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PKPD Considerations in Design and Development of T-cell Dependent Bispecific mAbs

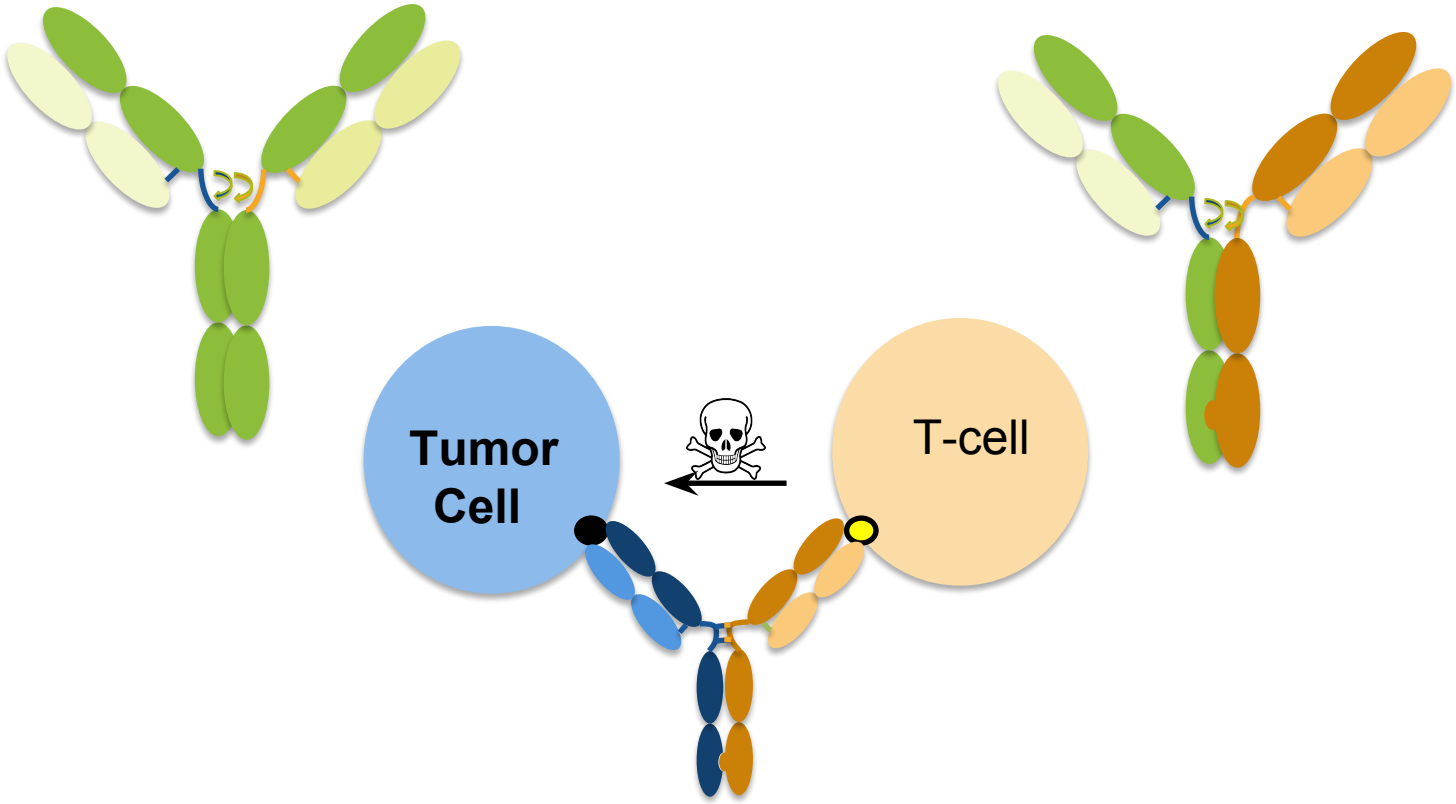
Saileta Prabhu
ASCPT Webinar

14 June 2018

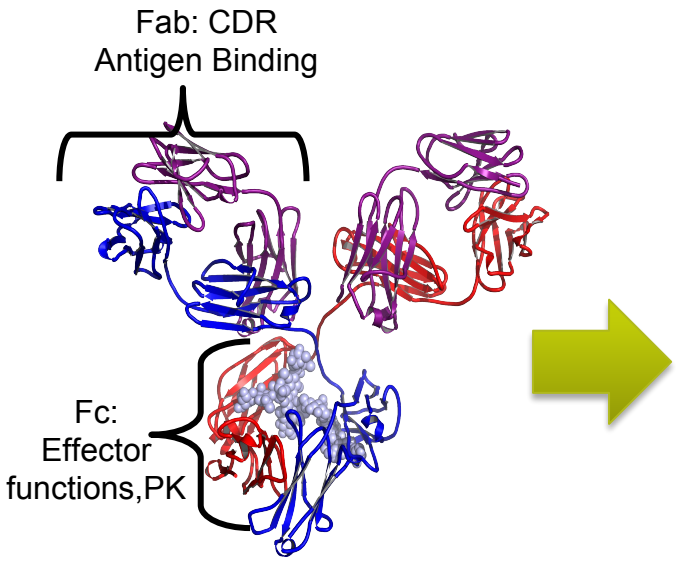
Agenda

Biodistribution and PKPD of therapeutic mAbs

PKPD Considerations for T-cell Dependent Bispecifics



Elimination mechanisms of mAbs



Nonspecific clearance

-pinocytosis/endocytosis ⇒ proteolysis;

-governed by FcRn, FcγRs, charge, and pI

Specific clearance due to antigen binding

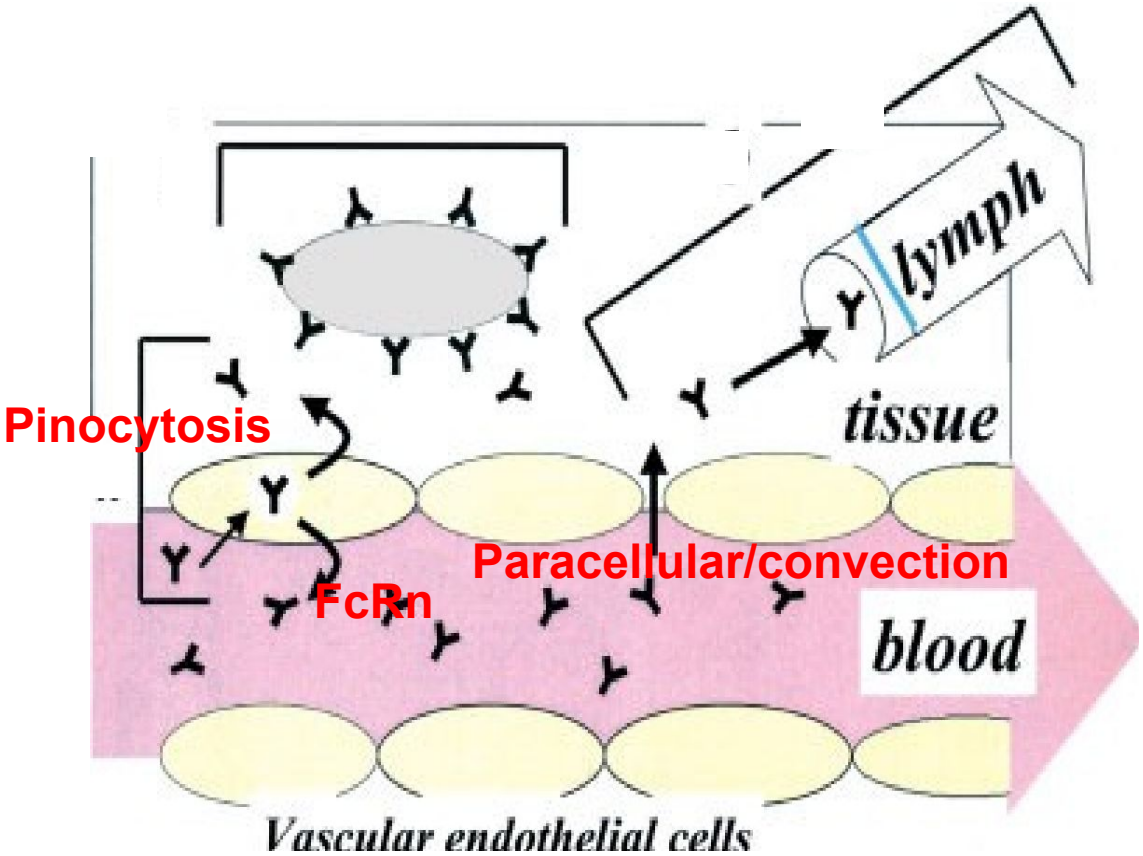
-governed by antigen biology, expression and kinetics

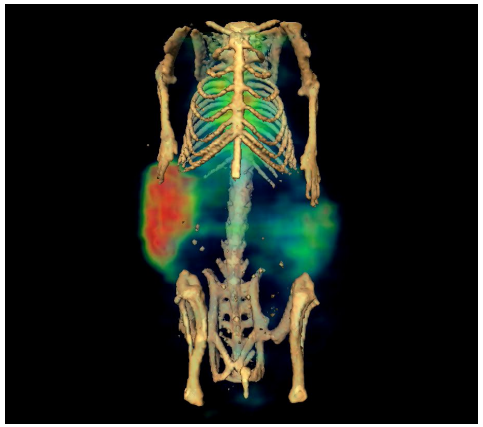
Immunogenicity

-clearance of immune complexes by FcgRs

Mechanism of distribution of mAbs

Antibodies are largely confined to the vascular space due to their size





Distribution of indium-labeled anti-huCD3/Her2 in a mouse tumor model

D. Mandikian, B. Shen, V. Yip, L. Nazarova, H. Anezinos, A. Boswell

Lobo and Balthasar, 2008; Boswell, 2014

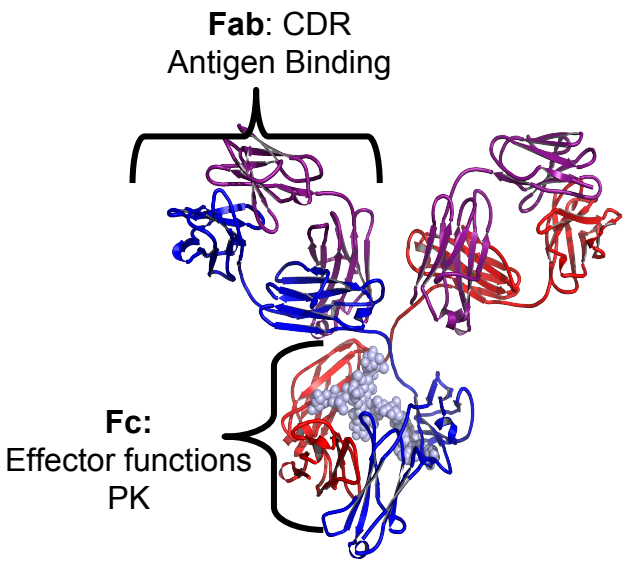
Antibody distribution and elimination is a function of its structure, th antigen, and MOA/pharmacology of the antigen and antibody

mAb structure

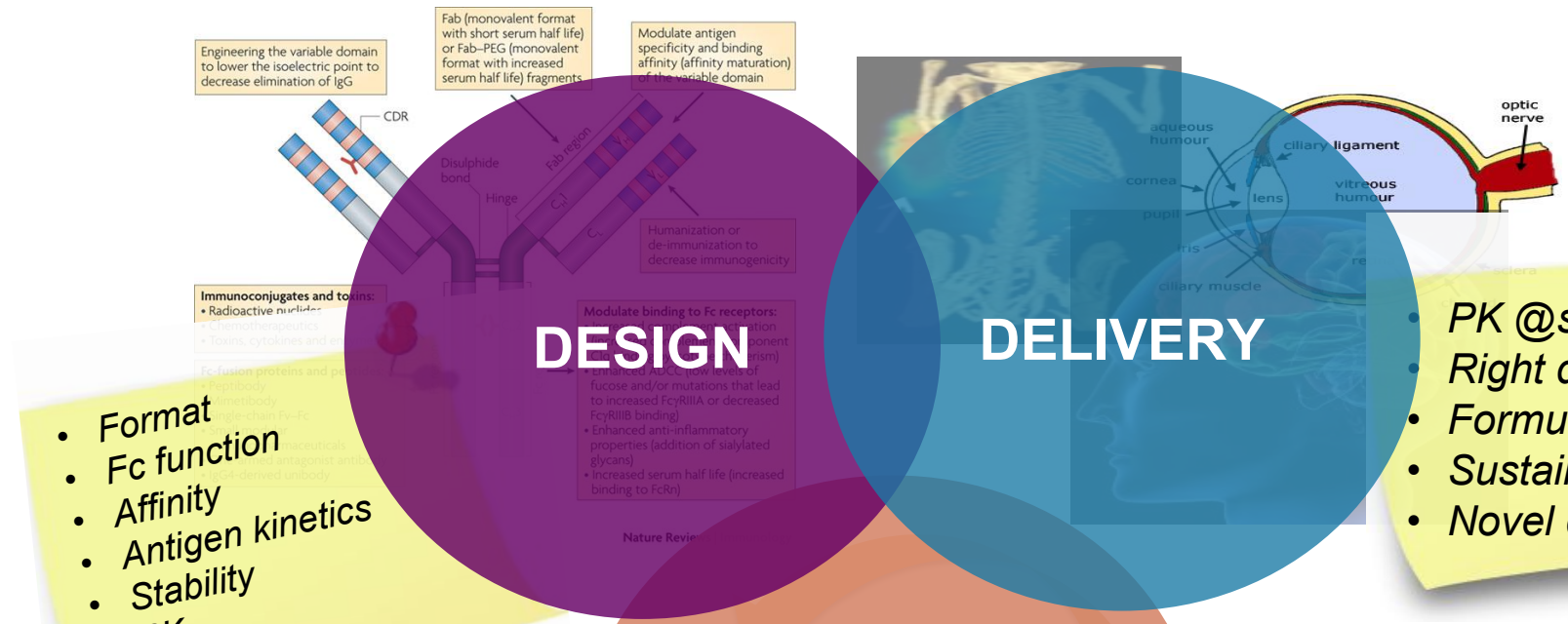
Size
Structural variants eg. glycans
Affinity to antigen, FcγR, FcRn
Depleting or blocking MOA
Species differences

Antigen

Turnover kinetics (half-life)
Expression levels/concentration
Soluble or membrane bound
Down/up-modulated or re-expressed
Pharmacological activity
Species differences



Our vision-To design, develop, and deliver novel medicines to patients using quantitative pharmacology

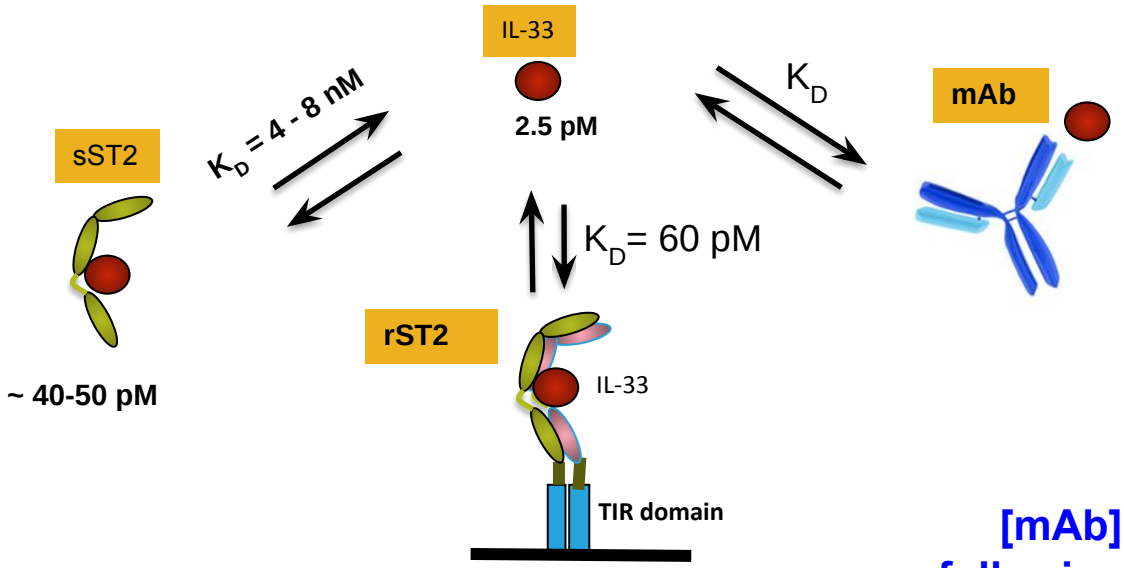


- Format
- Fc function
- Affinity
- Antigen kinetics
- Stability
- PK
- SAR

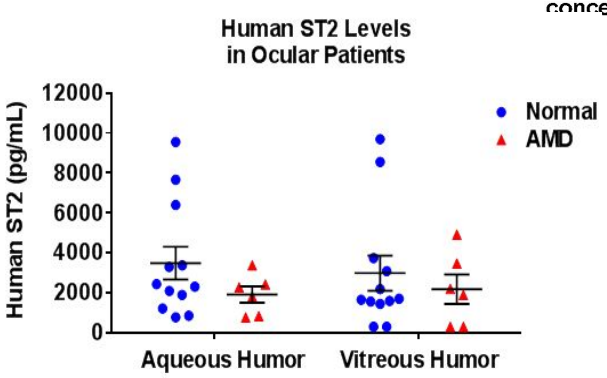
- *PK @site of action*
- *Right dose, route*
- *Formulation*
- *Sustained delivery*
- *Novel delivery systems*

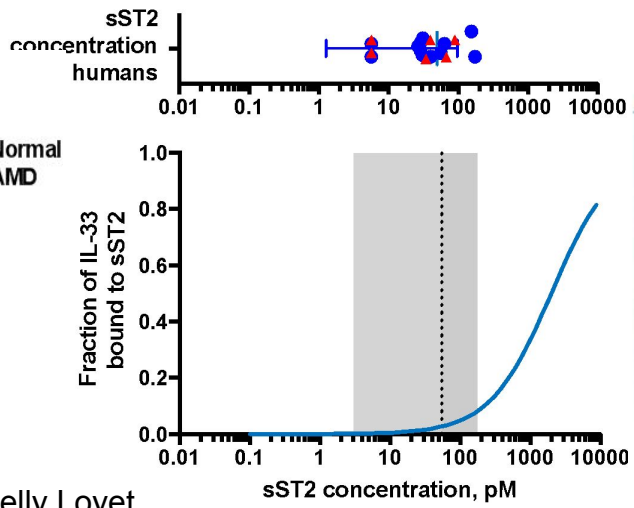
- *Translational PKPD*
- *FIH dose/regimen*
- *Toxicokinetics*
- *Proof-of-concept*
- *Therapeutic Index*
- *Clinical development*

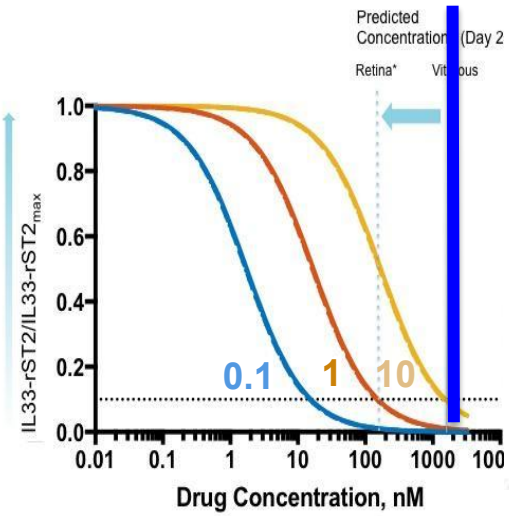
Soluble ST2 in vitreous is not a sink for IL33

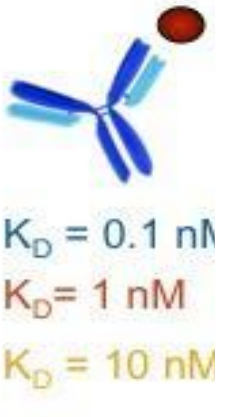


[mAb] on D28 following 10 mg IVT







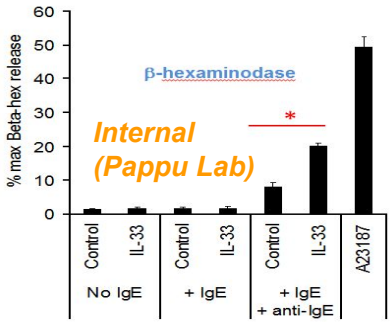


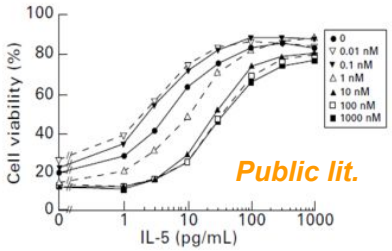
Levina Goon, Laetita Comps-Agrar, Kelly Loyet

Isabel Figueroa

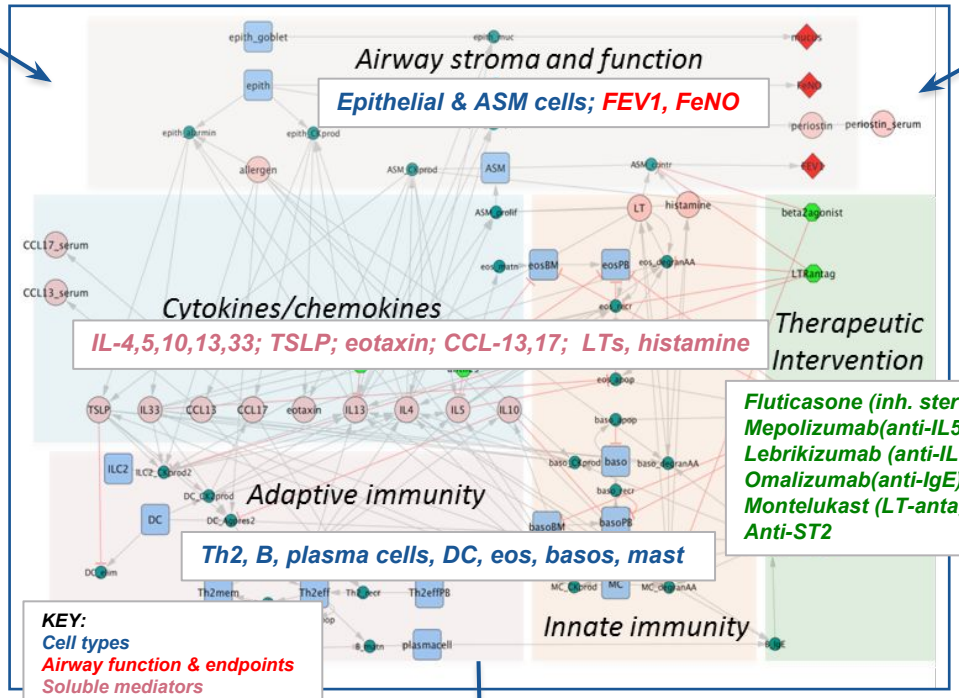
Mechanism based asthma disease model supporting Genentech pipeline for target validation, molecule selection & biomarker evaluation

Mechanistic Data
(in-vitro & in vivo, ex vivo)



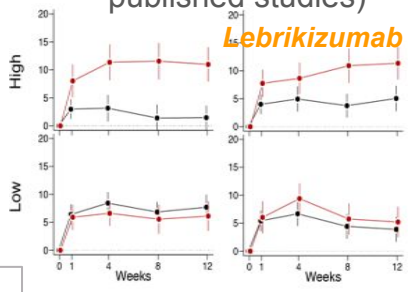


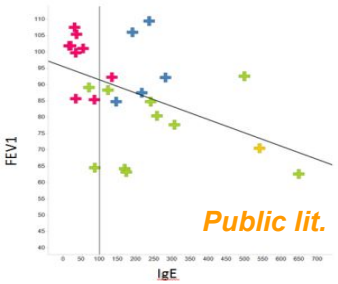
CONSTRUCT



CALIBRATE

Clinical Data
(50+ in–house and published studies)





VALIDATE

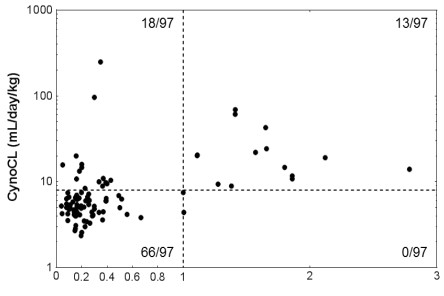
Model based research and analyses-
anti-ST2 PD biomarker, iJAK dose selection

Kapil Gadkar

Current PK screening strategy for selection of lead mAb candidate

A. Baculovirus Assay (BV)

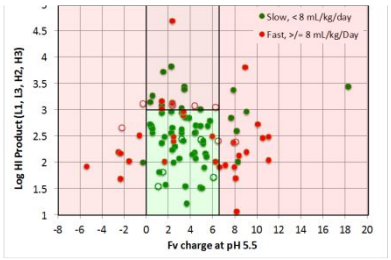
Hoetzel, 2012



- BV>1: 100% probability of fast clearance (reject candidates)
- BV<1: 80% probability of slow clearance

Hoetzel, 2014

B. *In silico* Charge and Hydrophobicity Tool (iCAT)

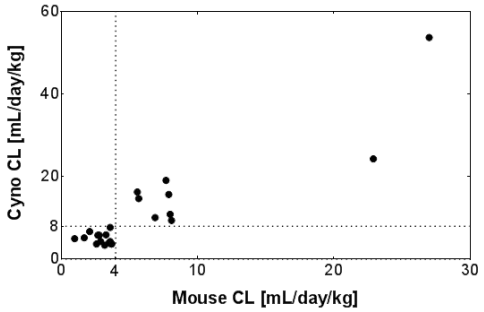


Sharma, 2015

- Fv Domain charge at pH 5.5 and a calculated HI sum of CDRs for LC1, LC3, and HC
- Normal Fv 5.5 Charge/Low HI: 85% probability of slow clearance

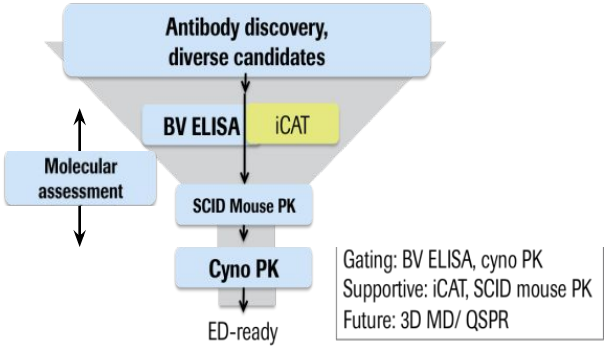
Sharma, 2014

C. PK in SCID mouse vs cyno PK



- Useful tool prior to cyno studies
- mAbs with mouse CL < 4 mL/day/kg showed CL < 8 ml/day/kg in cynos)

D. Screening Strategy



- Normal Fv Charge and BV < 1: 90% probability of slow clearance
- Currently working on *in silico* tools to identify charge patches
- Generally recommend having a back-up mAb engineering strategy

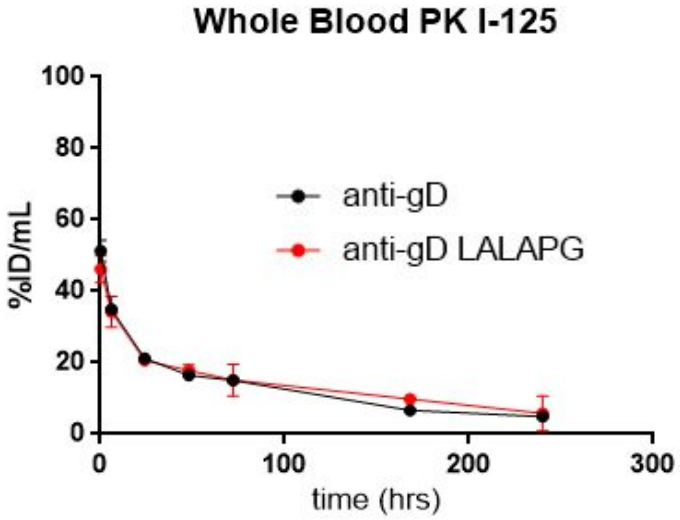
Jeff Lutman, Kapil Gadkar, Amrita Kamath, Daniela Bumbaca, Carol Cullen, Vikas Sharma, Yuda Zhu, Isidro Hoetzel, Paul Carter, Paul Fielder

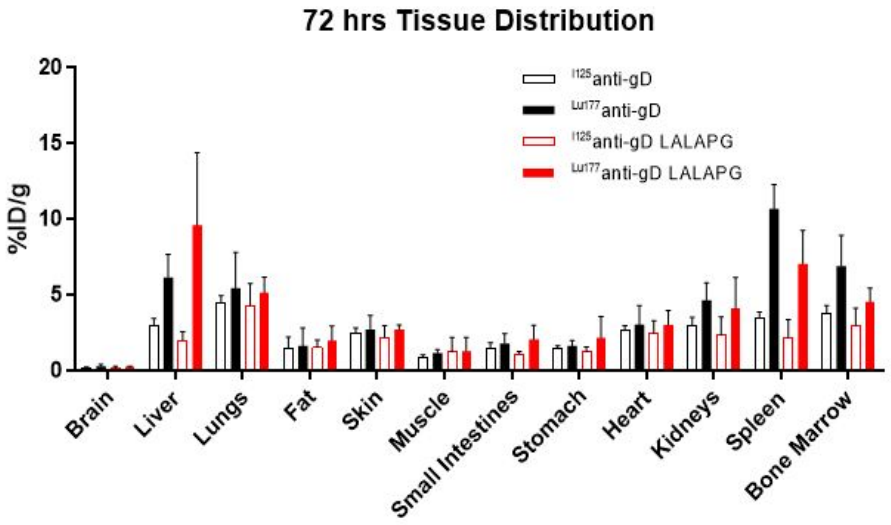
Areas for expansion: bispecific mAbs, murine mAbs, mAbs for ophtha, mechanism of atypical CL, slow CL, in silico structure-modeling.

Role of FcgRs in mAb biodistribution and clearance

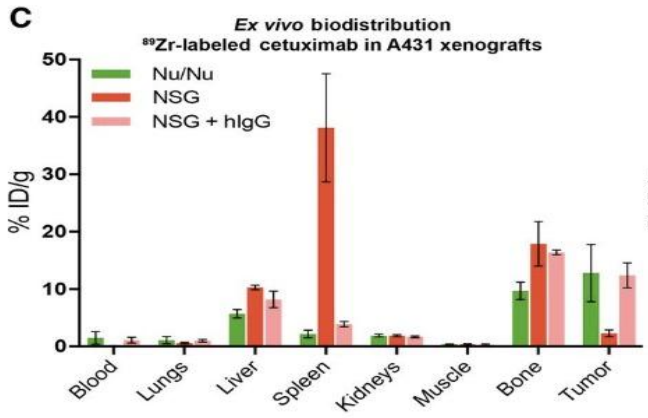
Limited understanding/data

Danielle Mandikian and Andy Boswell

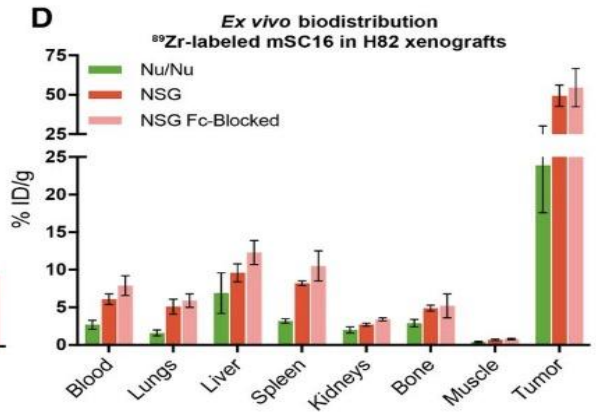




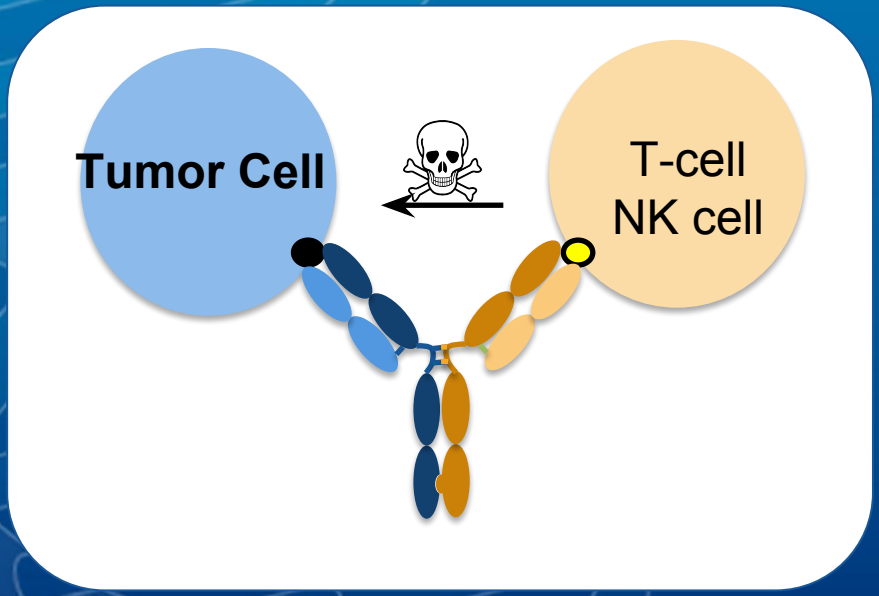
Humanized mAb

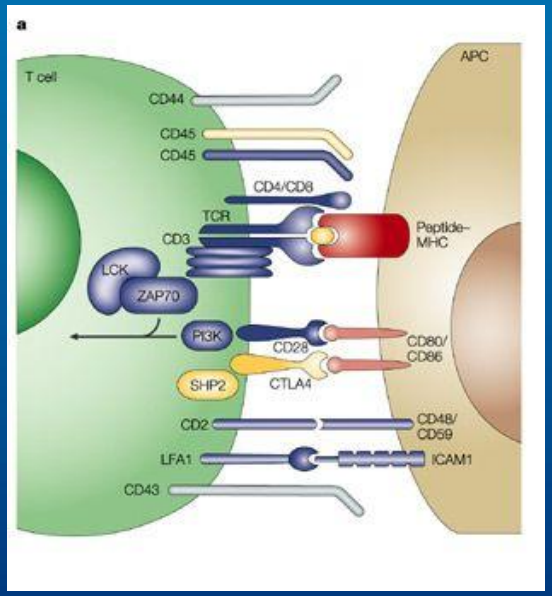


Murine mAb



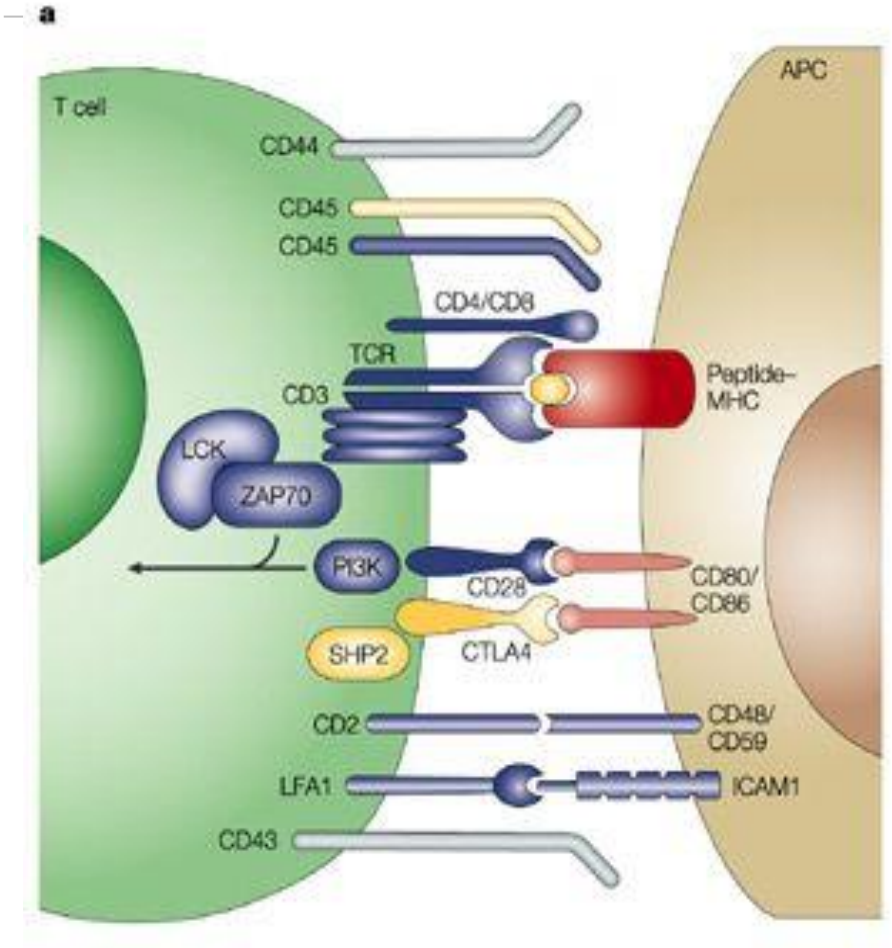
Sai Kiran Sharma et al. Cancer Res 2018;78:1820-1832



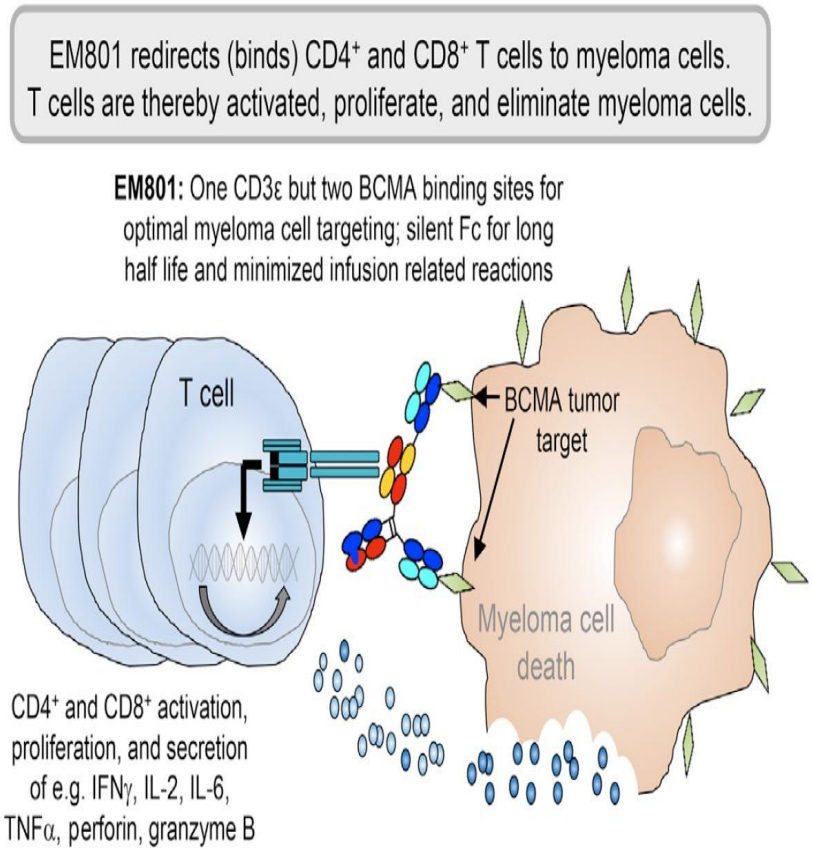


PKPD considerations for T-cell Dependent Bispecifics (TDBs)

Immunological Synapse: MOA of TDBs



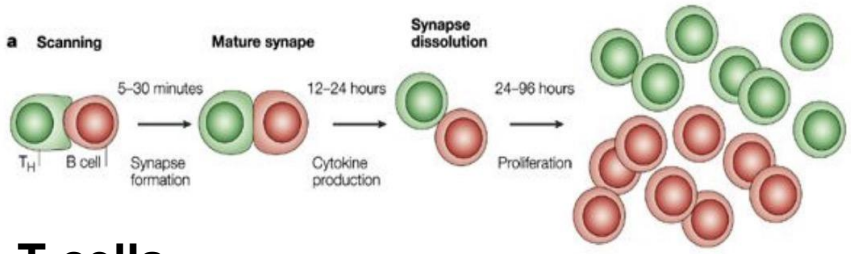
Huppa and Davis, 2003



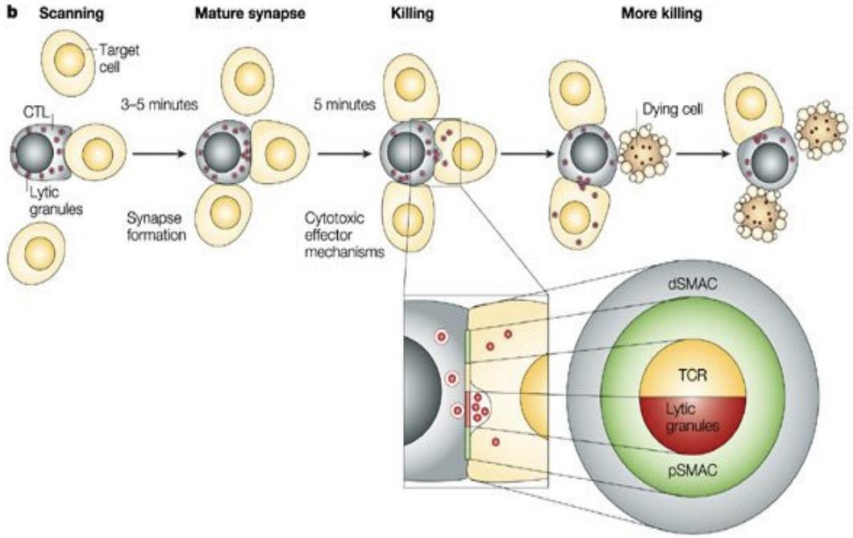
Seckinger, 2017

Spatiotemporal dynamics in immunological synapse

CD4+ T cells



CD8+ T cells

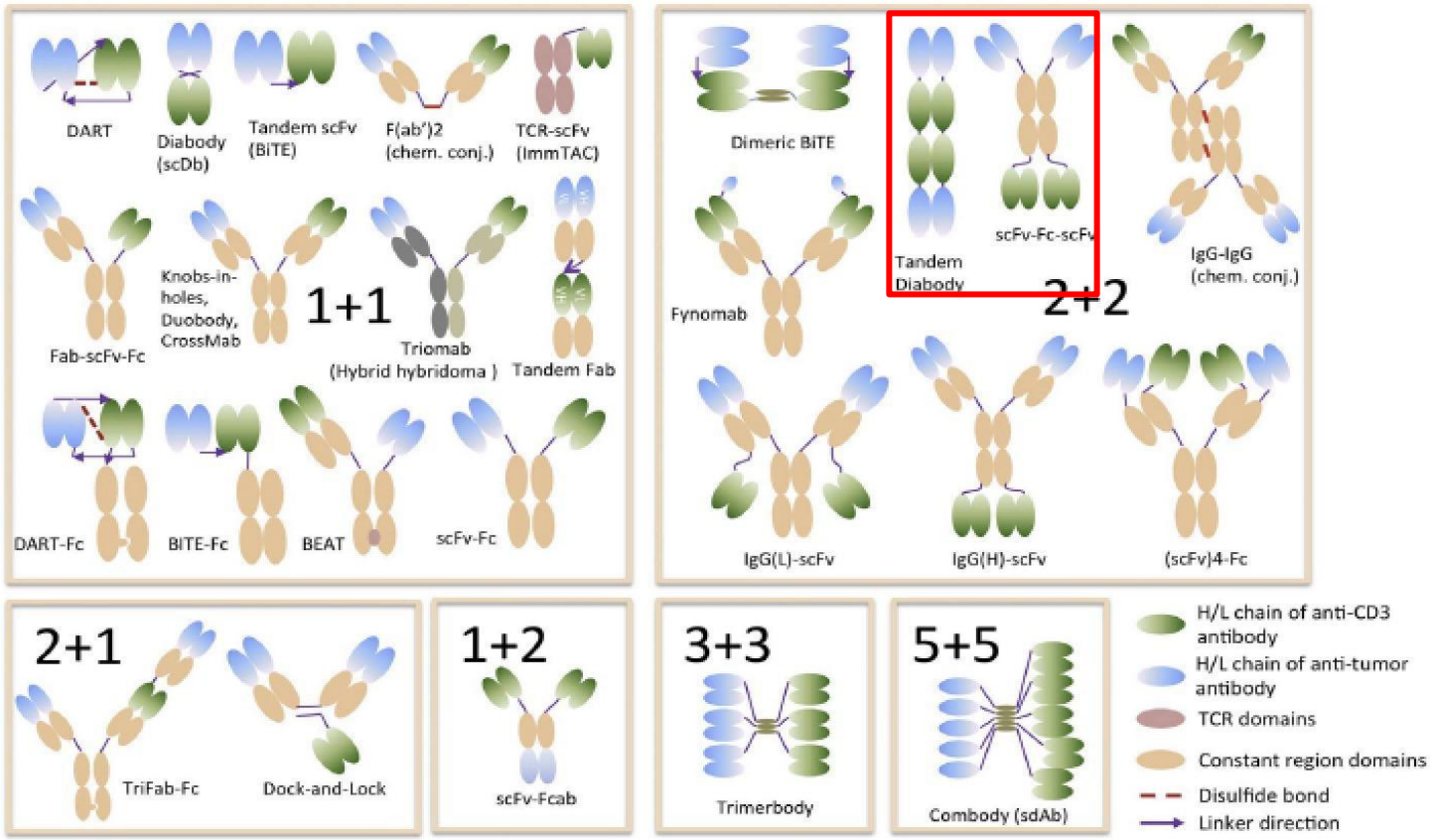


Huppa and Davis, 2003

Bispecific Formats for TDBs

Z. Wu, N.V.. Cheung

Pharmacology and Therapeutics 182 (2018) 161–175



- No Fc or silent Fc in all formats except Catumaxomab
- Monovalent CD3 binding for all except Tandem Diabody and scFv-Fc-scFv
- CD3 affinity ranges from 1-200 nM
- Tumor ag binding is monovalent or bivalent (helps avidity)
- Elimination half-life ranges from 2-8 hr (BiTE), 7-22hr (scFv-Fc-scFv) to days (for TDBs with Fc)

TDBs in Clinical Development

Wu and Jimeno, 2018

Z. Wu, N.V.. Cheung

Pharmacology and Therapeutics 182 (2018) 161–175

Table 1
T-BsAb in clinical development[a].

Tumor antigen	Name	Clinical phase[b]	αCD3 clone used[c]	Formats	References
BCMA	AMG 420 (a.k.a. duvortuxizumab, BI 836909)	I (2015/NCT02514239)	n.a.	BiTE	(Hipp et al., 2017)
CD123	JNJ-63709178	I (2016/NCT02715011)	n.a.	hIgG	(Gaudet et al., 2016)
CD123	MGD006	I (2014/NCT02152956)	proprietary	DART	(Chichili et al., 2015; Huang & Johnson, 2014)
CD123	XmAb14045	I (2016/NCT02730312)	n.a.	Fab-scFv-Fc	(Chu, Pong, et al. 2014)
CD19	AFM11	I (2014/NCT02106091)	UCHT1 (h)	TandAb	(Reusch et al., 2015)
CD19	MGD011 (a.k.a. JNJ-64052781)	I (2016/NCT02743546)	XR32 (h)	DART-Fc	(Liu et al., 2016)
CD19	MT103 (blinatumomab)	Approved	L2K	BiTE	(Dreier et al., 2002, 2003; Löffler et al., 2000; Mølhøj et al., 2007)
CD20	Bi20 (FBTA05)	I/II (2010/NCT01138579)	2611 6 (r)	m/rIgG	(Stanglmaier et al., 2008)
CD20	CD20-TDB (a.k.a. BTCT4465A, RG7828)	I (2015/NCT02500407)	UCHT1 (h)	hIgG	(Sun et al., 2015)
CD20	REGN1979	I (2014/NCT02290951)	n.a.	hIgG	(Smith, Olson, et al. 2015) **From VelocImmune mice**
CD33	AMG-330	I (2015/NCT02520427)	n.a.	BiTE	(Friedrich et al., 2014; Harrington et al., 2015; Laszlo, Gudgeon, Harrington, & Walter, 2015)
CEA	CEA TCB (RG7802, RO6958688)	I (NCT02324257 and NCT02650713)	Proprietary	TriFab-Fc	(Bacac et al., 2016)
CEA	MEDI-565 (a.k.a. AMG-211)	I (2011/NCT01284231)	L2K (de)	BiTE	(Oberst et al., 2014)
CLEC12A, a.k.a. CLL-1	MCLA-117	I (2017/NCT03038230)	Proprietary	hIgG	(Bakker, Van Loo, & Logtenberg, 2014; Van Loo, Doornbos, Dolstra, Shamsili, & Bakker, 2015)
EpCAM	AMG110 (a.k.a. MT110, solitomab)	I (2008/NCT00635596)	L2K (de)	BiTE	(Brischwein et al., 2006; Hermann et al., 2010)
EpCAM	Catumaxomab	Approved	2611 6 (r)	m/rIgG	(Chelius et al., 2010; Ruf et al., 2004; Zeidler et al., 1999)
GPA33	MGD007	I (2014/NCT02248805)	n.a.	DART-Fc	(Moore et al., 2014)
GPC3	ERY 974	I (2016/NCT02748837)	n.a.	hIgG	(Ishiguro et al., 2016)
Her2	Ertumaxomab	II (2007/NCT00522457)	2611 6 (r)	m/rIgG	(Haense et al., 2016)
Her2	GBR1302	I (2016/NCT02829372)	n.a.	BEAT	(Croset et al., 2014)
HLA-A2/gp100	IMCgp100	Ib/II (2015/NCT02535078)	n.a.	TCR-αCD3	(Liddy et al., 2012)
p-cadherin	PF-06671008	I (2016/NCT02659631)	XR32 (h)	DART-Fc	(Root et al., 2016)
PSMA	BAY2010112 (AMG212, pasotuxizumab)	I (2012/NCT01723475)	Proprietary	BiTE	(Friedrich et al., 2012; WHO, 2014)
PSMA	MOR209/ES414	I (2014/NCT02262910)	n.a.	scFv-Fc-scFv	(Hernandez-Hoyos et al., 2016)

[a] This table excludes trials using pre-arm ATC.
[b] Clinical trial stage shows the most advanced clinical phases for the molecule to date. The year of the trial is based on the date published on clinicaltrials.gov.
[c] n.a. denotes clones whose information is not disclosed in the references given; proprietary denotes clones whose information is available in the patent issued or patent pending, as cited in the references; (h):humanized; (r):rat; (de):deimmunized.

PKPD considerations for TDBs

Antigen(s)

TDB

PK

Immunogenicity

DESIGN

DELIVERY

Activation at **site of action** (tumor microenvironment)

Route: IV vs SC

DEVELOPMENT

PKPD/Biomarkers FIH

Dose/Regimen

Therapeutic Index

Immunogenicity

Drug-Drug Interactions

Combinations

Comparability

Indications

Factors unique to TDBs

Cell-Cell Interactions

(Conditional) Agonist

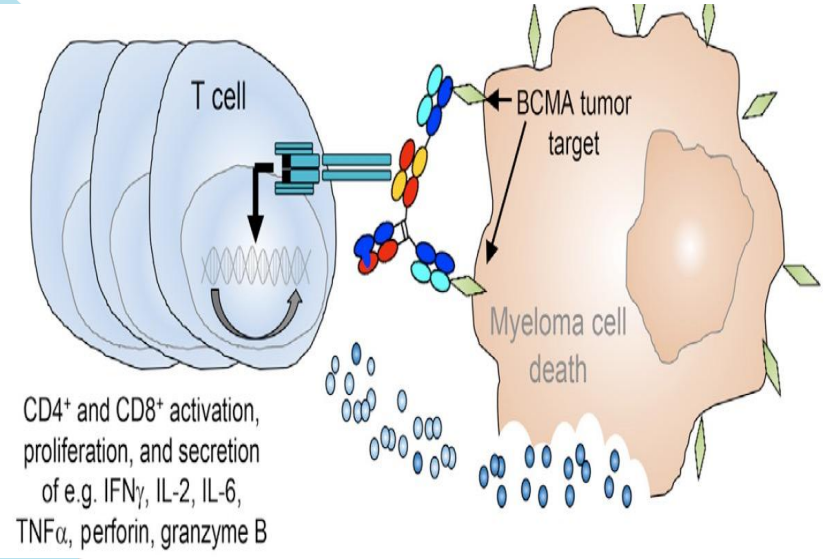
PKPD considerations for DESIGN of TDBs

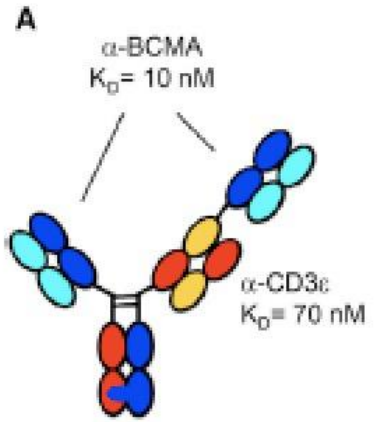
Antigen(s): CD3 and tumor antigen
Kinetics
Expression levels and profile
Pharmacology
Epitope

TDB:
Affinity, avidity, kon/koff
Valency
Format
Conformation
Linker stability
Fc functions

PK-Fc, size, format

Immunogenicity
Structure
MOA

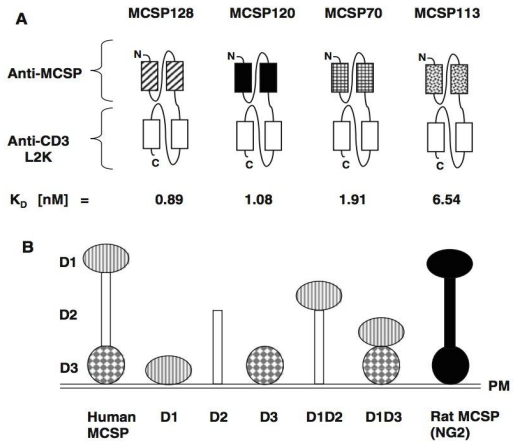


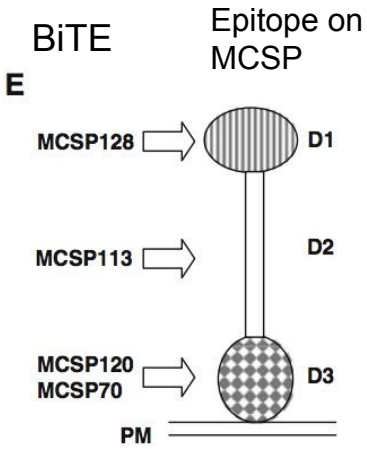


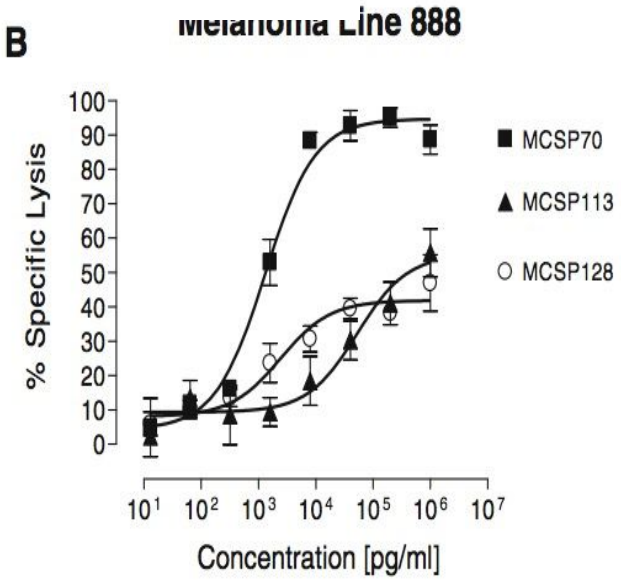
Seckinger, 2017

Epitope distance to the target cell membrane determines the potency of T cell-mediated lysis by BiTE antibodies specific for MCSP or EpCAM*

Cancer Immunol Immunother (2010) 59:1197–1209



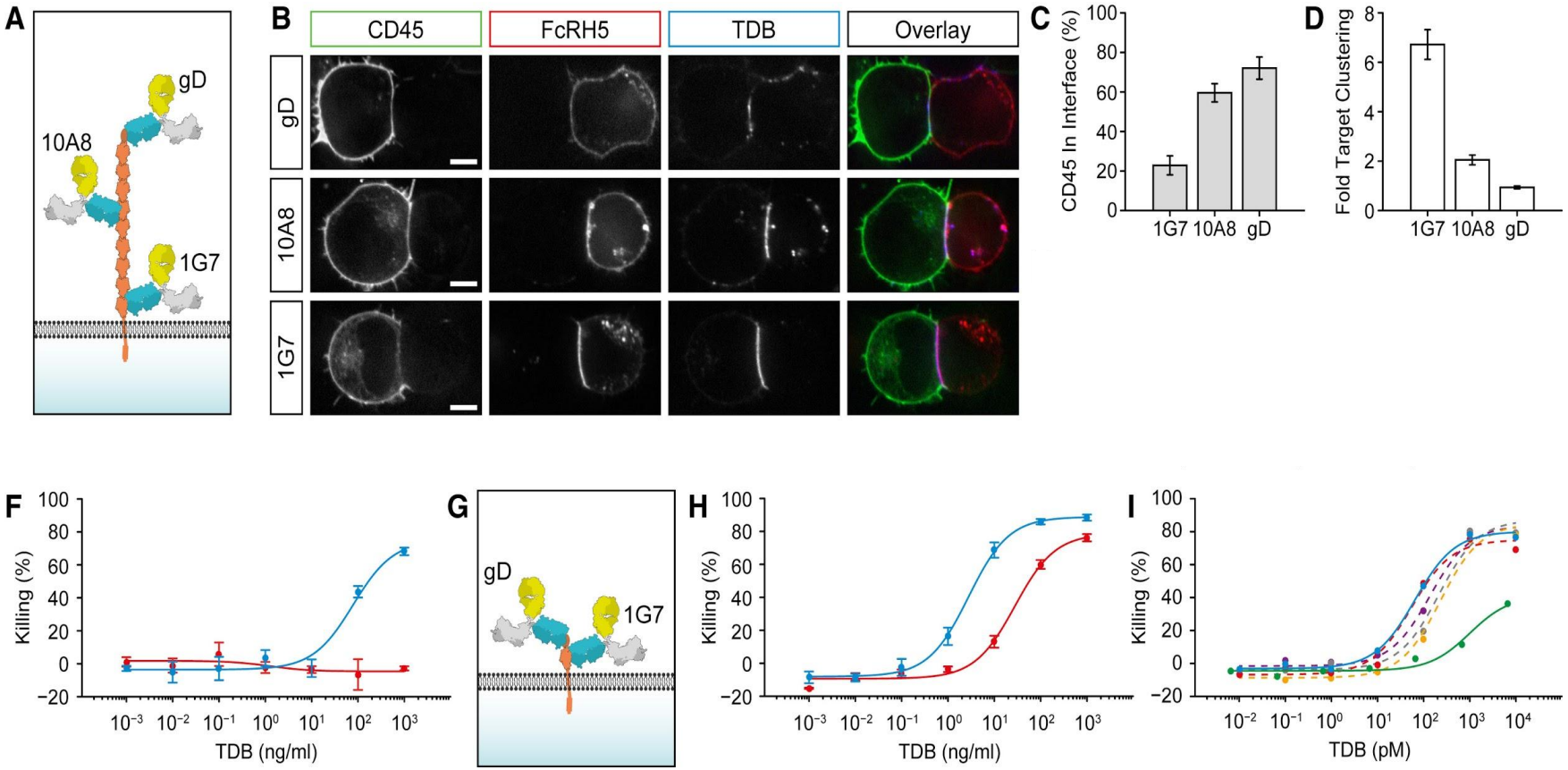




MCSP: Melanoma chondroitin sulfate proteoglycan (melanoma antigen); *EpCAM data not shown

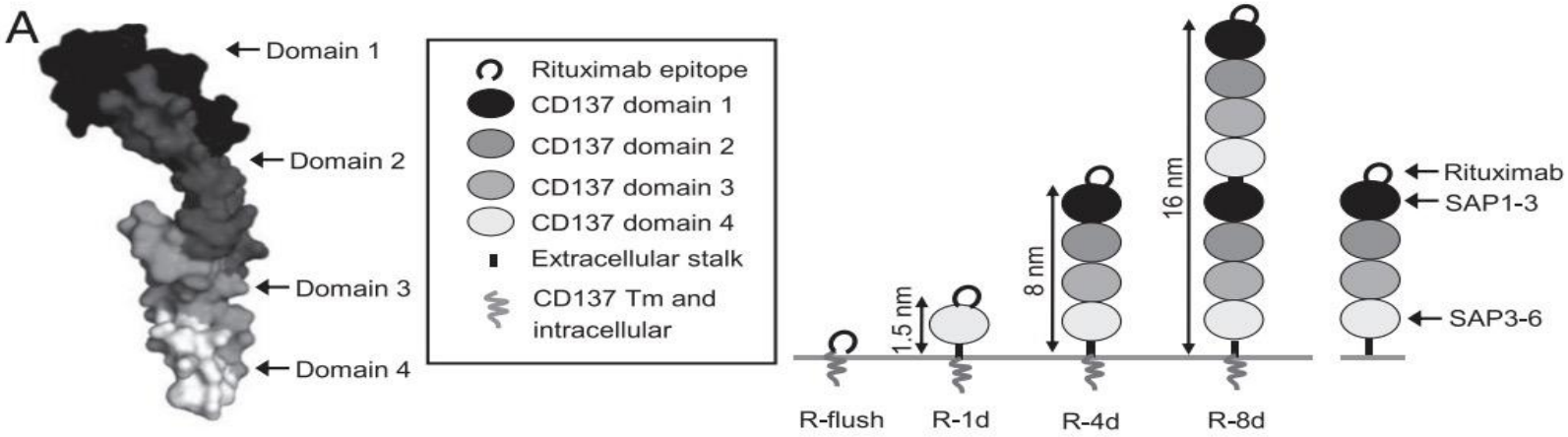
Bluemel, 2010

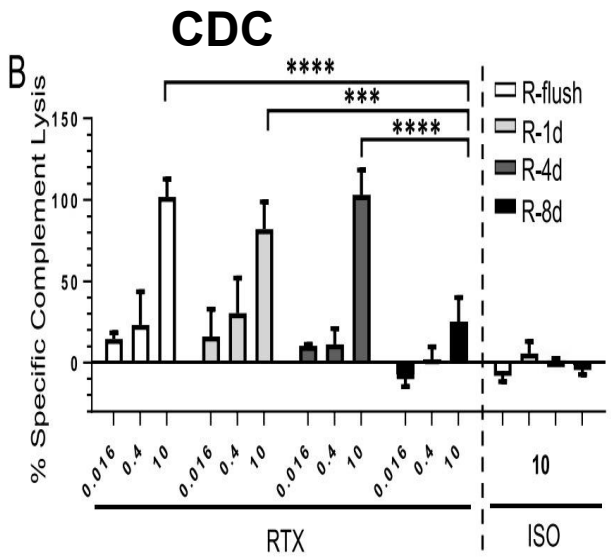
Membrane-Proximal Epitope Facilitates Efficient T Cell Synapse Formation by Anti-FcRH5/CD3 and Is a Requirement for Myeloma Cell Killing

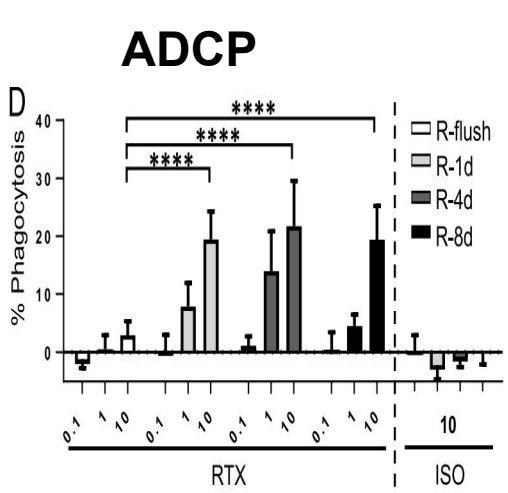


Li 2017

Antibody Distance from the Cell Membrane Regulates Antibody Fc-mediated Effector Mechanisms







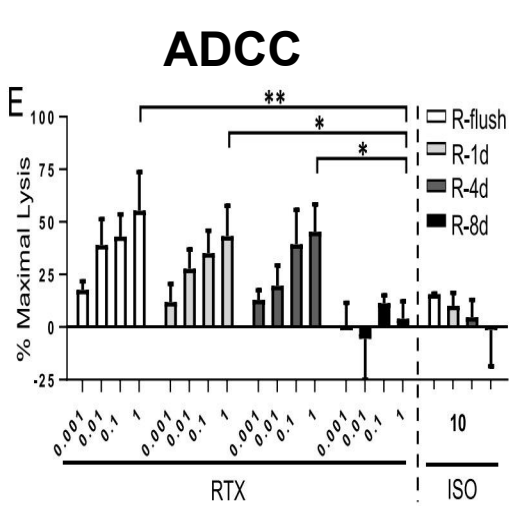
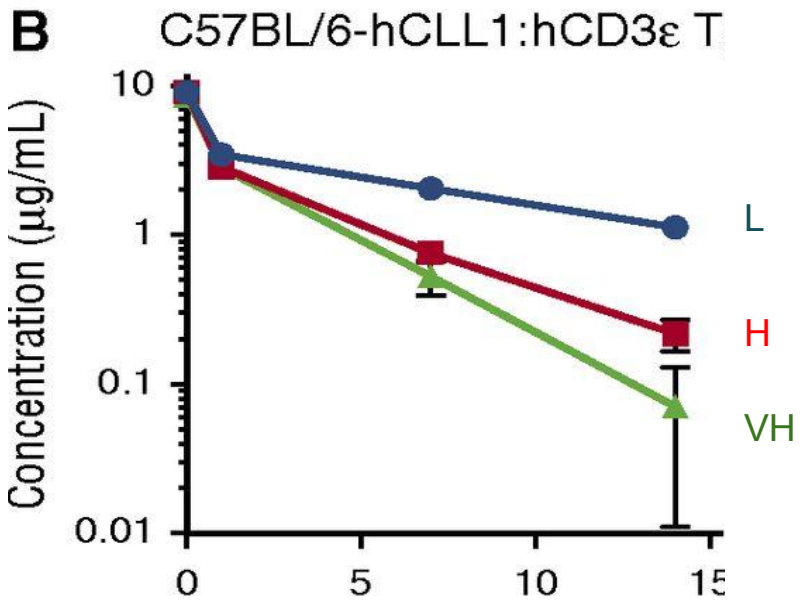


FIGURE 2. Comparison of cytotoxic activity engaged by RTX when targeting CHO-S cells displaying the RTX epitope at different distances from the

Cleary, *J. Immunol.*, 2017

Impact of CD3 affinity on PK and cytokine levels

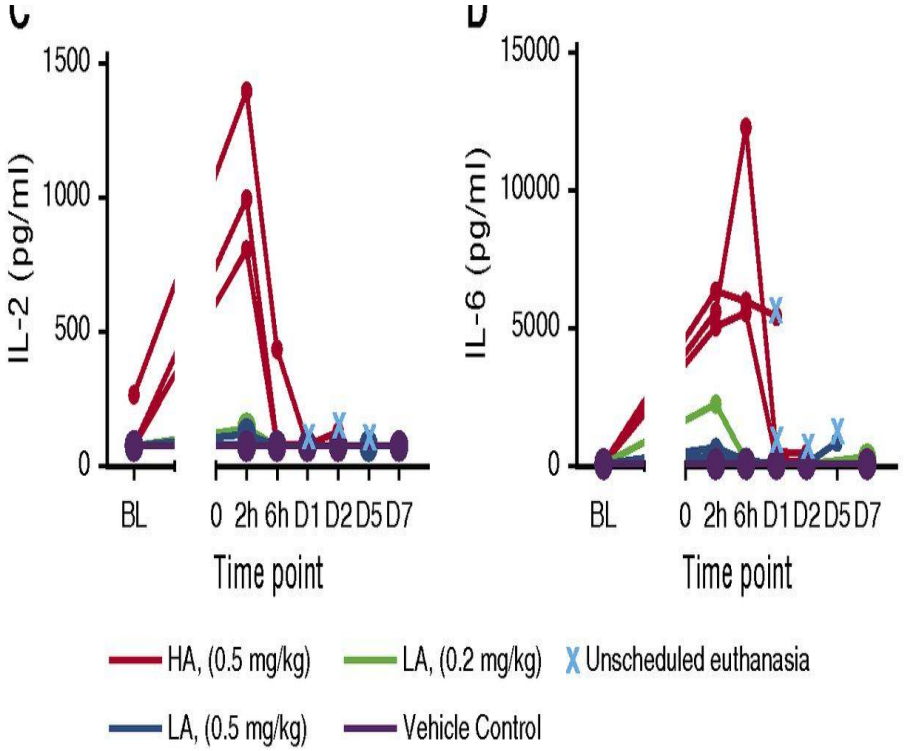
PK in Tg mice



C57Bl/6 2xTg Group	Cmax (µg/ml)	AUC all (day*µg/mL)	CL (mL/day/kg)
CLL/CD3L	9.1	47.1	10.6
CLLCD3H	9.1	21.3	23.5
CLL/CD3VH	8.5	18.1	37.4

Cytokine Profiles in Cyno

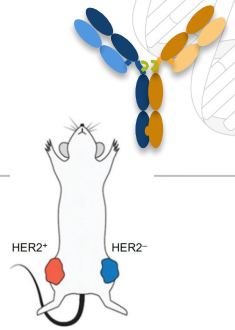
Cytokine levels in cynomolgus monkeys receiving CLL1/CD3H (HA) and CLL1/CD3L (LA).



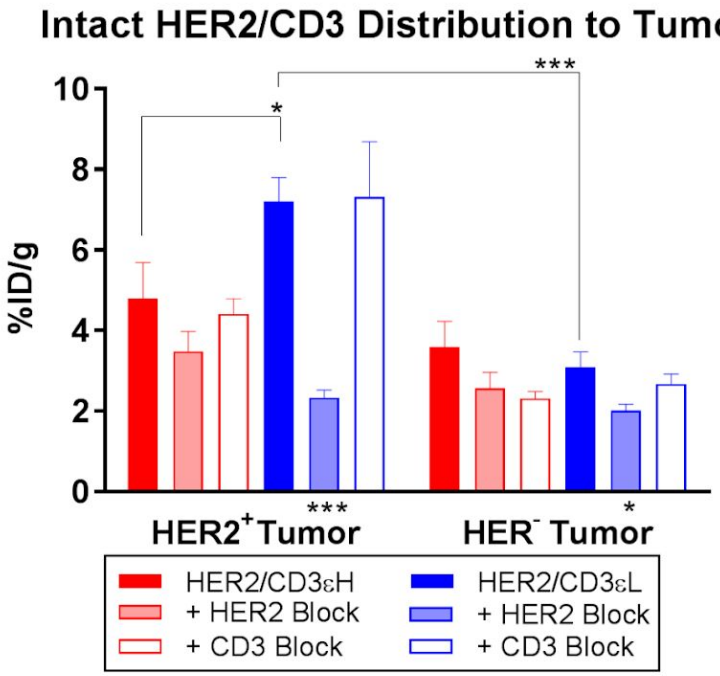
Does binding on/off rates impact activity?

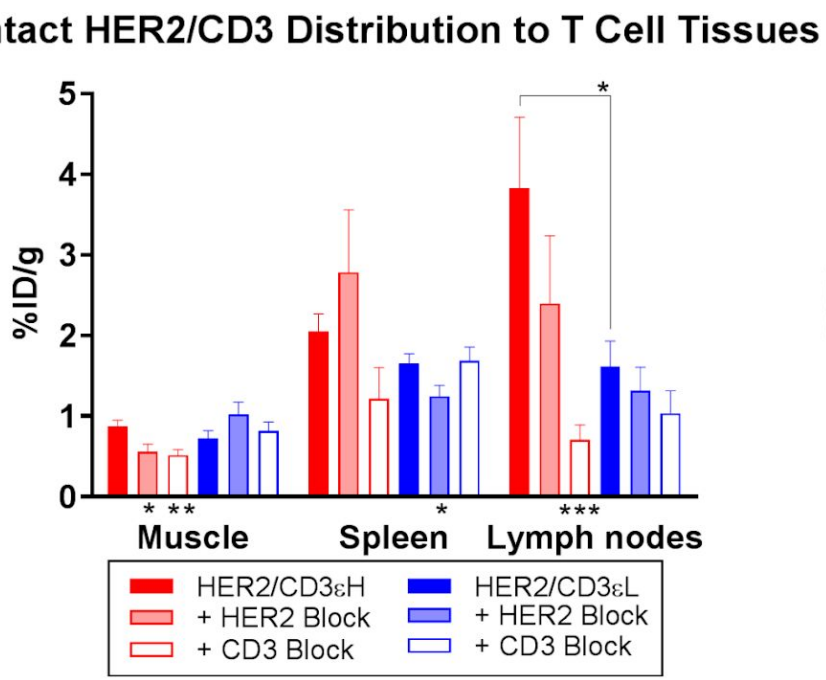
Steven R. Leong et al. Blood 2017;129:609-618

Relative affinities of CD3/(Her2) compete for distribution to targets



Example: HER2/CD3 TDBs dosed in huCD3TG mice, inoculated with dual tumors

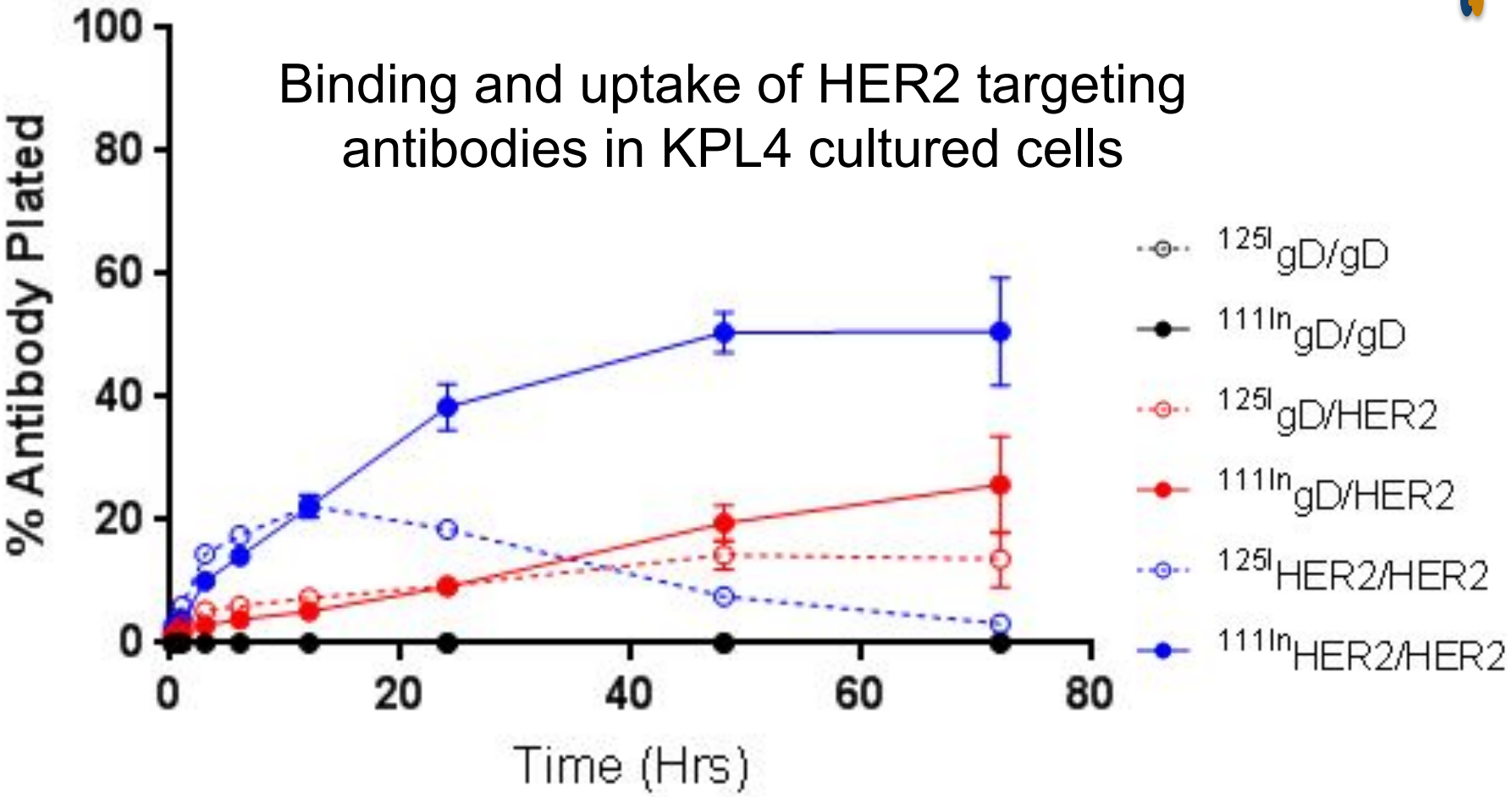




Increased CD3 affinity leads to decreased tumor distribution and increased secondary lymphatic tissue distribution

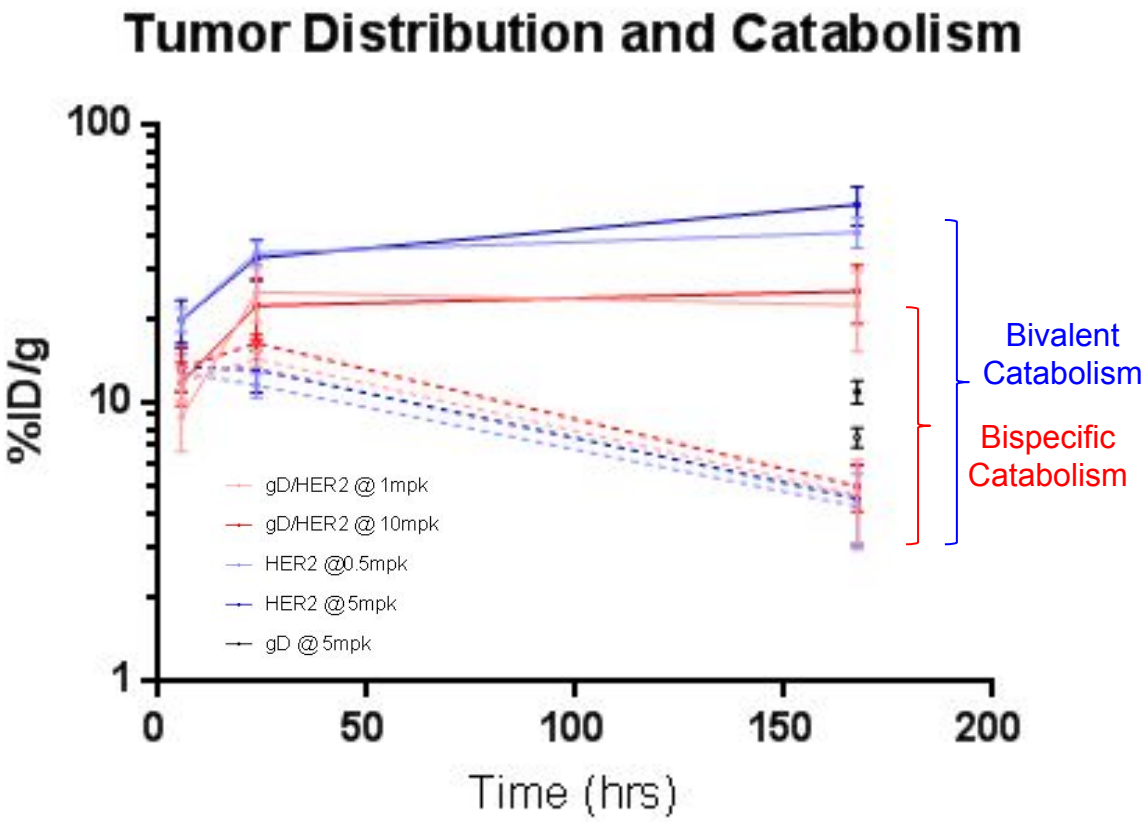
Mandikien, 2018

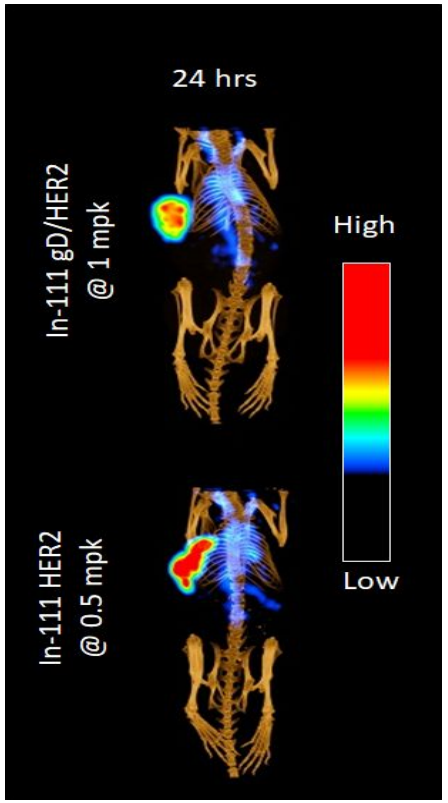
Valency: Binding and antibody internalization is reduced for bispecific antibodies compared to bivalents



Reduced valency leads to reduced internalization *in vivo*

Example: Anti-HER2 knob in hole antibodies dosed in KPL4 tumor bearing mice, normalized to HER2 epitope

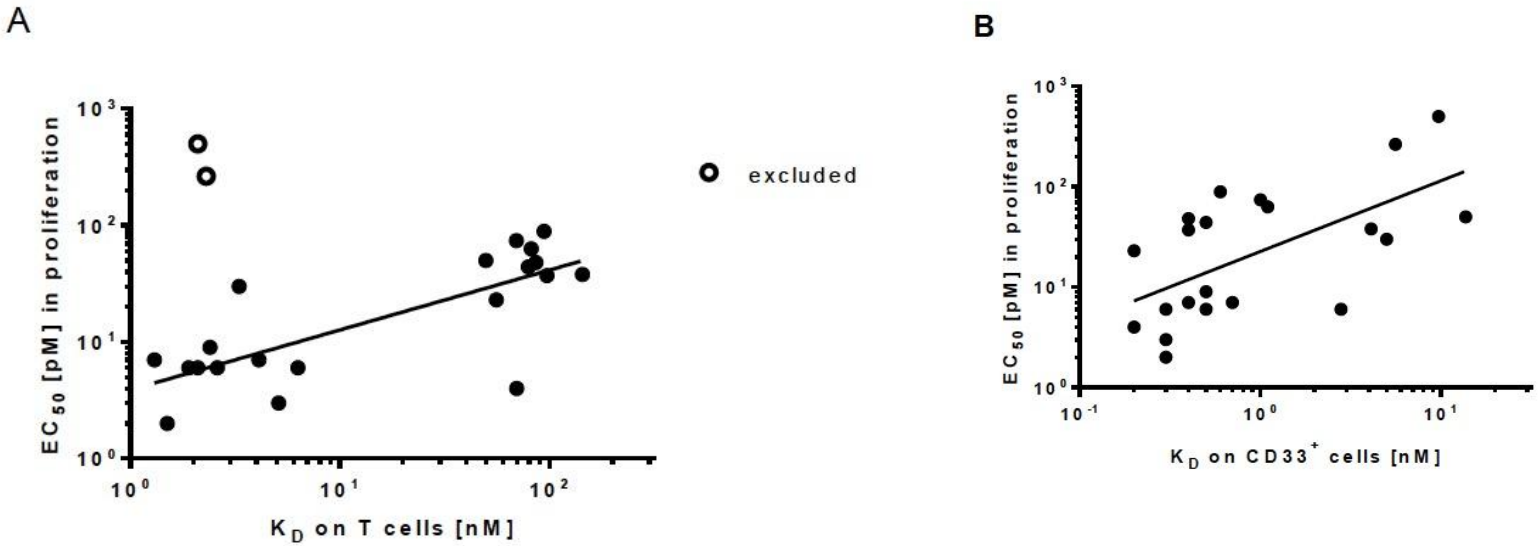




Bispecific Antibodies show slight differences in tumor penetration and appear at higher concentrations on the periphery.

Danielle Mandikian, Madeleine Ramos and Andy Boswell

With anti-CD33/CD3 TandAb, bivalent binding for both CD3 and CD33 correlated with activity

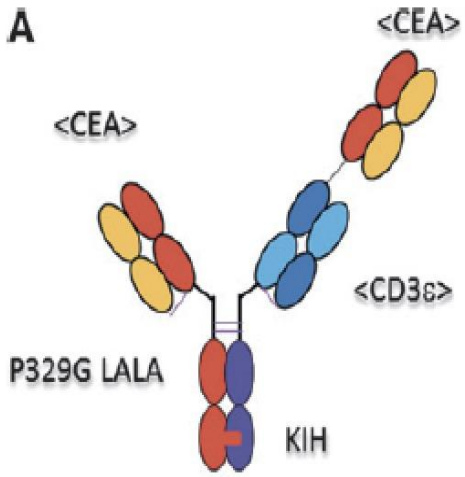


Correlation of CD3 and CD33 affinity with EC_{50} in T-cell proliferation assays

Data points from proliferation assays shown in Supplemental Figure 2 were plotted as a function of K_D on T-cells (**A**) or CD33+ cells (**B**). Slope in **A** is 0.5±0.1, r=0.764, p<0.0001. Data points excluded in A due to cell viability <50% at completion of the experiment. Slope in B is 0.7±0.2, r=0.622, p=0.002.

Reusch, 2016

Anti-CEA/CD3 TCB (IgG-Fab) format leverages bivalent binding and avidity



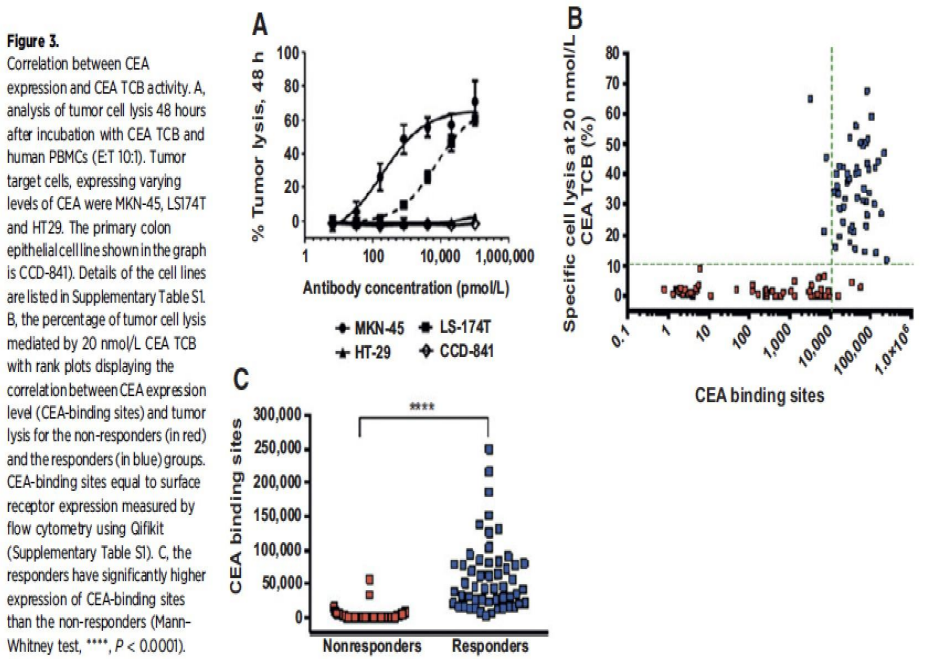
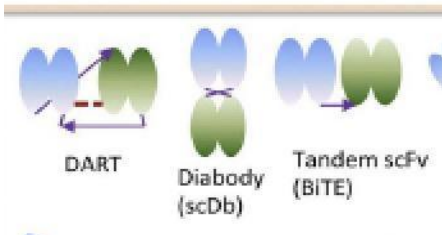


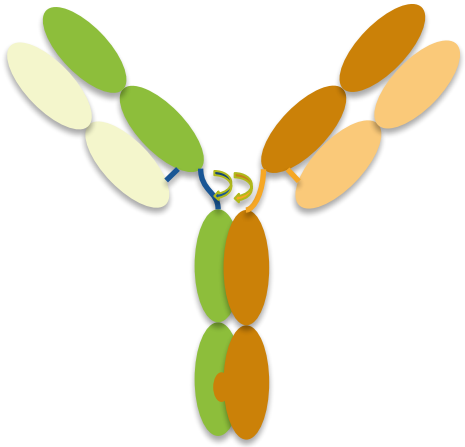
Figure 3.
Correlation between CEA expression and CEA TCB activity. A, analysis of tumor cell lysis 48 hours after incubation with CEA TCB and human PBMCs (E:T 10:1). Tumor target cells, expressing varying levels of CEA were MKN-45, LS174T and HT29. The primary colon epithelial cell line shown in the graph is CCD-841). Details of the cell lines are listed in Supplementary Table S1. B, the percentage of tumor cell lysis mediated by 20 nmol/L CEA TCB with rank plots displaying the correlation between CEA expression level (CEA-binding sites) and tumor lysis for the non-responders (in red) and the responders (in blue) groups. CEA-binding sites equal to surface receptor expression measured by flow cytometry using Qifikit (Supplementary Table S1). C, the responders have significantly higher expression of CEA-binding sites than the non-responders (Mann-Whitney test, ****, $P < 0.0001$).

Does format impact TDB PKPD and distribution?

Size, valency



Size, valency, Fc (PK only)



Size, valency, Fc (PK), Avidity

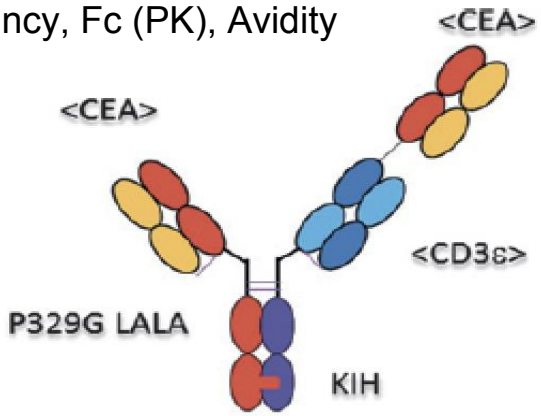
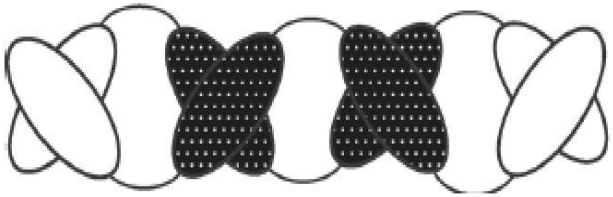


Figure 1.
Structure and binding of CEA TCB. A, structural cha
to CD3e (monovalent binding mode), and the Fc w
adenocarcinoma cells (MKN45, EC_{50} of binding 10
SD based on triplicates.

Size, valency, Fc, Avidity



CD3 CD33 CD3

PKPD considerations for Design of TDBs

Closing remarks

DESIGN

Affinity, avidity, format, epitope, valency, flexibility/conformation are key design parameters-need further systematic studies to delineate effects on activity, PK, distribution.

Antigen epitope and size likely critical.

PK: long half-life is desirable feature

Immunogenicity: no clinical data yet but given novel formats and “immune activation” MOA, immunogenicity should be critically assessed

Next generation TDB designs will likely include additional immune functions (co-stimulation), and/or NK cells or gamma delta T cells. Also, “threading the needle” between normal expression vs tumor expression will be an important design challenge.

PKPD considerations for Development of TDBs

DEVELOPMENT

- **PKPD/Biomarkers**
- **FIH/Dose Escalation**
- **Dose/Regimen**
- Therapeutic Index
- Immunogenicity
- Drug-Drug Interactions
- **Combinations**
- Comparability
- Indications

Anti-CD20/CD3 PKPD (Model)

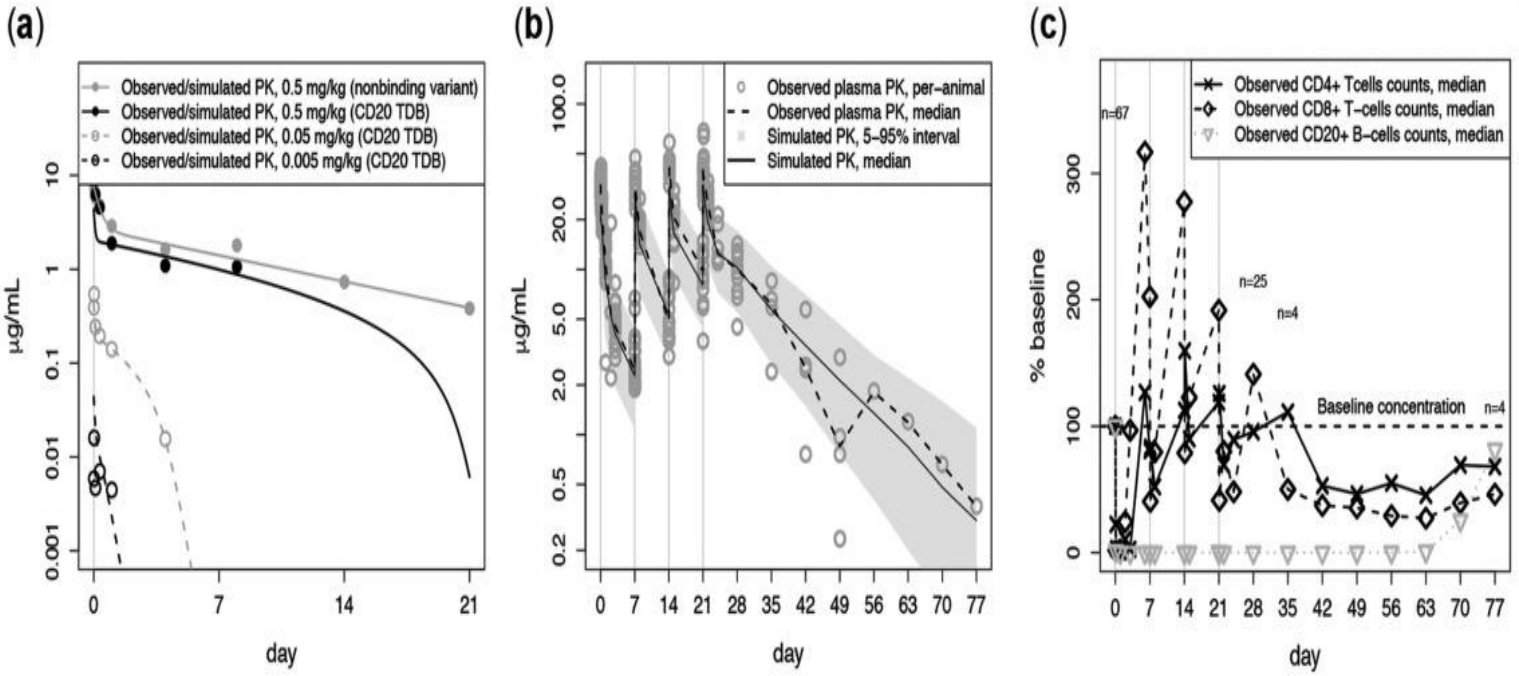


Figure 2 Murine PK and cynomolgus monkey PK/PD data with model simulations. (**a**) Two-compartment nonlinear PK model, as described by Eqs. (1) and (2), with $CL_2^0 = 0$ and $\lambda_1 = 0$, fitted to murine data. Key estimated parameters for the CD20 TDB are $CL_1 = 16$ mL/day/kg, $K_M = 0.015$ μg/mL, and $V_{max} = 12$ μg/day. The solid gray curves indicate model fitted to PK data from the low-affinity CD3 TDB variant UCHT1 [1] (V_{max} fixed to zero). (**b**) Comparison of mixed-effects model simulations (median PK profile and interanimal variability) generated using fitted mixed-effects parameters summarized in **Table 2**, and observed PK data for 1 mg/kg i.v. bolus and slow push repeat dosing cohorts. Solid line and shaded region correspond to simulated median and 5–95% intervals. Circles are observed drug concentrations in plasma and dashed line corresponds to median observations at each timepoint. (**c**) Corresponding changes in median peripheral blood CD4+, CD8+ T-cell counts, and CD20+ B-cell counts. Number of animals (*n*) for which T- and B-cell levels were measured is indicated at t = 0, 28, 35, and 77 days. In all panels, vertical lines indicate dosing times.

Ferl 2018

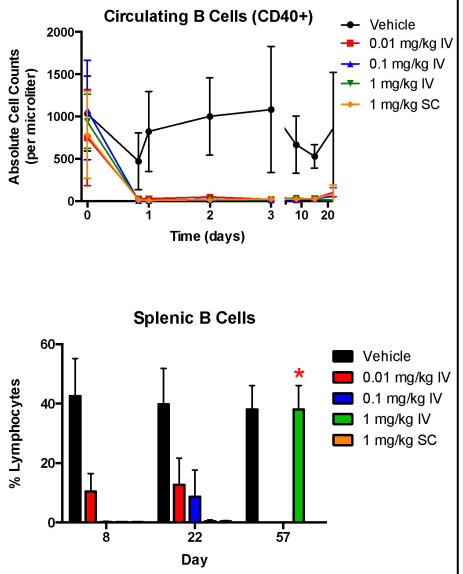
PKPD in Cynomolgus Monkeys with anti-CD20/CD3

Target engagement/PD biomarker

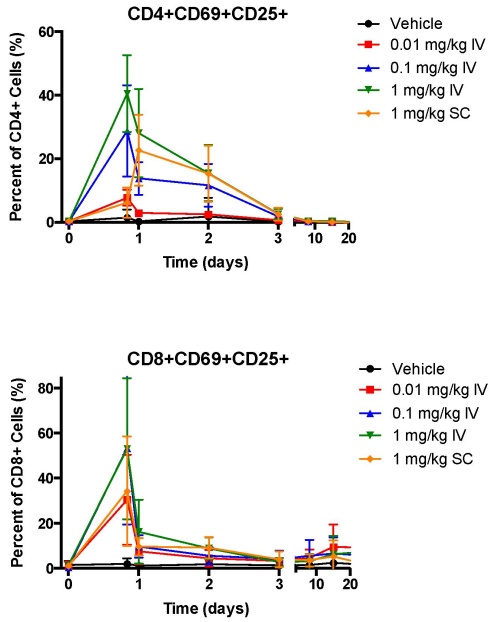
Target engagement biomarker

Safety biomarker

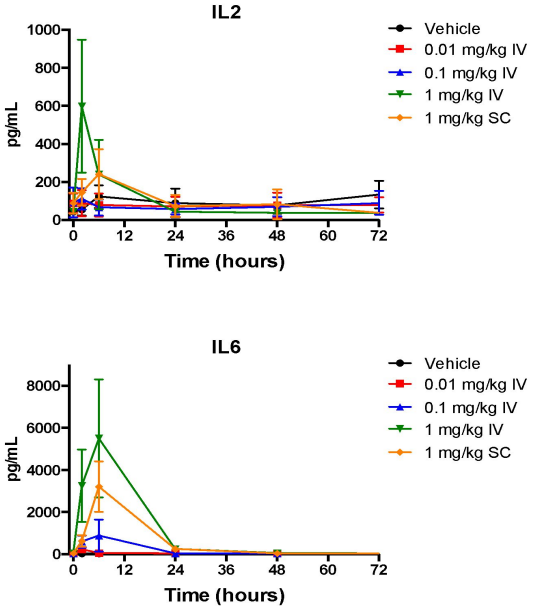
B-Cell Depletion



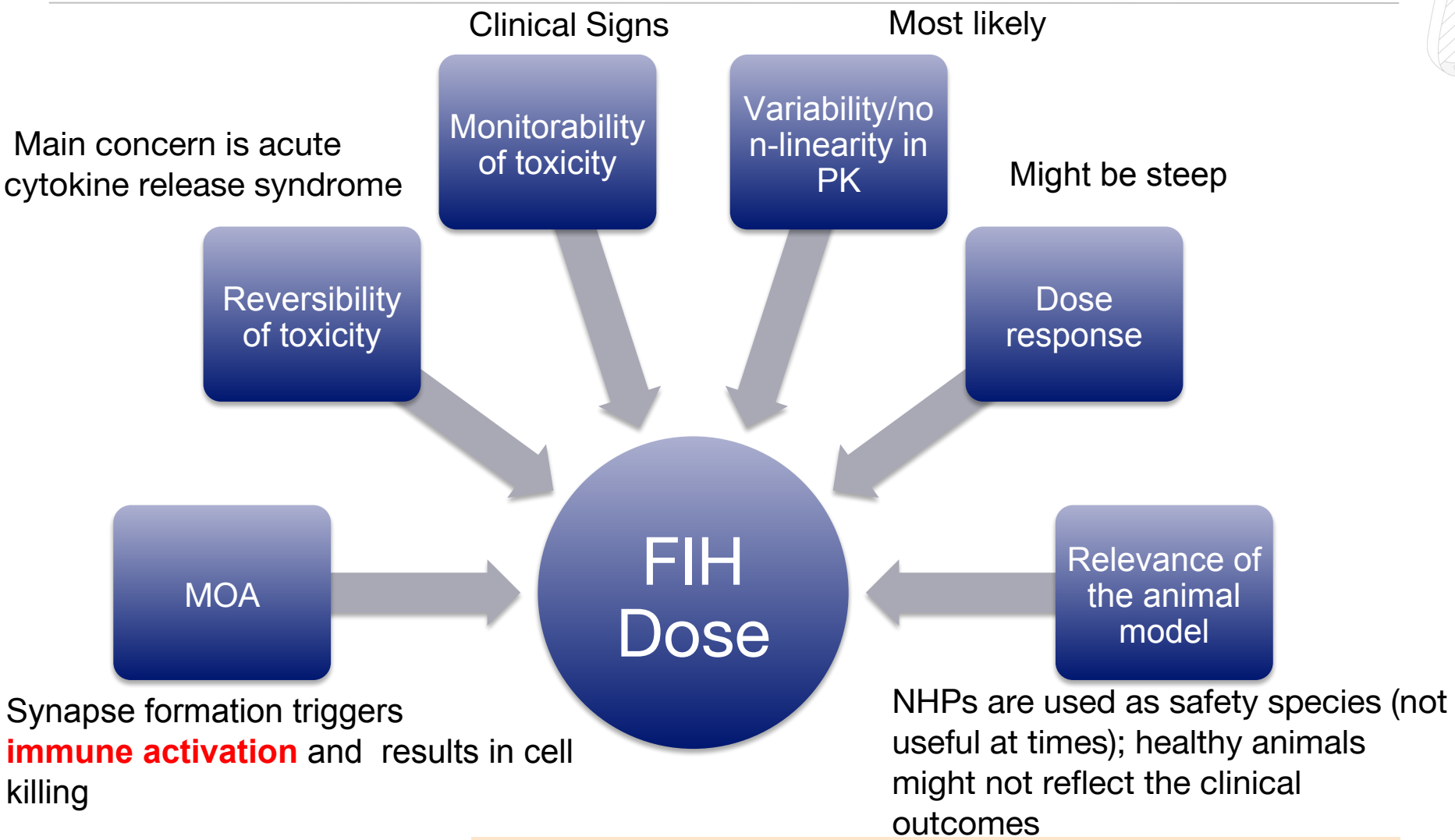
T-Cell Activation



Cytokine Release



Multiple variables contribute to the determination of approach for (TDBs) FIH dose selection



Meric Ovacik

FIH dose selection is usually based on MABEL

Adapted from Siddharth Sukumaran, NBC 2017

FiH Dose - *in vitro* approach

***In vitro* cell killing, T cell activation or cytokine release are the main assays**

- ✓ Either i-) PMBC or ii-) PBMC and target expressing cell line
- ✓ EC_{20} – EC_{30} were used as the projected C_{max} to determine FiH dose (in conjunction with V_C)
- ✓ Most sensitive endpoint (e.g. cell killing or T cell activation) or most sensitive safety endpoint (T cell activation or cytokine release) were used
- ✓ Safety factors and RO information were used as supporting data

Saber et al. ,2017

Dose Escalation Considerations

Findings and recommendations from Saber et al, 2017

Typically dose escalation 3+3; for TDBs single patient cohort before switching to 3+3.

Protocol Amendments:

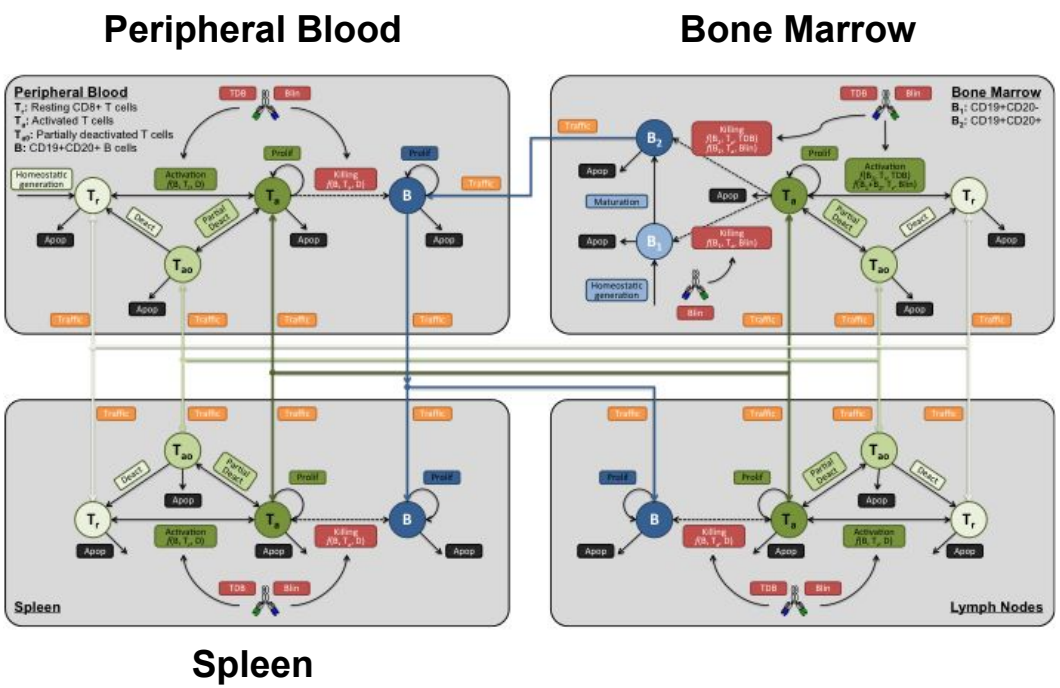
- increase duration of infusion
- include a step dose
- use medication prophylactically for IRR/CRS

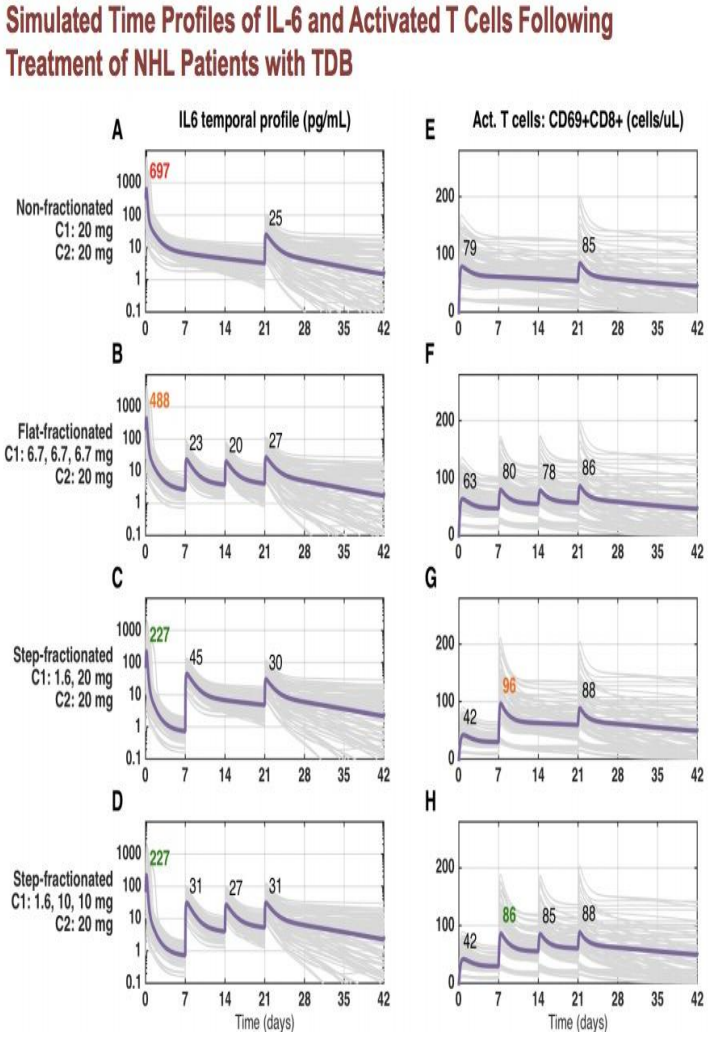
For approximately half (12 out of 27 or 44%) of the antibodies examined the FIH doses were in microgram ranges and corresponded to up to 50% RO. These doses were 100s-1000s fold less than doses given to patients with acceptable/manageable toxicities and the period of time to complete the dose-finding trial was up to 5 yr (range of approximately 1–5 yr). While obtaining safety data is the main goal of Phase 1 trials, patients enrolling in clinical trials for cancer drugs have generally exhausted available therapies and enter with the hope of benefiting from the study. A clinical trial design that minimizes exposure to sub-therapeutic doses while maintaining safety is desired for these patients. This goal may be achieved by optimal FIH dose selection or through non-traditional FIH trial designs which permit intra-patient dose escalation when the FIH doses are low, such as ≤ 50% RO using Equation B.

Meric Ovacik

Saber et al. ,2017

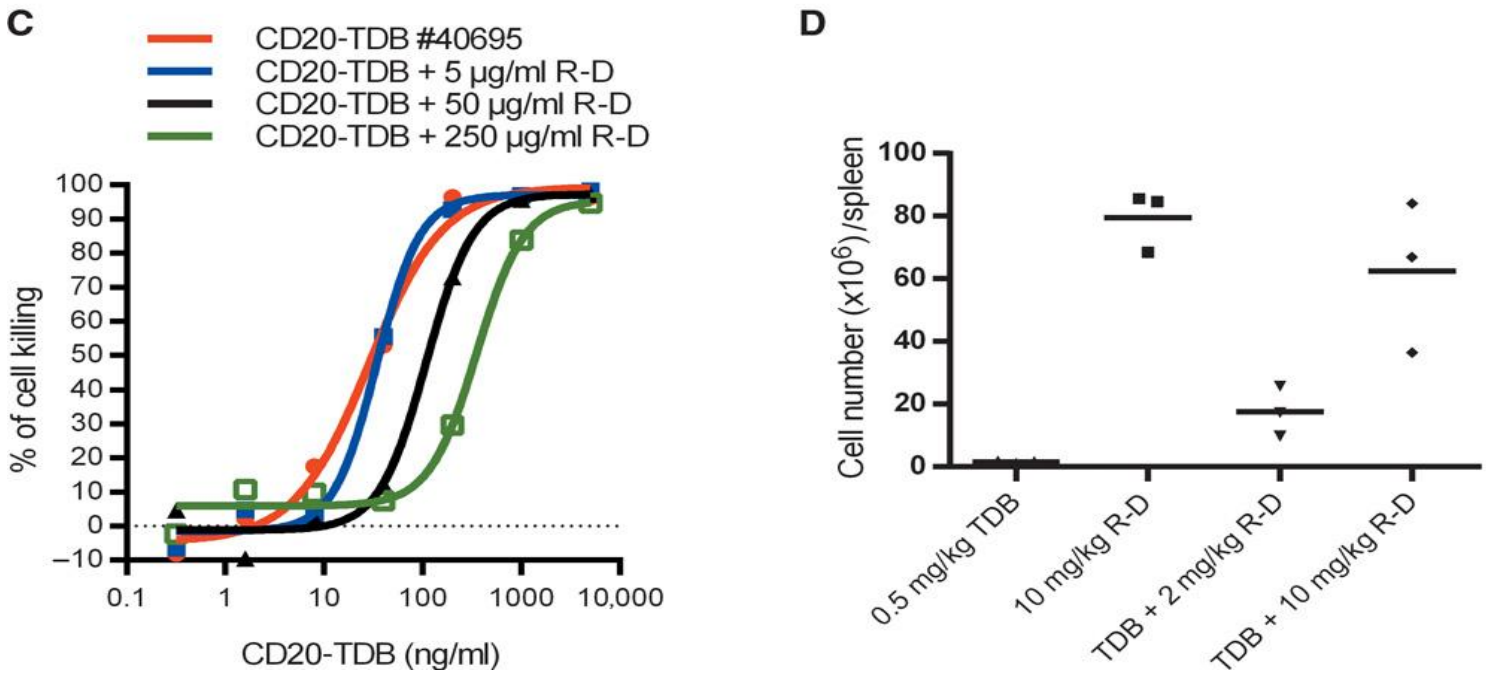
Quantitative systems pharmacology (QSP) model of Anti-CD20/CD3 to characterize cycle 1 dose schedules





Iraj Hosseini and Saroja Ramanujan

Rituxan (effectorless) increases the EC50 for B-cell killing



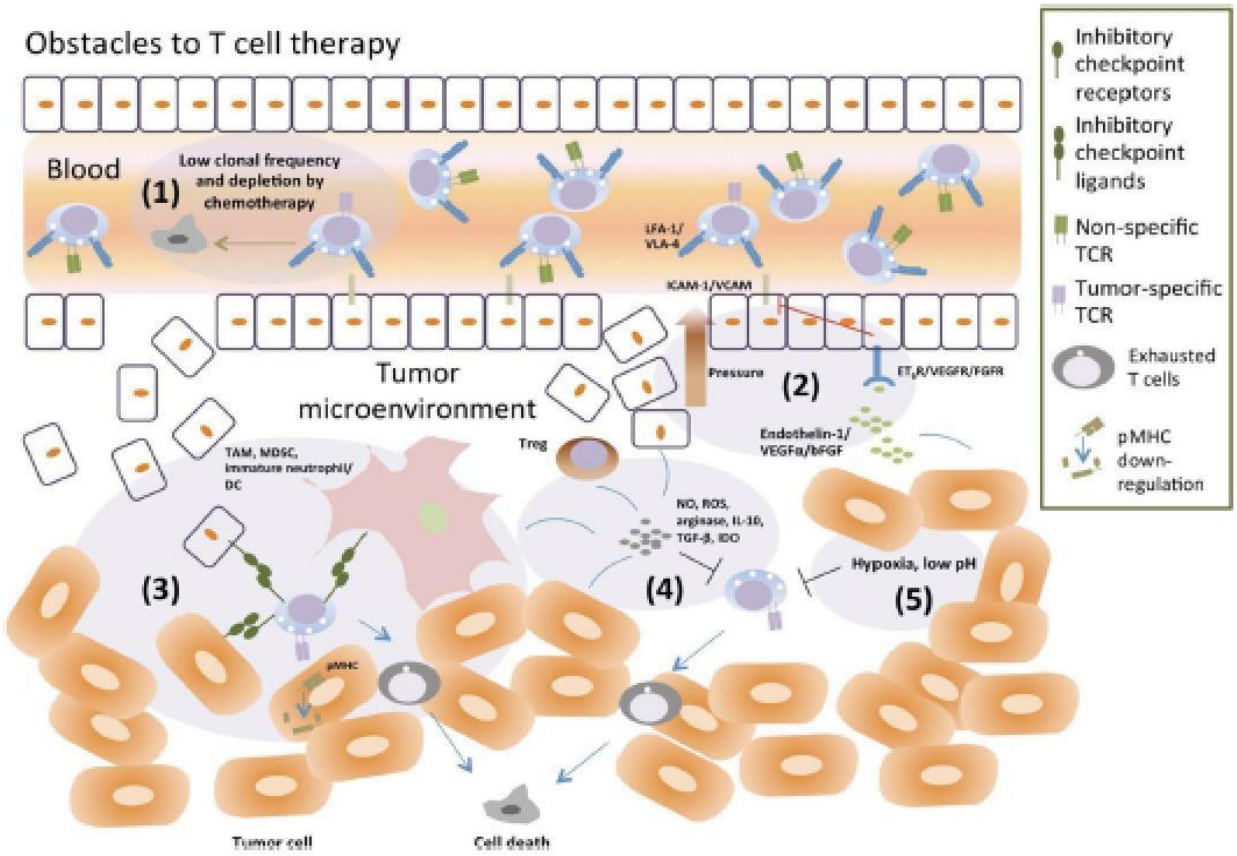
C) PBMCs isolated from healthy donors were first incubated with rituximab-DANA (R-D) at the concentration indicated for 1 hour, and then CD20-TDB was added. After 48 hours, B cell killing was determined by FACS. (**D**) huCD20-huCD3 double-transgenic mice were treated once intravenously at the dose indicated; for combination treatment, mice were pretreated intravenously with rituximab-DANA, and CD20-TDB (0.5 mg/kg) was injected intravenously 30 min later. Spleens were collected at day 7, and B cell counts were determined by FACS. Bars in the plots indicate mean values, with *P* values calculated by unpaired *t* test (*n* = 3 mice per group).

Published by AAAS

Use of Combos with TDBs

Z. Wu, N.V., Cheung

Pharmacology and Therapeutics 182 (2018) 161–175



Combinations: Agents that increase tumor infiltrating lymphocytes, decrease T cell/tumor cell immunosuppression; cytokines/chemokines; anti-VEGF, vaccines.

PKPD considerations for T-cell bispecifics

Closing remarks

DEVELOP-MENT

PKPD: Well established serum PKPD relationships (eg. T cell activation); need tissue PKPD assessments

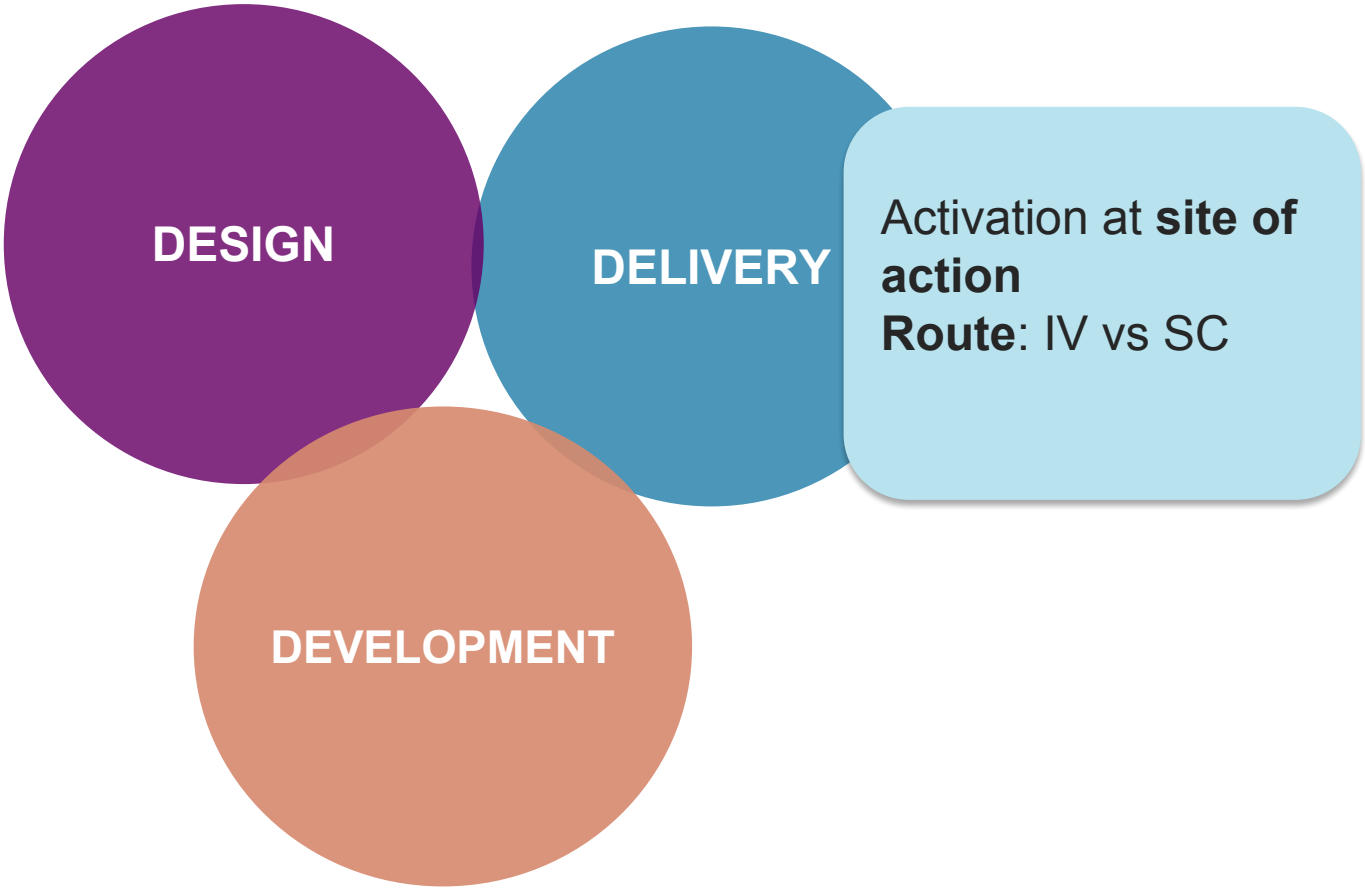
FIH: use of in vitro T cell activation assays

Dose/Regimen: Dose escalation and fractionation

DDI: Presence of Herceptin or Rituxan/Gazyva

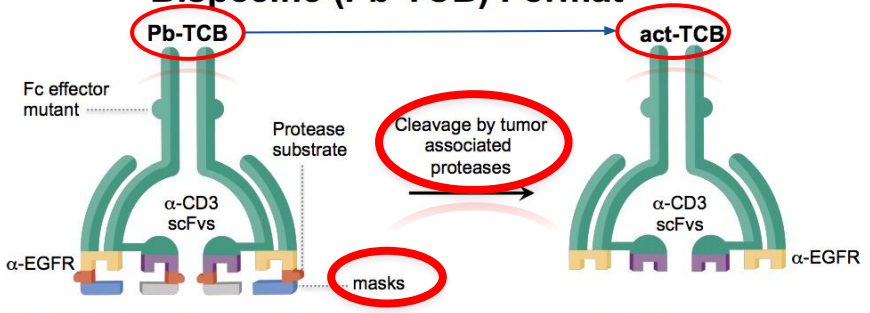
Combinations: Agents that increase TILs, decrease T cell/tumor cell immunosuppression; cytokines/chemokines; anti-VEGF.

PKPD considerations for Delivery of TDBs



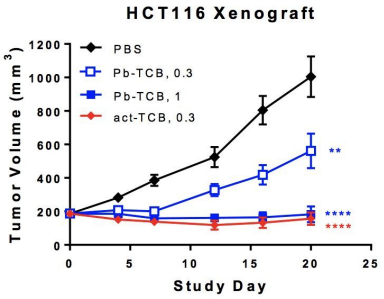
Activation of T-cell (effector cell) at site of action (tumor)

Figure 2: CytomX Probody T Cell-Engaging Bispecific (Pb-TCB) Format



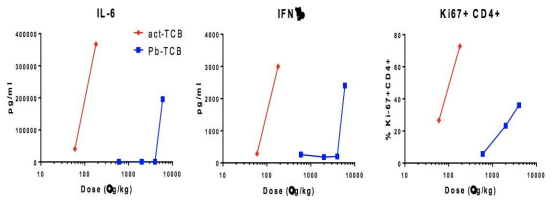
- Full IgG bispecific format to maximize exposure and half-life
- Fc-effector impaired to minimize cross linking to FcγR bearing cells
- Format optimized for a-CD3 affinity, mask strength and cleavable substrates
- act-TCB represents protease activated, unmasked TCB

Figure 7: EGFR/CD3 Pb-TCB is Efficacious in HCT116 Established Tumor Model



Female NSG mice (n=8/group) were implanted SC with 2 million HCT116 cells on day -15. Three days later, mice were injected IP with human PBMCs at a T cell/tumor inoculum ratio of 1:1. Test and control articles were administered IV at 0.3 mg/kg or 1 mg/kg, weekly. TV is presented as mean ±SEM.

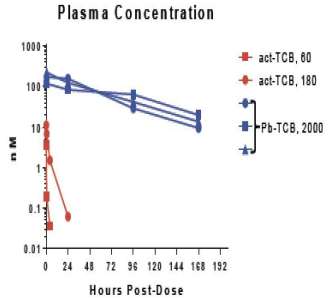
B Pb-TCB shifts dose-response for cytokine release and T cell activation relative to act-TCB



Cytokine analysis was performed with a Luminex® suspension array system on serum samples. Data presented were obtained at 8 hours post-dose.

Flow cytometry was performed on permeabilized samples. Data presented were obtained at 72 hours post-dose.

Figure 9: Tolerated Pb-TCB Exposure is > 10,000-fold Higher than Tolerated Exposure of act-TCB in Cynomolgus Monkeys



Plasma concentration of act-TCB and Pb-TCB was measured by ELISA using anti-id capture and anti-huFc detection. Time points after 4 hours for act-TCB dosed at 60 µg/kg and 24 hours for act-TCB dosed at 180 µg/kg were BLQ. Tolerated exposure represents area under the curve (AUC) of Pb-TCB (448 day*nM) dosed at 2000 µg/kg and act-TCB (0.04 day*nM) dosed at 60 µg/kg.

Boustany, 2017

PKPD considerations for T-cell bispecifics

Closing remarks

DESIGN

Affinity, format, epitope, valency are key design parameters-need further systematic studies to delineate effects on activity

PK: long half-life is desirable feature

Immunogenicity: no clinical data yet but given novel formats and "immune activation" MOA, immunogenicity should be considered

Next generation TDBs design will likely include additional immune functions (co-stimulation), and/or NK cells or gamma delta T cells

DEVELOP-MENT

PKPD: Well established PKPD relationships (eg. T cell activation); PKPD at site of action will be critical
FIH: use of in vitro T cell activation assays
Dose/Regimen: Dose escalation and fractionation
DDI: Presence of Herceptin or Rituxan/Gazyva
Combinations: Agents that increase TILs, decrease T cell/tumor cell immunosuppression; cytokines/chemokines; anti-VEGF.

DELIVERY

Improving "delivery" of TDBs will likely be part of next generation TDBs; eg. SC route or "mask" TDBs

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appendix

PK(PD) Considerations for Comparability Strategy

Non-linearity in PK

Variability in PK (low doses generally tested)

PK dependent on time variant-PD marker

Presence of impurities (eg. CD3 homodimers)

Key References

Wu and Jimeno, 2018

Bargou, 2008

Blumel, 2010

Huppa and Davis, 2004

Li, 2017

Sun 2015

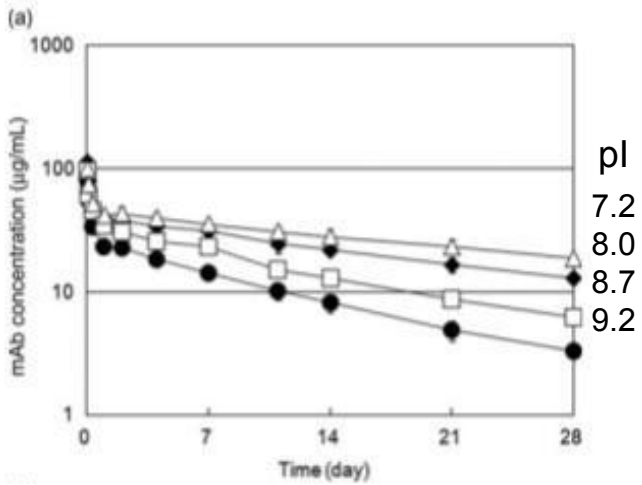
Leong, 2017

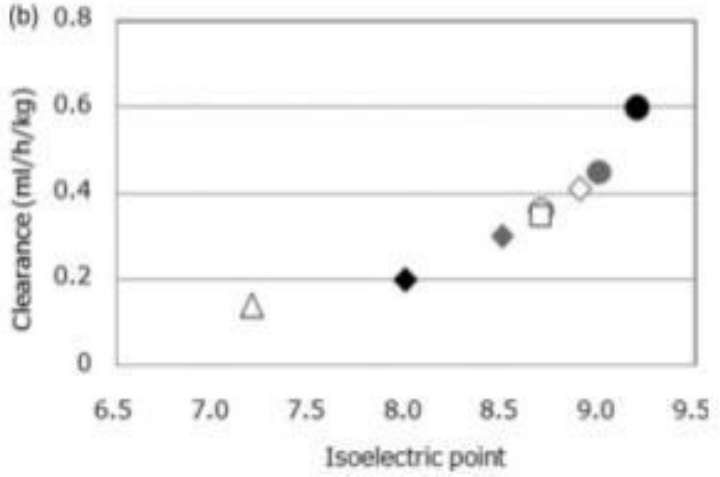
Saber, 2017

Rod Prell, 2017 AAPS presentation

Sid Sukumaran, 2016 AACR presentation

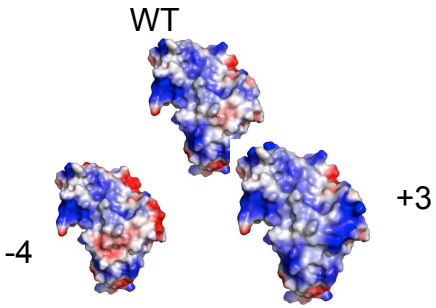
Changes in charge and/or pI may affect PK



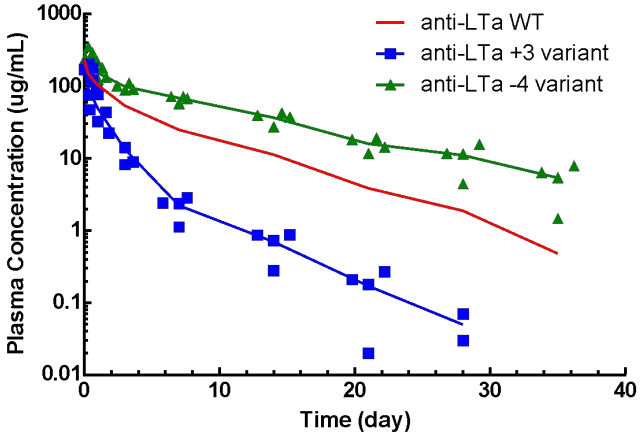


Igawa, 2010

Anti-Lymphotoxin alpha (LTa) Variants



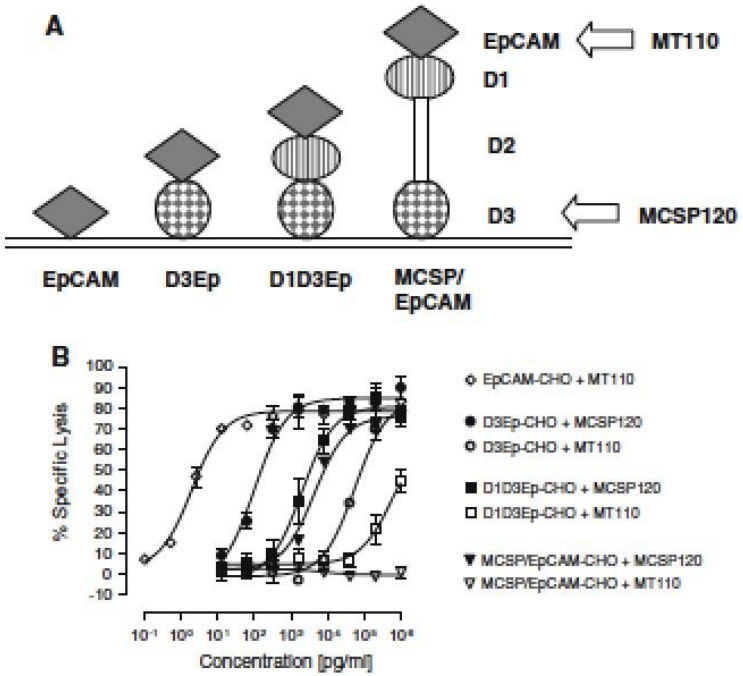
Single IV Bolus @ 10 mg/kg in Cynos



How do we use this to design great molecules?
Factors to consider: FcRn/FcgR binding, charge, pI, hydrophobicity, 3D structure.

Igawa, 2010; Yadav, 2015

BiTE potency is influenced by antigen size



C

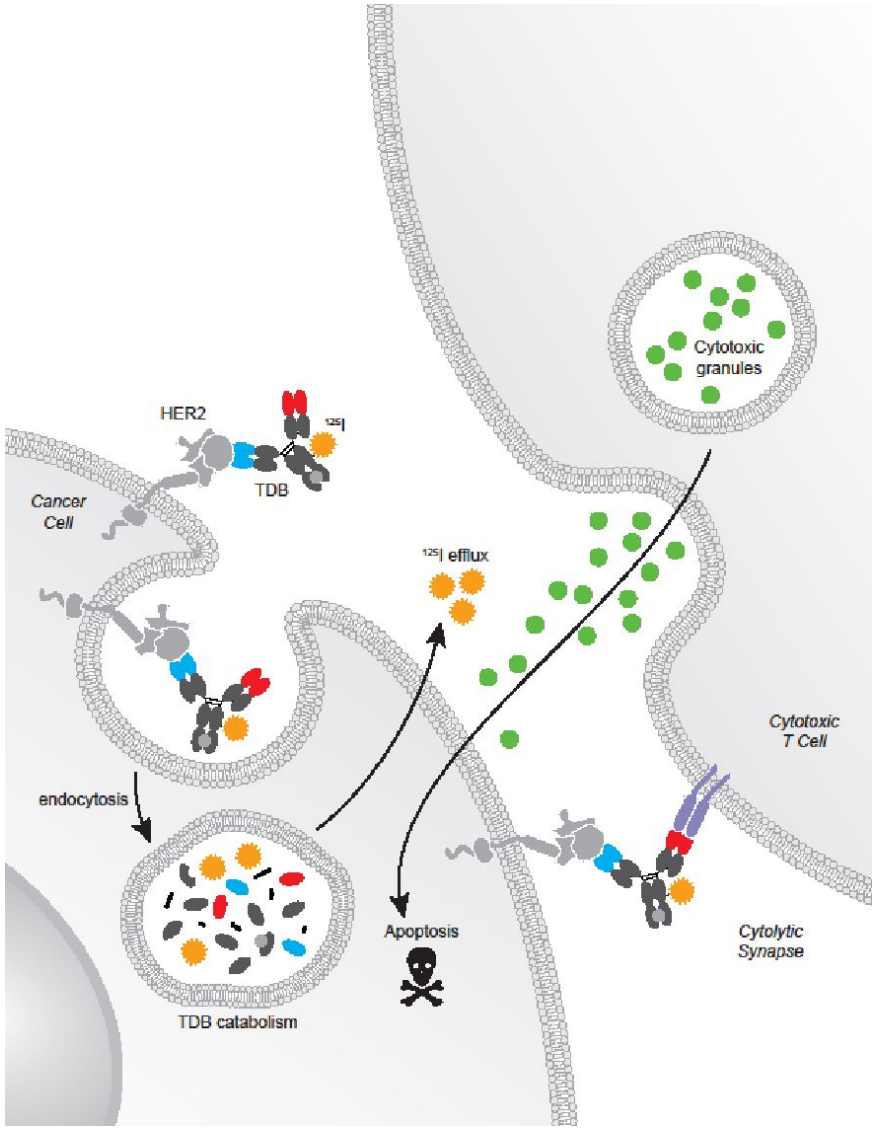
Transfected Cell Line	EpCAM-CHO		D3Ep-CHO		D1D3Ep-CHO		EpCAM/ MCSP-CHO	
BiTE	MT110	MCSP120	MT110	MCSP120	MT110	MCSP120	MT110	MCSP120
Max. Lysis (%)	78	n.d.	84	85	68	81	0.0	76
EC_{50} (ng/ml)	0.003	n.d.	54.95	0.101	576.47	1.95	$>10^3$	4.39

Fig. 6 The effect of antigen size on redirected lysis of transfected CHO cells by domain D3-specific BiTE antibody MCSP120. **a** CHO cell lines expressing EpCAM/MCSP fusion proteins or EpCAM alone were used as targets. **b** Dose–response analysis of redirected lysis for D3-specific BiTE antibody MCSP120 or EpCAM-specific BiTE antibody MT110 of CHO lines stably expressing fusion EpCAM/MCSP proteins or EpCAM. **c** Quantitation of assay results for maximal lysis and half maximum lysis (EC_{50})

MCSP: Melanoma chondroitin sulfate proteoglycan (melanoma antigen)

Blumel, 2010

Retention at the cell surface is key for TDB MOA



TDBs need to be on the surface in order to form functional cytolytic synapses

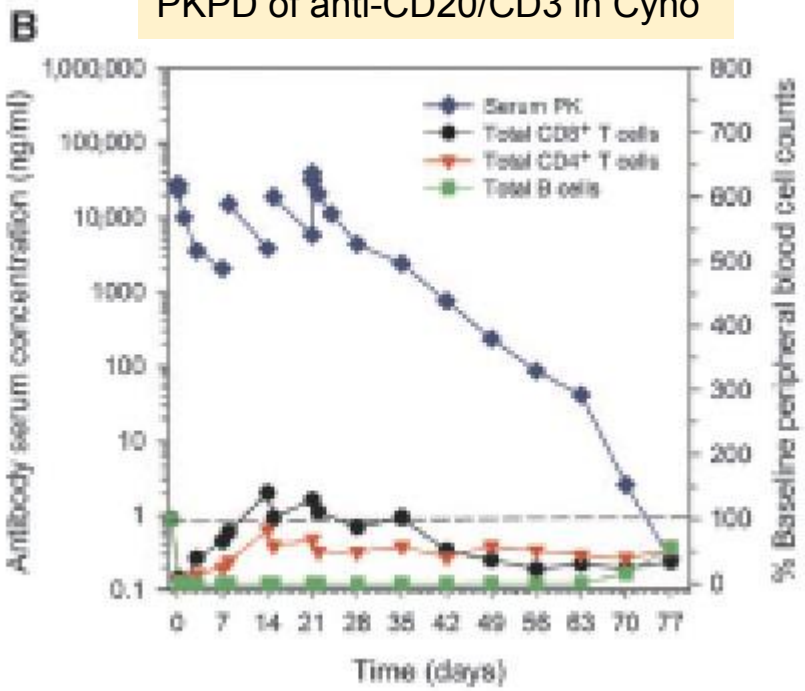
Surface half-life of tumor antigen

Many antibody therapeutics are against receptors shown to have increased internalization

Its unknown if switching into bispecific format will impact the internalization rate of antibody bound receptors

PKPD and Biomarker Strategy

PKPD of anti-CD20/CD3 in Cyno

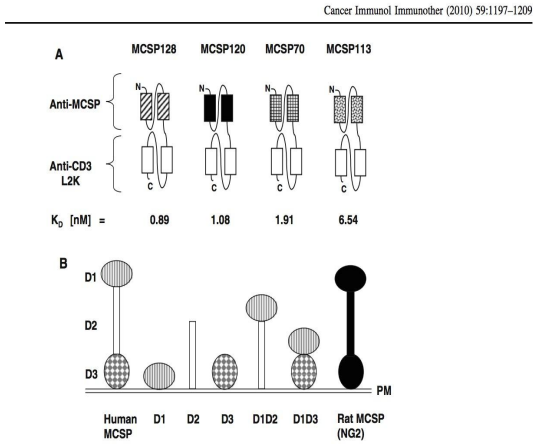


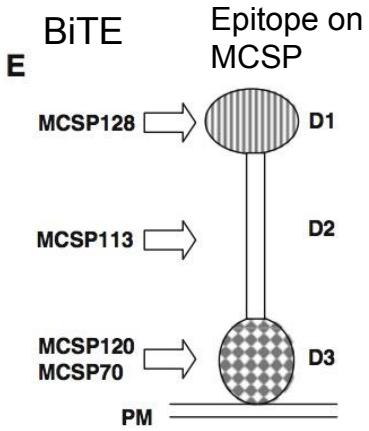
1) Safety: IL6 is being considered as the surrogate for CRS.

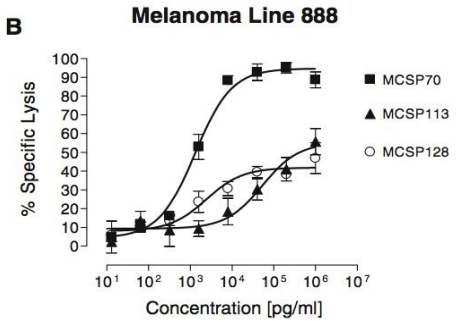
2) PD (target engagement):
T cell activation (CD69+, or CD25+ T cells), T cell margination, B cell depletion, increase in cytokines. They are not correlated with efficacy but they all show that the drug is active.

3) Resistance biomarker: no definitive marker yet

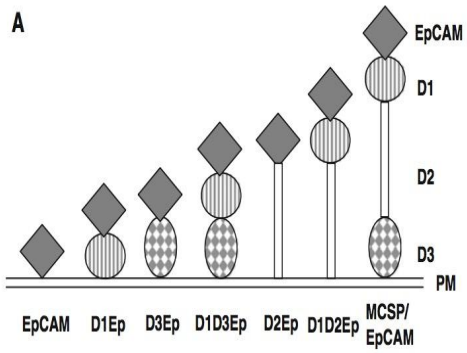
Epitope distance to the target cell membrane determines the potency of T cell-mediated lysis by BiTE antibodies specific for MCSP or EpCAM

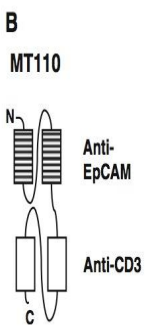


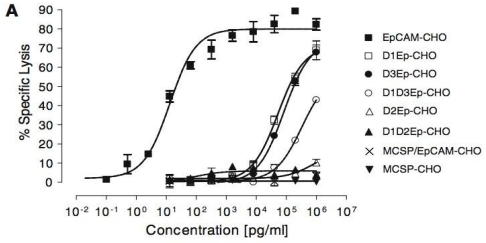




Potency of EpCAM-specific BiTE antibody MT110 decreased with increasing distance of EpCAM to target cell membrane







B

Transfected Cell Line	EpCAM-CHO	D1Ep-CHO	D3Ep-CHO	D1D3Ep-CHO	D2Ep-CHO	D1D2Ep-CHO	MCSP/EpCAM-CHO
No. Experiments	3	3	3	3	3	3	3
Max Lysis (%)	82 (± 3.3)	54 (± 12.4)	67(± 6.8)	61 (± 4.8)	16 (±13)	0	0
EC_{50} (ng/ml)	0.014 (± 0.002)	151.5 (± 78)	50 (± 25)	323 (± 34)	>10^3	>10^3	>10^3

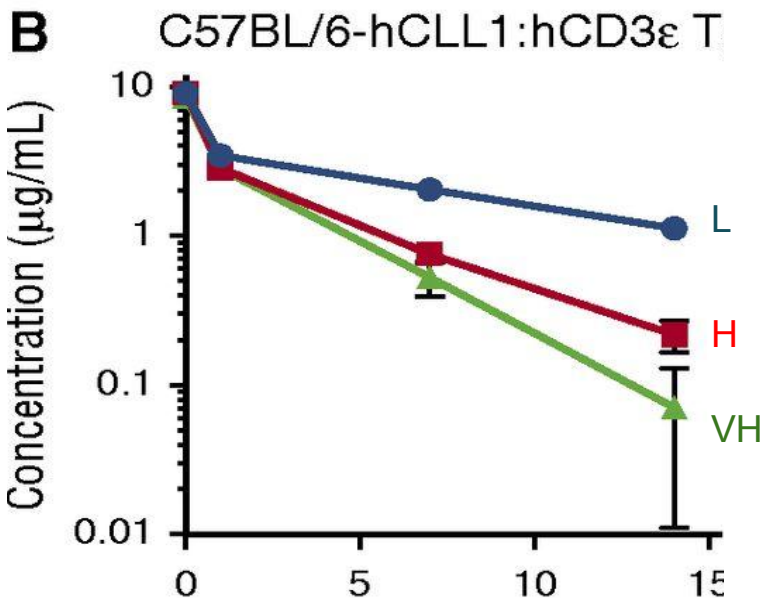
Fig. 5 Redirected lysis of CHO lines expressing EpCAM/MCSP fusion proteins by BiTE antibody MT110. **a** MT110 dose–response analysis of redirected lysis of CHO lines stably expressing fusion proteins between EpCAM and MCSP. **b** Quantitation of assay results for maximal lysis and half maximum lysis (EC_{50}). Standard deviations of the mean are shown from three independent experiments

MCSP: Melanoma chondroitin sulfate proteoglycan (melanoma antigen)

Blumel, 2010

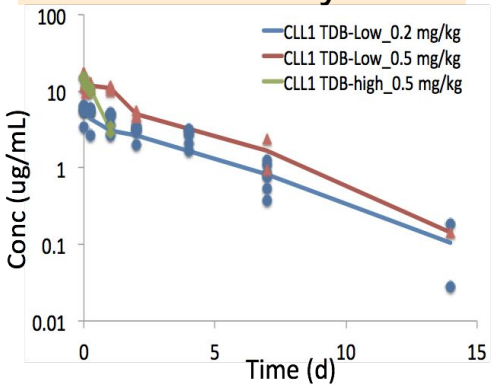
Impact of CD3 affinity on PK and cytokine levels

PK in Tg mice

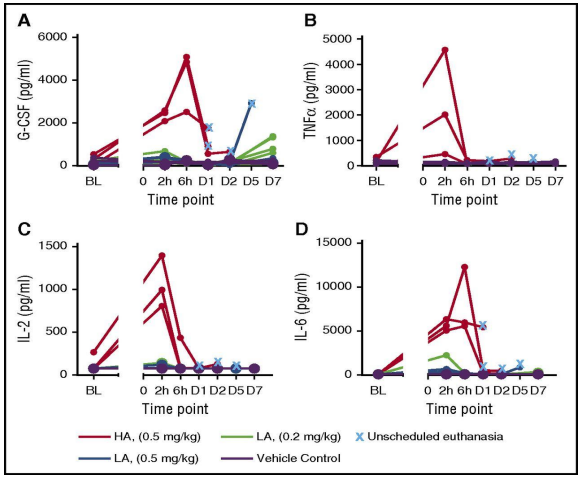


C57Bl/6 2xTg Group	Cmax (µg/ml)	AUC all (day*µg/mL)	CL (mL/day/kg)
CLL/CD3L	9.1	47.1	10.6
CLLCD3H	9.1	21.3	23.5
CLL/CD3VH	8.5	18.1	37.4

PKPD in Cyno



Cytokine levels in cynomolgus monkeys receiving CLL1/CD3H (HA) and CLL1/CD3L (LA).



Does binding on/off rates impact activity?

Steven R. Leong et al. Blood 2017;129:609-618

Anti-CD20/CD3 PKPD (Model)

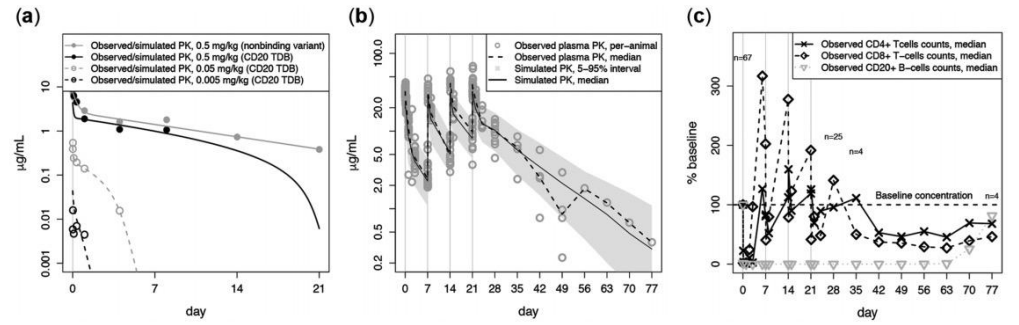


Figure 2 Murine PK and cynomolgus monkey PK/PD data with model simulations. (**a**) Two-compartment nonlinear PK model, as describ by Eqs. (1) and (2), with $CL_2^0 = 0$ and $\lambda_1 = 0$, fitted to murine data. Key estimated parameters for the CD20 TDB are $CL_1 = 16$ mL/day/k $K_M = 0.015$ μg/mL, and $V_{max} = 12$ μg/day. The solid gray curves indicate model fitted to PK data from the low-affinity CD3 TDB varia UCHT1 [1] (V_{max} fixed to zero). (**b**) Comparison of mixed-effects model simulations (median PK profile and interanimal variability) generat using fitted mixed-effects parameters summarized in **Table 2**, and observed PK data for 1 mg/kg i.v. bolus and slow push repeat dosi cohorts. Solid line and shaded region correspond to simulated median and 5–95% intervals. Circles are observed drug concentrations plasma and dashed line corresponds to median observations at each timepoint. (**c**) Corresponding changes in median peripheral blo CD4+, CD8+ T-cell counts, and CD20+ B-cell counts. Number of animals (*n*) for which T- and B-cell levels were measured is indicat at t = 0, 28, 35, and 77 days. In all panels, vertical lines indicate dosing times.

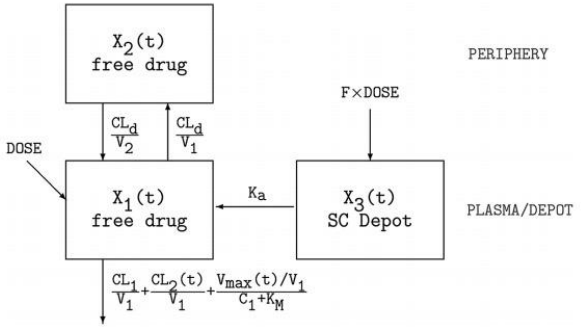


Figure 1 Schematic representation of an augmented two-compartment PK model with subcutaneous absorption, where $X_1(t)$ is the central plasma compartment and $X_2(t)$ represents peripheral tissue, both using units drug in μg. $X_3(t)$ represents the subcutaneous (s.c.) depot used for describing s.c. dosing. CL_1 and V_1 represent linear, nonsaturable drug clearance and central volume of distribution. CL_d and V_2 represent distribution clearance and peripheral tissue volume of distribution. K_a represents the fractional absorption rate of drug from the s.c. depot (1/time) and F is fractional bioavailability ($0 \le F \le 1$). $CL_2(t)/V_1$ and $(V_{max}(t)/V_1)/(C_1 + K_M)$ are ostensibly correlated with fractional B-cell and T-cell-mediated drug disposition/elimination, respectively, where $CL_2(t) = CL_2^0 \cdot e^{-\lambda_2 t}$ and $V_{max}(t) = V_{max}^0 e^{-\lambda_1 t}$.

Table 2 Summary of population model parameters (cf. **Figure 1**) for Model VI

Parameter	Definition	Units	Pop. Mean	SE	%CV	SE
K_a	Subcutaneous absorption rate	1/day	1.33	0.23	—	—
Z	Bioavailability = $1/(1+e^{-Z})$	dimensionless	1.66	0.56	—	—
CL_1	Nonsaturable mAb elimination	mL/day/kg	7.05	1.4	25	13
CL_2^0	Initial linear time-varying elimination rate	mL/day/kg	63.8	41	82	91
λ_2	Decay constant for CL_2	1/day	10.1	8.7	—	—
CL_d	mAb distribution clearance	mL/day/kg	31.3	6.9	69	15
V_1	Central distribution volume	mL/kg	44.4	3.5	41	6
V_2	Peripheral distribution volume	mL/kg	47.3	7.2	31	15
V_{max}^0	Initial nonlinear saturable elim rate	μg/mL	1280	678	41	16
λ_1	Decay constant for V_{max}	1/day	0.144	0.048	—	—
K_M	Michaelis–Menten constant	μg/mL	19.6	10	—	—

In a *post hoc* step, CL_1, CL_2^0, CL_d, V_1, and V_2 were normalized by median body weight (3.12 kg) across all animals. Note that subcutaneous bioavailability = $1/(1+e^{-Z}) = 0.84$. Pop. Mean, Population Mean; %CV, apparent percent coefficient of variation (interindividual variability); SE, standard error (a measure of precision for fitted population means and apparent percent coefficients of variation).

Ferl 2018

FDA Oncology analysis of CD3 bispecific constructs and FiH dose selection" Saber et al. ,2017

Regardless of sponsor's strategy; Saber et al.

1)	estimated FiH dose based on three methods
2)	defined a hypothetical dose escalation schedule
3)	used MTD from available clinical data to evaluate whether FiH dose will result in MTD or how many dose escalations until MTD is reached

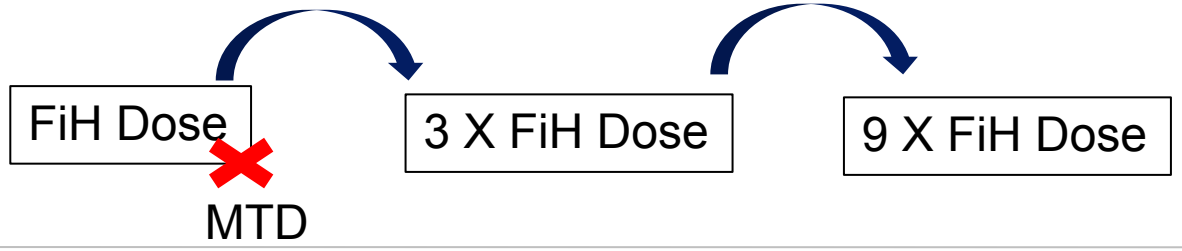
FiH Dose Estimation

A) $$PA = \frac{C}{EC_{50} + C}$$

B) $$RO = \frac{C}{K_D + C}$$

C) $1/10^{th}$ NOAEL
$1/6^{th}$ HNSTD using BW or BSA

Dose Escalation



Meric Ovacik

C = projected C_{max} in humans, EC_{50}= EC_{50} from the most sensitive assay, K_D= Reported value, RO=Receptor Occupancy, PA= Pharmacologic activity,
NOAEL= No observed adverse effect level HNSTD= Highest non-severely toxic dose BW= Body weight BSA= Body surface area

Key findings from Saber et al. ,2017

FiH Dose based on RO %	X	above the human MTD
FiH Dose based on NOAEL or HNSTD		
FiH Dose based on *in vitro* Activity	✓	10%-30% PA acceptable/ manageable toxicities
in vitro Activity Studies (Human Cells)	Wide range of EC_{50}	
Animal Toxicology	HNSTD reached with 90% of constructs.	