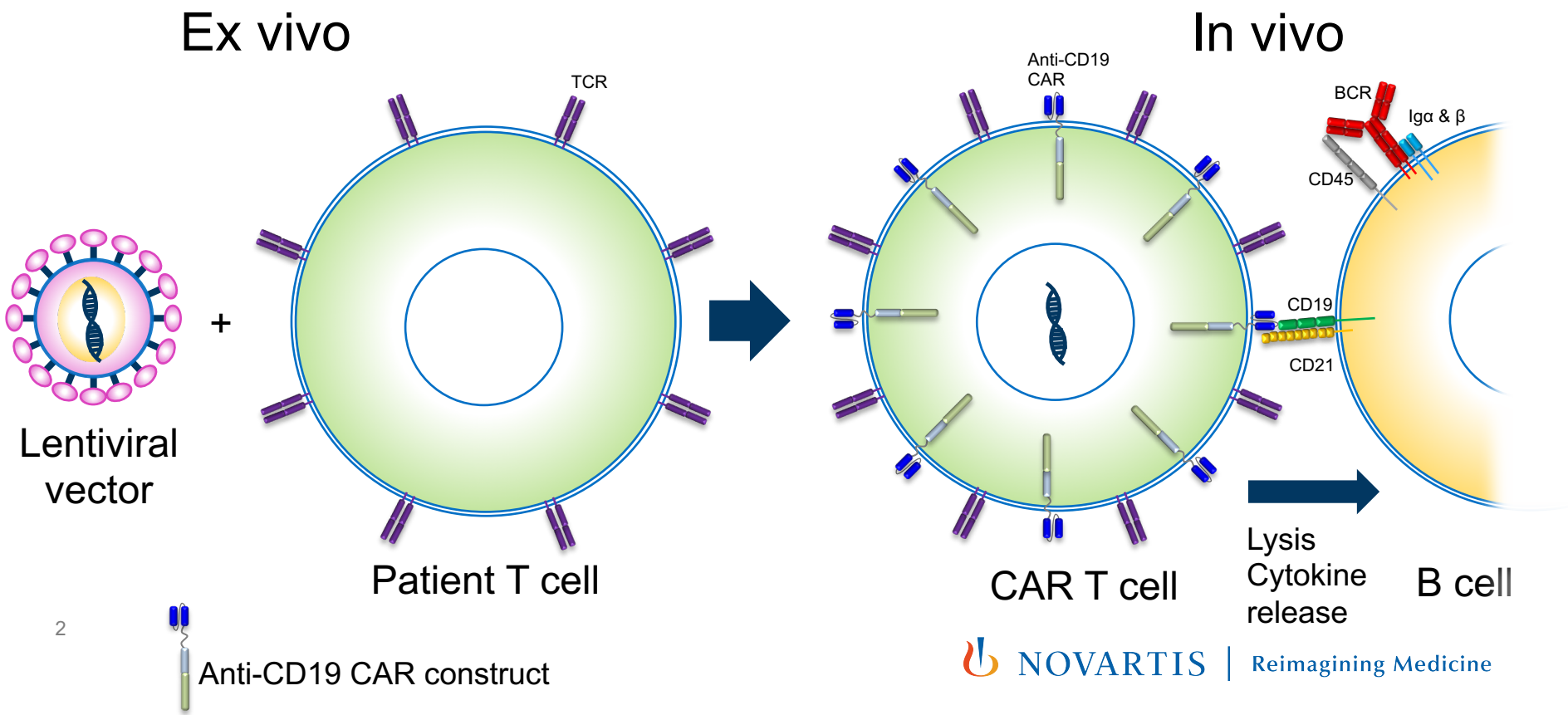
Novartis TM PKS
Bioanalytics

A cell based immunogenicity assay to detect antibodies against chimeric antigen receptor

Bernd Potthoff
November 18th, 2020

NOVARTIS | Reimagining Medicine

Chimeric Antigen Receptor 19 (CAR19)

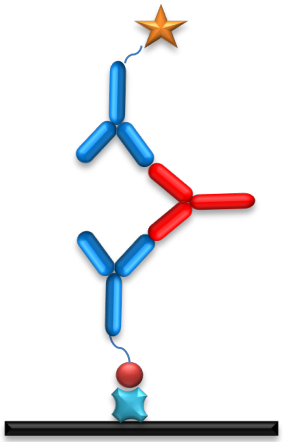


NOVARTIS | Reimagining Medicine

Detecting Immunogenicity

Bridging ELISA / homogeneous ECLIA

Antibody

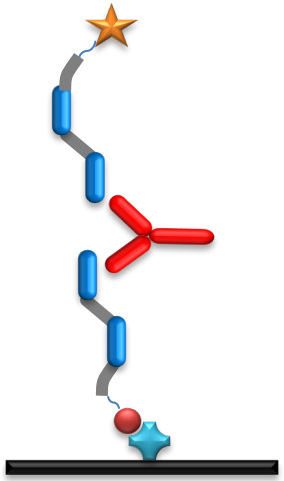


labeled drug
providing signal in assay

ADA or positive control antibody

labeled drug
bound to ELISA plate

Soluble extracellular
Chimeric Antigen Receptor (CAR)



Assay signal corresponds to presence of immunogenicity

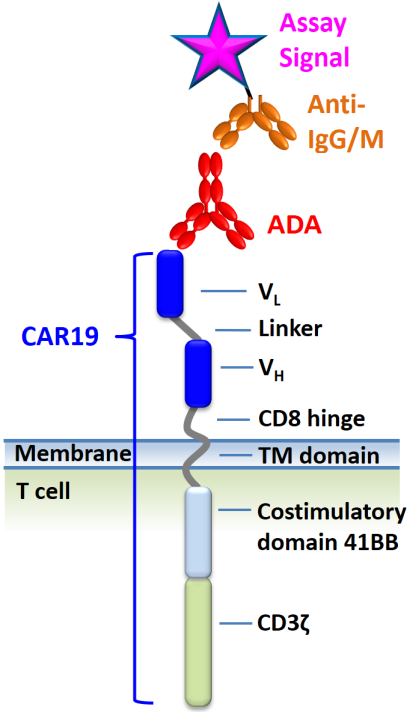
Why CAR19 needed a different approach

- Insoluble extracellular receptor parts
  - Risk of aggregate /precipitate formation in LBA, non-ADA dependent dimerization etc.
- Soluble CAR19 ≠ complete extracellular domain
  - Risk of not identifying immunogenicity against missing epitopes
- Potentially immunogenic interactions with other membrane proteins[1] / tertiary structural differences in membrane vs. LBA
- Risk of masking immunogenicity epitopes by labeling

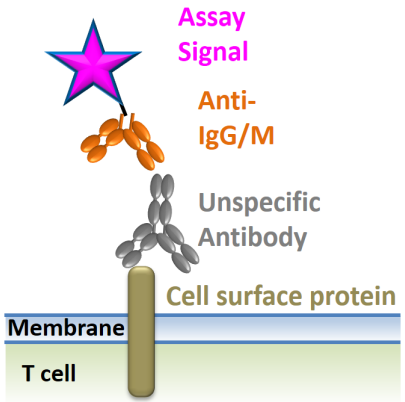
1. A conformational epitope expressed upon association of CD3-epsilon with either CD3-delta or CD3-gamma is the main target for recognition by anti-CD3 monoclonal antibodies. A Salmerón, et al. J Immunol November 1 1991,147 (9) 3047-3052

Concept of a cell based ADA assay

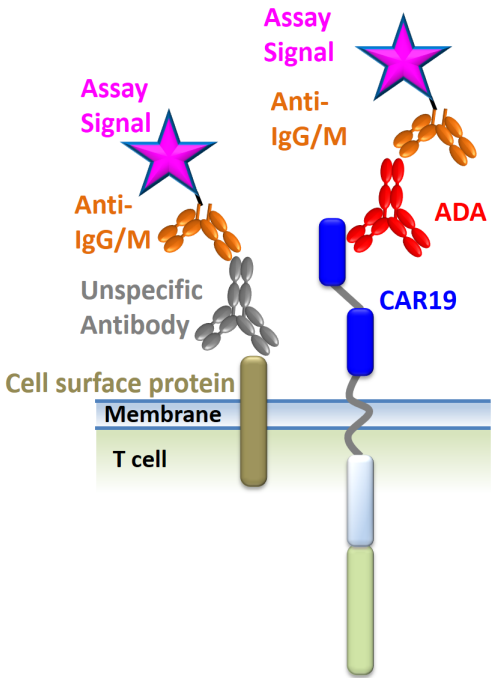
A: CAR specific



WT cells
B: Unspecific



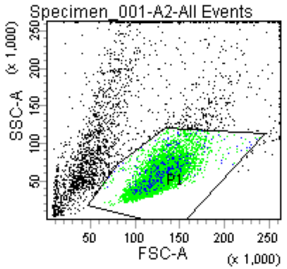
CAR expressing cells
C: Combination of both



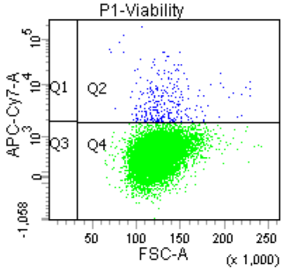
A can be calculated as **C – B**

Flow cytometer gating strategy

1. Cells



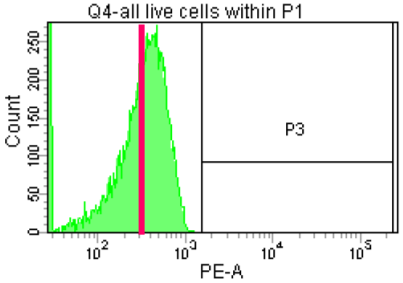
2. Live Cells



- Sodium azide containing assay buffer to prevent internalization
- Thorough resuspension necessary at all staining steps for good precision
- 90 seconds read & wash time / well

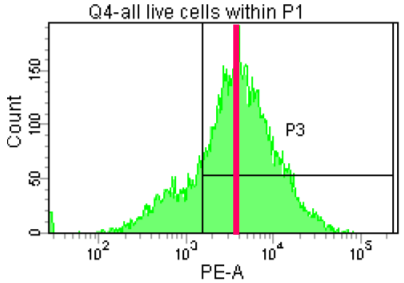
3. Histogram of assay signal

Naïve serum



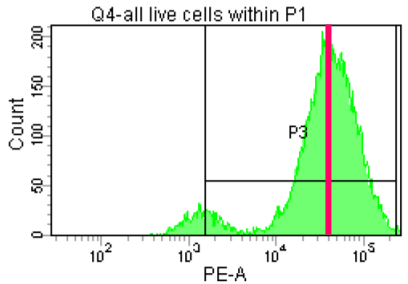
MFI 300

+300 ng/mL α-CAR



MFI 3’600

+80 µg/mL α-CAR



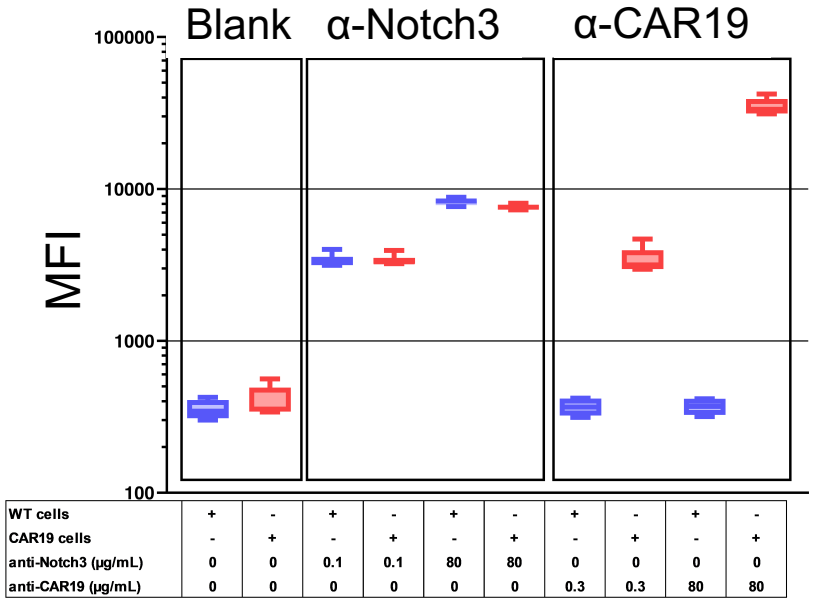
MFI 37’400

NOVARTIS | Reimagining Medicine

Integrating data from 2 cell lines

Non-CAR specific signal has to be comparable for calculation of CAR specific signal

- Second positive control against ubiquitously expressed receptor Notch3



WT cells

CAR19 cells

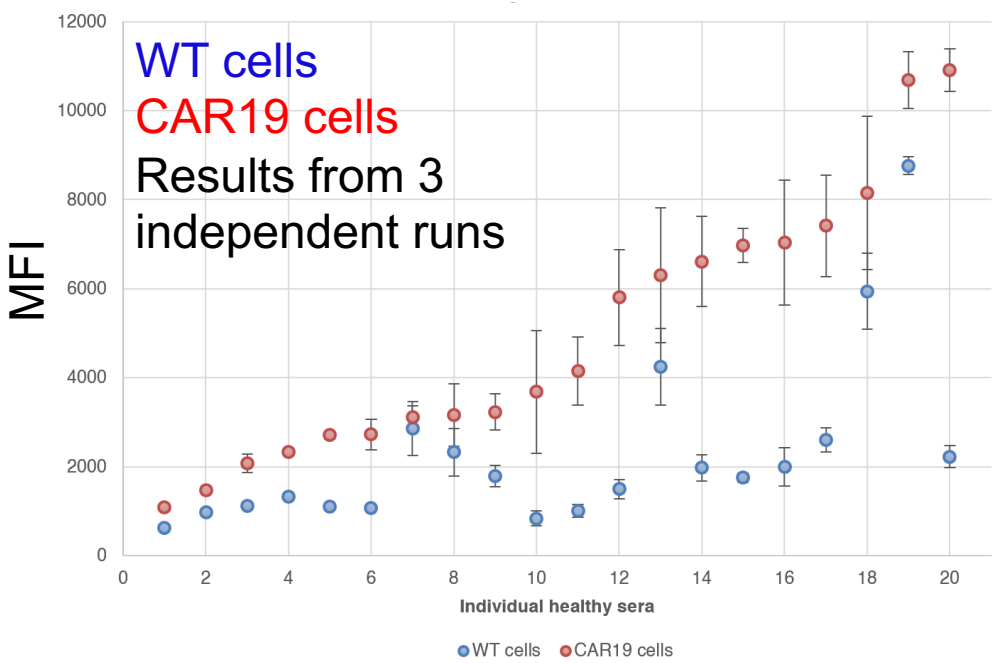
Results from 8 independent runs

➡ Comparable staining on both cell lines, good intra-and inter-run precision ✓

NOVARTIS | Reimagining Medicine

Pre-existing ADA & Cut point

Pre-existing antibodies in >80% of individual sera & also in IVIG purifications

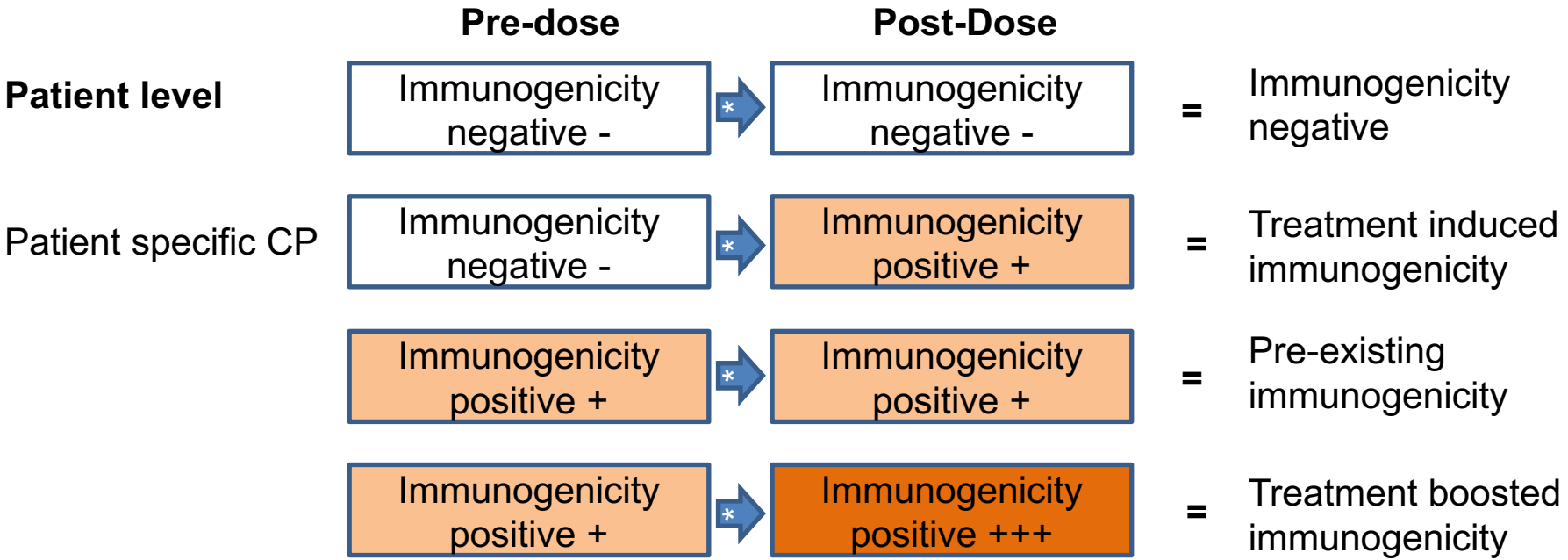


- No meaningful cut point with normal serum.
- Outlier removal procedures not useful
- Signal inhibition possible with CAR19 protein (confirmation)

- Final decision to calculate Cut point with Immunoglobulin depleted sera ✓

NOVARTIS | Reimagining Medicine

Data interpretation - Patient

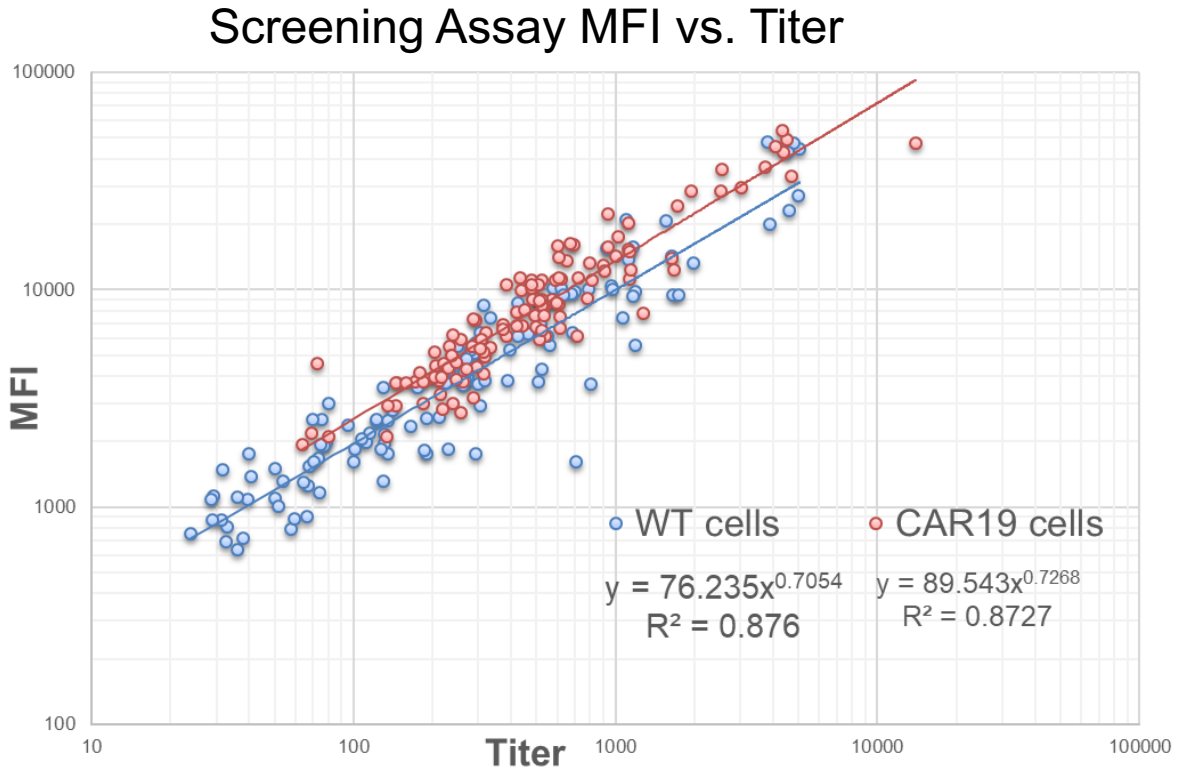


Mock data

Day / Month	A: WT cell signal	B: CAR cell signal	C: CAR specific signal (=B-A)	Sample IG positive	Patient IG boosted
Day -28	11479	13969	**2'491**	YES	**Predose**
Day -1	16506	19074	**2'568**	YES	**Predose**
Day 7	12770	14259	**1'489**	YES	**NO**
Day 14	6125	8288	**2'164**	YES	**NO**
Day 28	5751	7834	**2'083**	YES	**NO**
Month 2	5018	7160	**2'142**	YES	**NO**
Month 6	23833	83096	**59'263**	YES	**YES**
Month 12	14749	62637	**47'888**	YES	**YES**

Titration assay

- Dilution (1:X) of study samples until signal falls below Titer cut point
- Common TCP required for comparability
- Reliable Prediction of Titer based on Screening Assay signal possible
- Reduction of analyzed dilution steps from up to 14 to 6

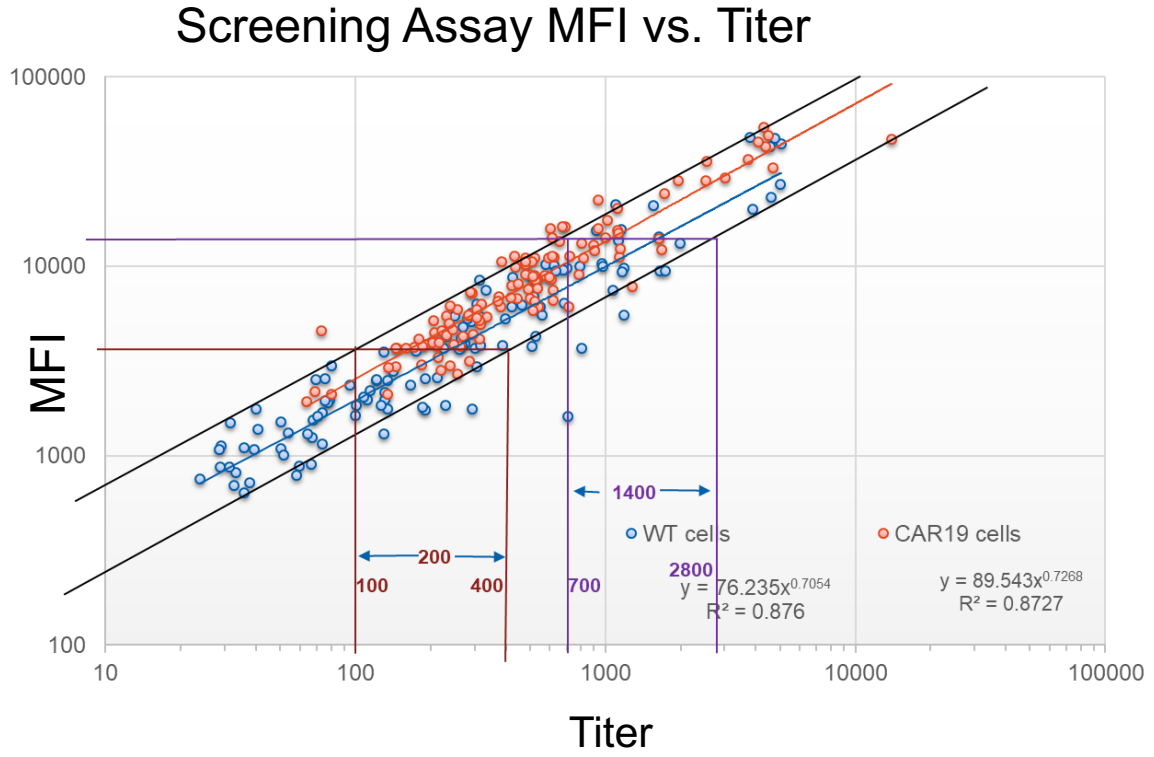


Correlation of Signal vs. Titer

Potential future application:

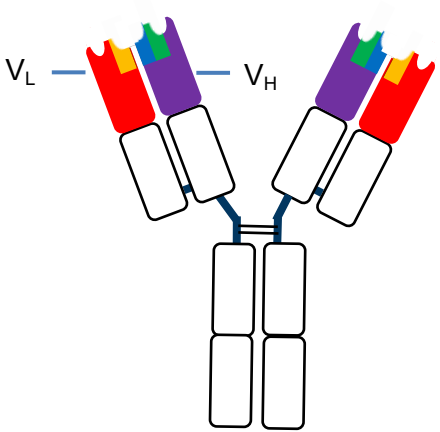
Estimate ADA response magnitude by screening assay only. This requires a screening assay not restricted by hard limit of maximum signal

Acceptable titer range of titer positive controls is
½ median titer to 2*median titer

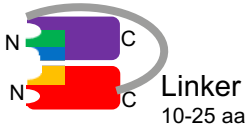


From source antibody to CAR

Source Antibody

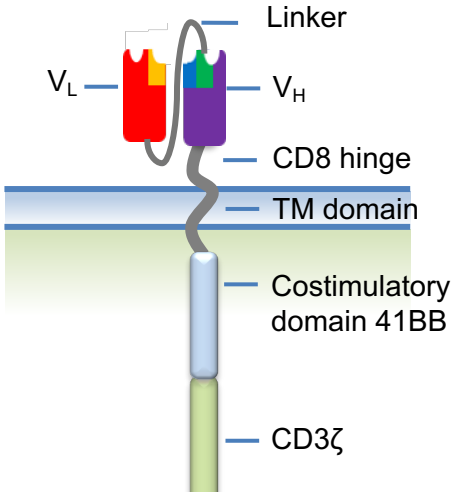


scFv



Recombinant, with artificial linker

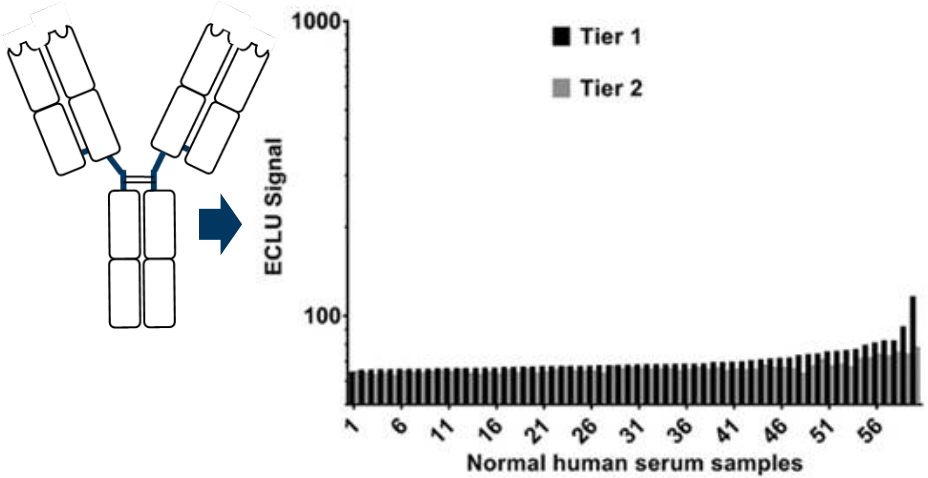
CAR19



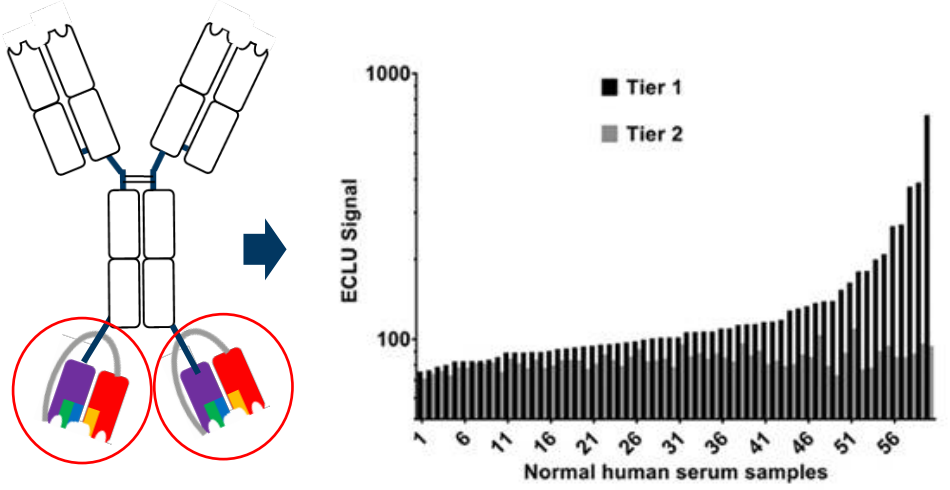
Source antibody bridging assays: risk of not detecting immunogenicity against scFV specific neo-epitopes or other extracellular domains

Pre-existing anti-scFVs antibodies

LBA Screening data for IgG



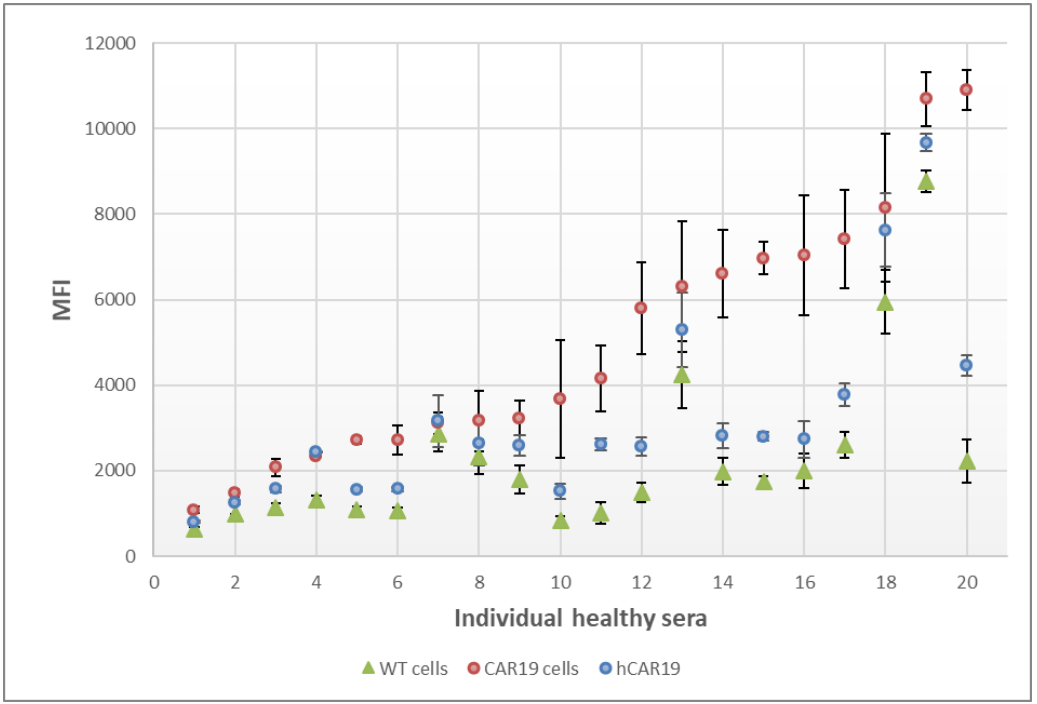
LBA Screening data for IgG + scFv



scFv containing drugs demonstrate a high rate of preexisting ADA.
Confirmed as specific IgG/M via confirmatory assay, different scFv, mass spec etc.

Figures adapted from Bivi N et al. (2019) “Investigation of pre-existing reactivity to biotherapeutics can uncover potential immunogenic epitopes and predict immunogenicity risk” mAbs, 11:5, 861-869,; https://doi.org/10.1080/19420862.2019.1612699

ADAs -Human anti mouse or anti-scFv?



Differences in pre-existing ADA binding mouse (red) vs. humanized (blue) scFv

Tisagenlecleucel and Yescarta are both based on murine α-human CD19 scFv, derived from antibody FMC63*

ADA assay assessing ADAs to source antibody FMC63§ observed lower IG incidence rate (~3%) possibly consequence of assay format and/or difference in linker (CD28 vs CD8)?

*Engineering and Design of Chimeric Antigen Receptors. Guedan et al. *Mol Ther Methods Clin Dev*. 2019 Mar 15; 12: 145–156.

§ https://www.fda.gov/media/108377/download

Comparison of CAR ADA assay formats

Detected ADAs & Features	Bridging ELISA with soluble CAR	Bridging ELISA with source Antibody (Yescarta)	Cell based assay (Kymriah)
Anti-VAR AD	✓	✓	✓
Anti-scFv	✓	X	✓
Anti-Hinge, anti-Linker	✓	X	✓
Anti-membrane protein interaction epitopes	X	X	✓
Anti-insoluble extracellular domains	X	X	✓
Label-Free	X	X	✓

Potthoff B et al- (2020) “A cell-based immunogenicity assay to detect antibodies against chimeric antigen receptor expressed by tisagenlecleucel, JIM, Vol. 476,112692, https://doi.org/10.1016/j.jim.2019.112692.

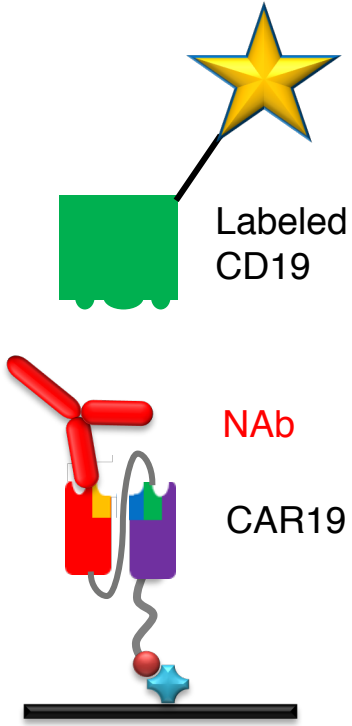
Outlook – Tiered strategy

- No observed correlation of ADA (pre-existing or treatment-induced) to clinical outcome
- No knowledge about neutralizing properties of ADA and potential impact on outcome

Options for future BA strategy:

1. Screening assay (CBA)
2. Titer assay **OR** prediction of ADA magnitude based on screening signal
3. Neutralizing assay (LBA?) with labeled CD19 as baseline signal

NAb assay concept



Thank you

NOVARTIS | Reimagining Medicine

Acknowledgement

Denise Sickert
Edwige Chokote
Andy Warren
Fraser McBlane
Sebastian Spindeldreher
Karen Thudium
Robert Dodge
Constantine Bitsaktsis
Jimmy Flarakos
Alessio Maiolica
Jim Glick
Rakesh Awasthi
Christian Joffroy
Grzegorz Terszowski