu, [...], John A. Todd [view all]

Published: April 4, 2013 • https://doi.org/10.1371/journal.pgen.1003444

For a subset of virtual patients, IL6 crosses the threshold to activate …which …tion

… disrupts …s) IL6 …signaling

Could be also used to model risk-adapted preemptive tocilizumab which is being tested in CART-trials

Kadauke (2024) 10.1200/JCO.20.02477

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Summary

- Expansion of T-cell-cytokine models can cover co-variability of a multitude of cytokines important for CRS
- Detailed IL6 modeling can help to explain between-patient differences in switching to an amplified / severe cytokine storm and action of anti-IL6 axis mitigation
- Looking beyond oncology / bsTCE models can be helpful to get inspiration for concrete models

Next steps

Repositories of immune digital twin literature, models and best practice

Anna Niarakis

CBI Toulouse Centre de Biologie Intégrative

RDA RESEARCH DATA ALLIANCE

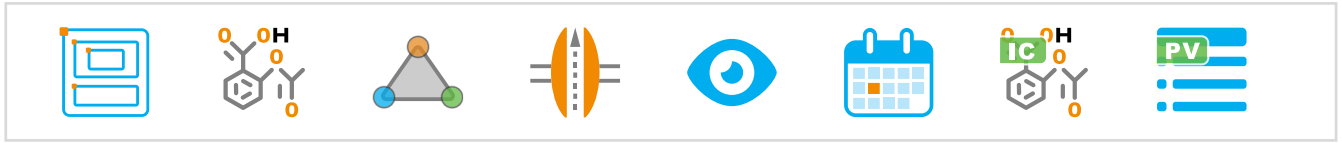
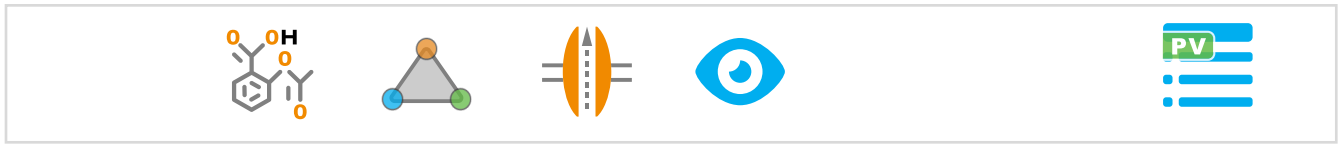
Building Immune Digital Twins

Systems immunogenicity of T-cell agonists

UNIVERSITY of NEBRASKA–LINCOLN

Tomáš Helikar

A fully modular approach PK-Sim / MoBi v12 (outlook)

Type	Module description	Building Blocks used in module
PKSIM	bsAb ADME and binding ○ Target expression ○ Protein binding partner ○ FcRn mediated recycling	
	Extended physiology ○ Tumor ○ Lymph nodes ○ SC depots	
	Receptors ○ TMDD ○ Kinetic proofreading	
	T-cell module and IS ○ Transmigration ○ Retention factor ○ Cell encounter + Adhesion ○ Activation + Proliferation	
	Cytokine release ○ IL6 release	

PK-Sim/Base module

Extension module

https://github.com/Open-Systems-Pharmacology/Suite/releases/tag/v12.0_beta1

Take home messages

1. Quantitative systems pharmacology (QSP) models can derisk various aspects of bispecific T-cell engager design and development

2. Emerging topics for future QSP models are immune synapse modeling, extended cytokine network and effects modeling and T-cell/bsTCE biodistribution modeling

3. Using modular workflows based on whole body PBPK and QSP extension modules allows for assembly of flexible „fit for purpose“ models streamlined to the question at hand

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Thank you