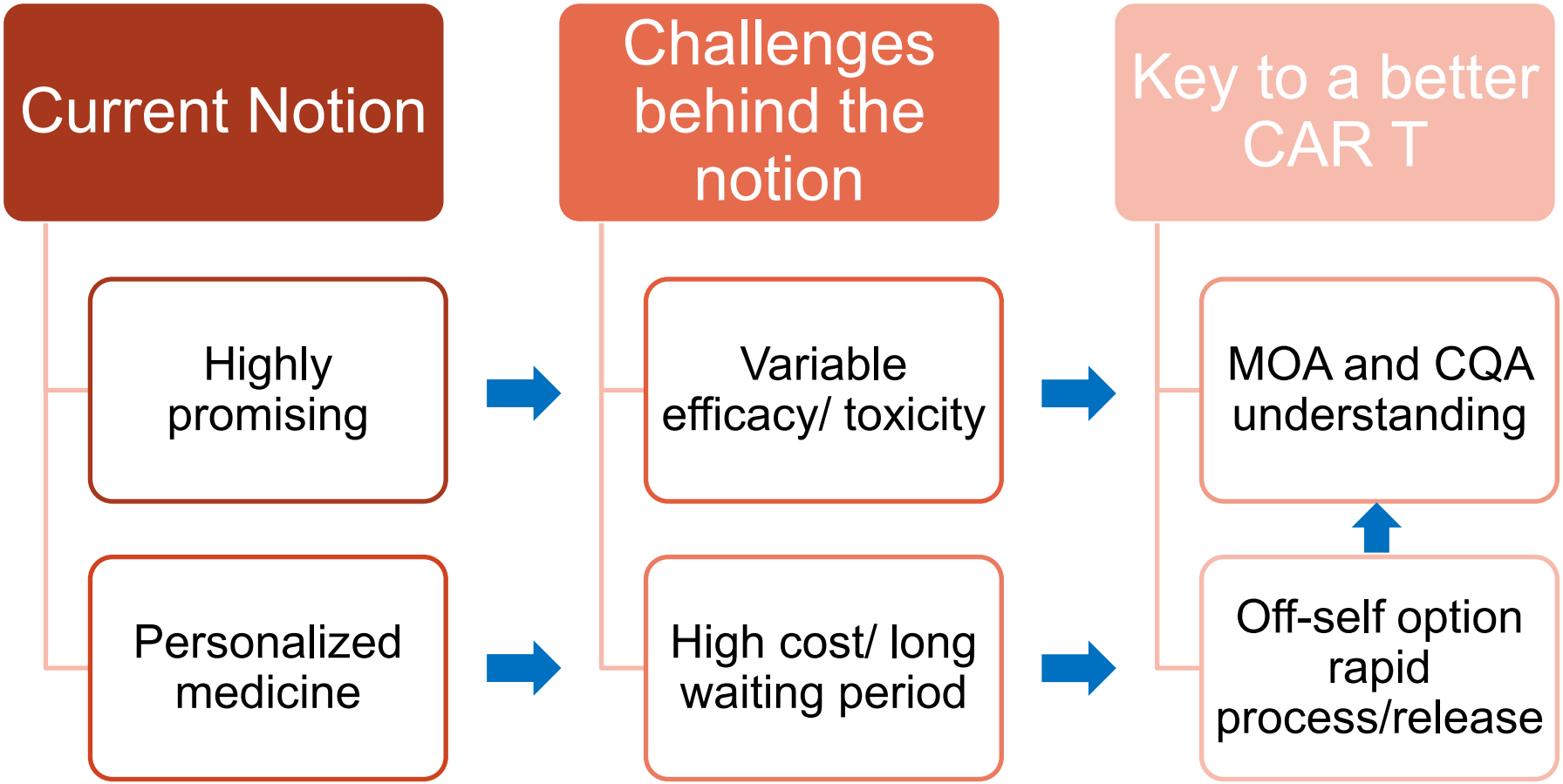
Better Health, Brighter Future

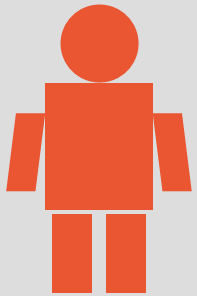
Advancing Product Analytics for CAR T Cell Therapies

Qiong (Chelsea) Xue
Cell Therapy
Takeda Pharmaceuticals

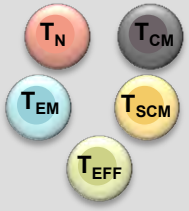
CAR T Cell Therapies



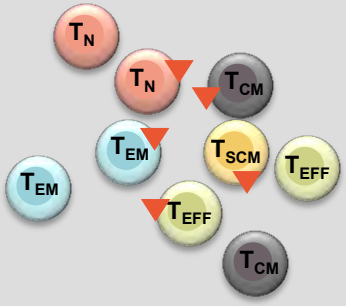
What's in the bag?



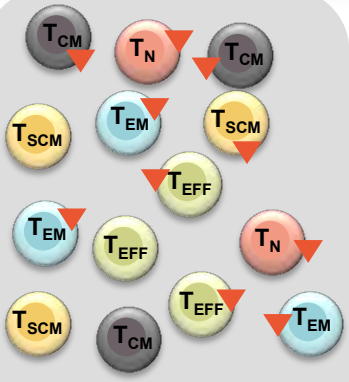
Blood collection



Activation



Transduction



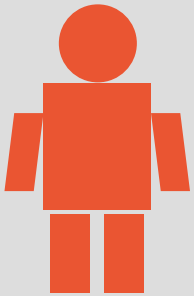
Expansion

Formulation

Manufacturing process

Complicated MOAs in patients:

- **Cytotoxic activity**: cytotoxines to promote cancer cell apoptosis
- **Proliferation**: interleukines to promote immue cell development and division
- **Immune cell activation**: inflammatory cytokines to promote inflammation and immune response; chemokines to recruit immune cells



Infusion back to patient

Complex compositions in final products:

- T cells and non-T cells
- Transduced and untransduced
- CD4/CD8 ratio
- T_N, T_{CM}, T_{EM}, T_{SCM}, T_{EFF}
- Th1, Th2, Th9, Th17
- Multiple cytokine/chemokine
- Different degree of exhaustion
- Different degree of senescence

From regulatory authorities - FDA

Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Investigational New Drug Applications (INDs)

- FDA guideline, July 2018

"Your summary should also include a description of **critical quality attributes (CQAs) that are relevant to the safety and biological activity** of the product as they are understood at the time of submission. … we recommend that **you evaluate a number of product characteristics during early clinical development to help you identify and understand the CQAs of your product.** This will also help ensure your ability to assess manufacturing process controls, manufacturing consistency, and product stability as product development advances."

From regulatory authorities - EMA

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

26 July 2018
EMA/CAT/GTWP/671639/2008 Rev. 1
Committee for Advanced Therapies (CAT)

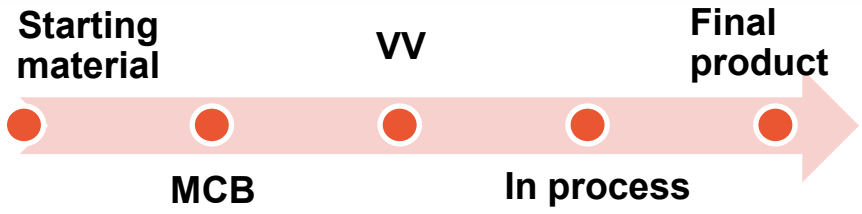
Guideline on quality, non-clinical and clinical aspects of medicinal products containing genetically modified cells

"Rigorous characterization of the genetically modified cell medical products (either alone or in combination with a medical device) is essential."

The characteristics including:

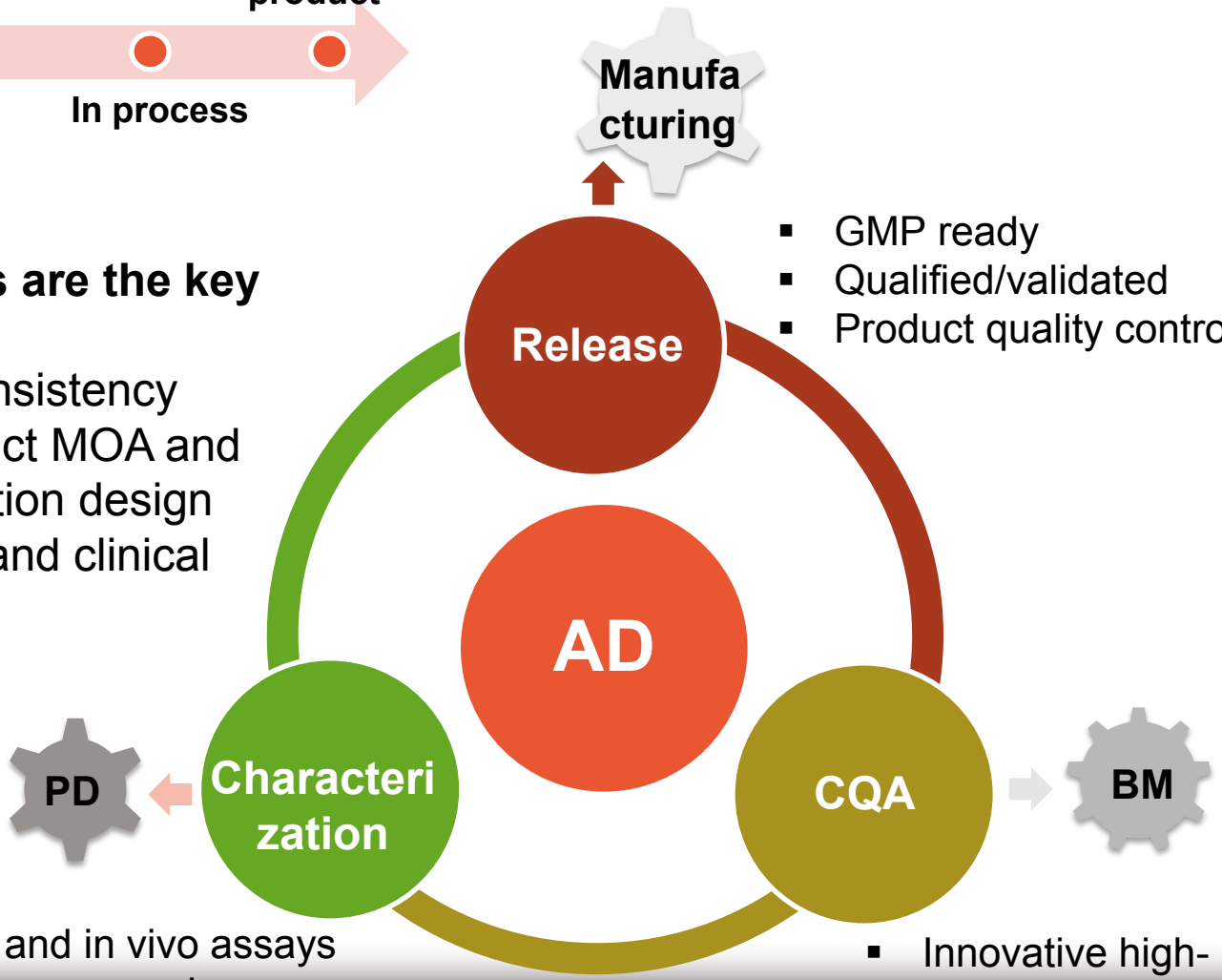
- Heterogeneity of the cell population (e.g. percentage of sub-populations)
- Proliferation and/or differentiation capacity of the genetically modified cells
- Cell functionality (other than proliferation/differentiation)
- Potency testing for products containing genetically modified T-cells against tumor cells (e.g. CAR-T cells) is preferably based on the cytotoxic potential of the T-cells)

Analytical development for cell therapies



Integrated analytics are the key

- Product quality
- Manufacturing consistency
- Understand product MOA and drive next-generation design
- Patient selection and clinical prediction



- GMP ready
- Qualified/validated
- Product quality control

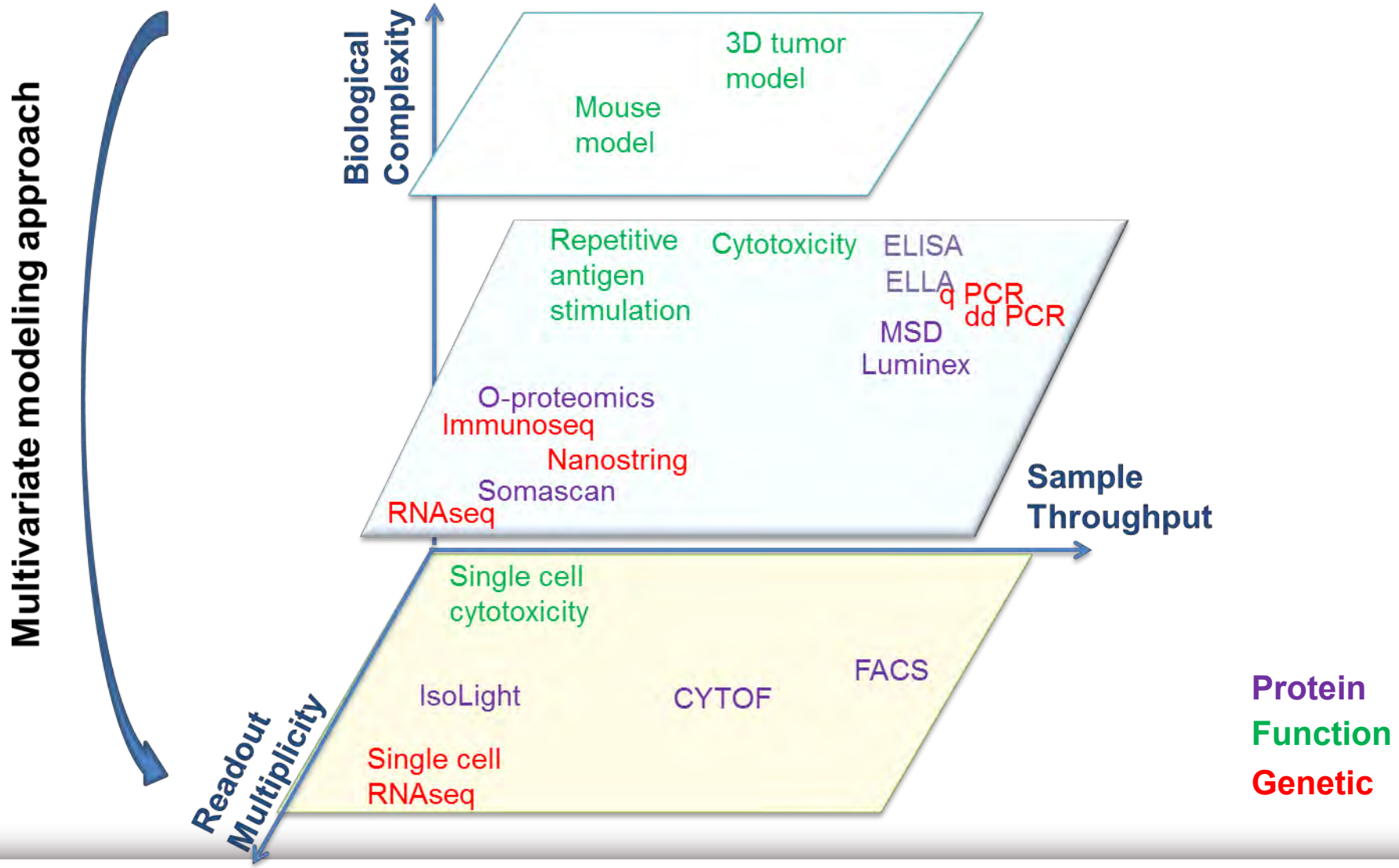
- In vitro and in vivo assays
- Assess process changes
- Ready to be included in IND

- Innovative high-dimensional platforms
- Enable correlative studies

Product analytics for release testing

	KYMRIAH® (tisagenlecleucel) Suspension for IV infusion	YESCARTA® (axicabtagene ciloleucel) Suspension for IV infusion
Appearance	Visual inspection	Visual inspection
Identity	Presence of CAR transgene, by q-PCR	Presence of scFv heavy chain variable region, linker and CD28 sequences
Dose	Viable cell count; CAR expression by flow cytometry	Viable cell count; anti-CD19 CAR expression
Potency	Release of IFNr in response to CD19-expressing target, by ELISA	Cell viability; anti-CD19 CAR expression
Purity/impurity	Percentage of viable T cells; percentage of viable CD19+ B cells; determination of residue beads; endotoxin	Gentamicin; endotoxin
Sterility	Pharmacopia (USP 71/EP/JP) growth method	Pharmacopia (USP 71/EP/JP) growth method
Mycoplasma	USP compendia method	USP compendia method; q -PCR
RCL	Determination of VSV-G DNA by q PCR; co-culture RCL	Co-culture RCR; presence of scFV heavy chain variable region, linker and CD28 sequences

Analytical platforms enabling comprehensive product characterization

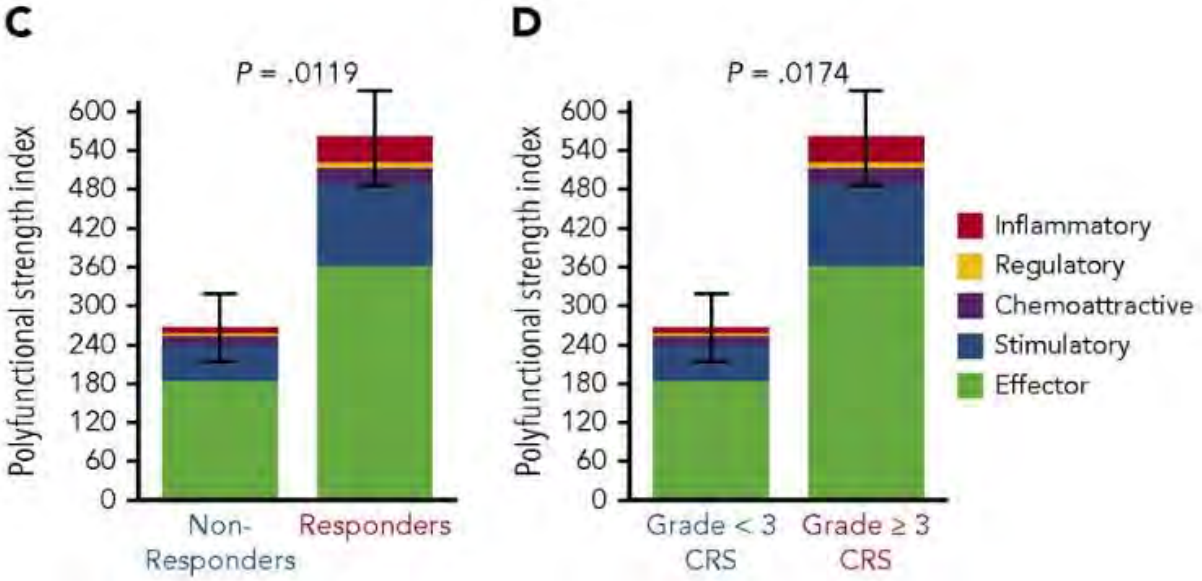


Single cell analysis reveals CQA of CD19 CAR T for NHL

blood®
Prepublished online June 12, 2018;
doi:10.1182/blood-2018-01-828343

Preinfusion polyfunctional anti-CD19 chimeric antigen receptor T cells associate with clinical outcomes in NHL

John Rossi,[1]* Patrick Paczkowski,[2]* Yueh-Wei Shen,[1] Kevin Morse,[2] Brianna Flynn,[2] Alaina



Comprehensive analytics uncover CTL019 CAR T CQA for CLL

nature medicine

LETTERS

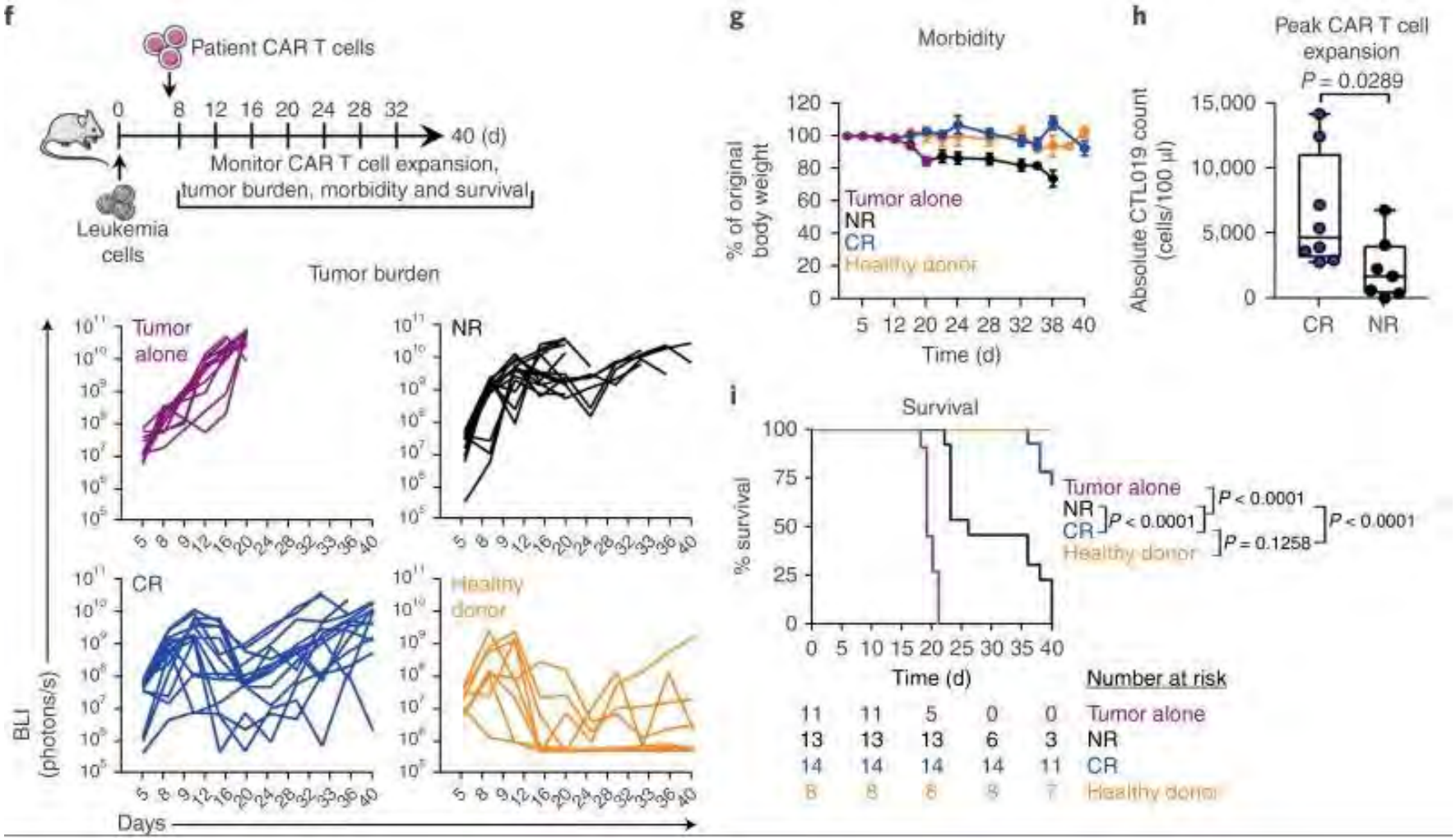
https://doi.org/10.1038/s41591-018-0010-1

Determinants of response and resistance to CD19 chimeric antigen receptor (CAR) T cell therapy of chronic lymphocytic leukemia

Joseph A. Fraietta[1,2,3], Simon F. Lacey[1,2,3,9], Elena J. Orlando[4,9], Iulian Pruteanu-Malinici[4], Mercy Gohil[2],

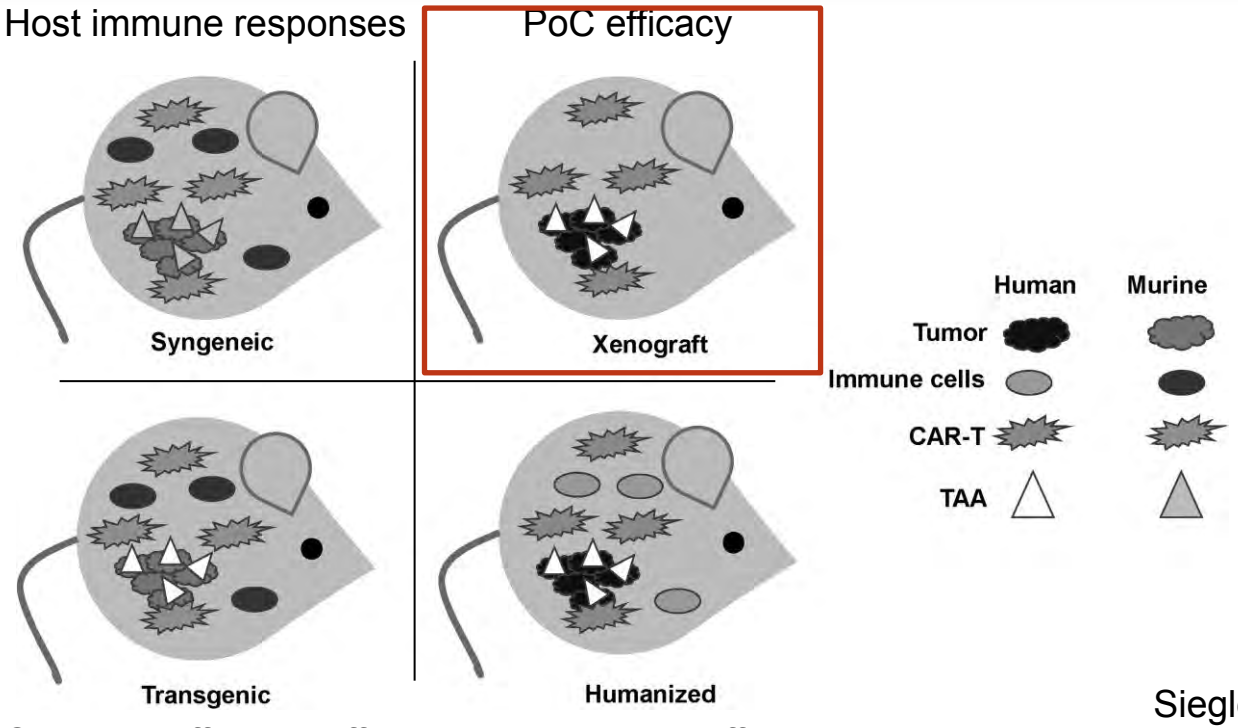
Transcriptional profiling - RNAseq	• CR: memory-related genes, IL-6/STAT3 signatures • NR: effector differentiation, glycolysis, exhaustion and apoptosis
Phenotypic - FACS	• CR: %CD8+CD45RO-CD27+ • NR: %CD8+PD-1+; %CD8+PD-1+TIM-3+; %CD8+PD-1+LAG3+
Functional – cytokine/ chemokine by Luminex	• CR: highly functional CAR T cells produced STAT3-related cytokines IL-6, IL-17, IL-22, IL31, CCL20

Capability of mouse models in differentiating an efficacious vs. non-efficacious products



Fraietta et al., Nature medicine, 2018

Mouse model used for product characterization



Siegler EL et al., 2018

Measurements:

1) Efficacy comparison between products generated from different processes
2) In vivo CK – serial blood sample for flow cytometry/qPCR
3) Cytokine/chemokine production
4) Tumor/tissue pathology

Moving beyond Kymriah and Yescarta

KYMRIAH® (tisagenlecleucel) Suspension for IV infusion

YESCARTA® (axicabtagene ciloleucel) Suspension for IV infusion

Hematopoietic

Autologous

Future CAR Ts

Solid tumor

- Armored CAR T — Memorial Sloan Kettering Cancer Center
- Smart CAR T — NOILE-IMMUNE BIOTECH

Allogeneic

- Healthy donor derived — cellectis EDITING LIFE; POSEIDA THERAPEUTICS; CRISPR THERAPEUTICS; Sangamo THERAPEUTICS
- iPSC derived — Fate THERAPEUTICS; Memorial Sloan Kettering Cancer Center
- γδ T cell derived — Adicet Bio; TC BIOPHARM; GAMMADELTA THERAPEUTICS

Additional analytical considerations for future CAR T therapies

New design of CAR T constructs:

- Expected effect need to be confirmed at the cellular level
- The regulation of transgene expression or the intended elimination of the genetically modified cells should be evaluated for proper function
- For secreted gene products the distribution and persistence of the transgene product should be included in the analysis

Genome editing:

- Induced off-target changes should be identified using appropriate bioinformatics tools for in silico screening as well as deep sequencing techniques. This is expected to include appropriate screening of genome-edited cells for off-target effects that may be missed by in silico prediction.
- The persistence of genome editing tools in the cells should be evaluated.

- Guideline on quality, non-clinical and clinical aspects of medicinal products containing genetically modified cells, EMA, July 2018

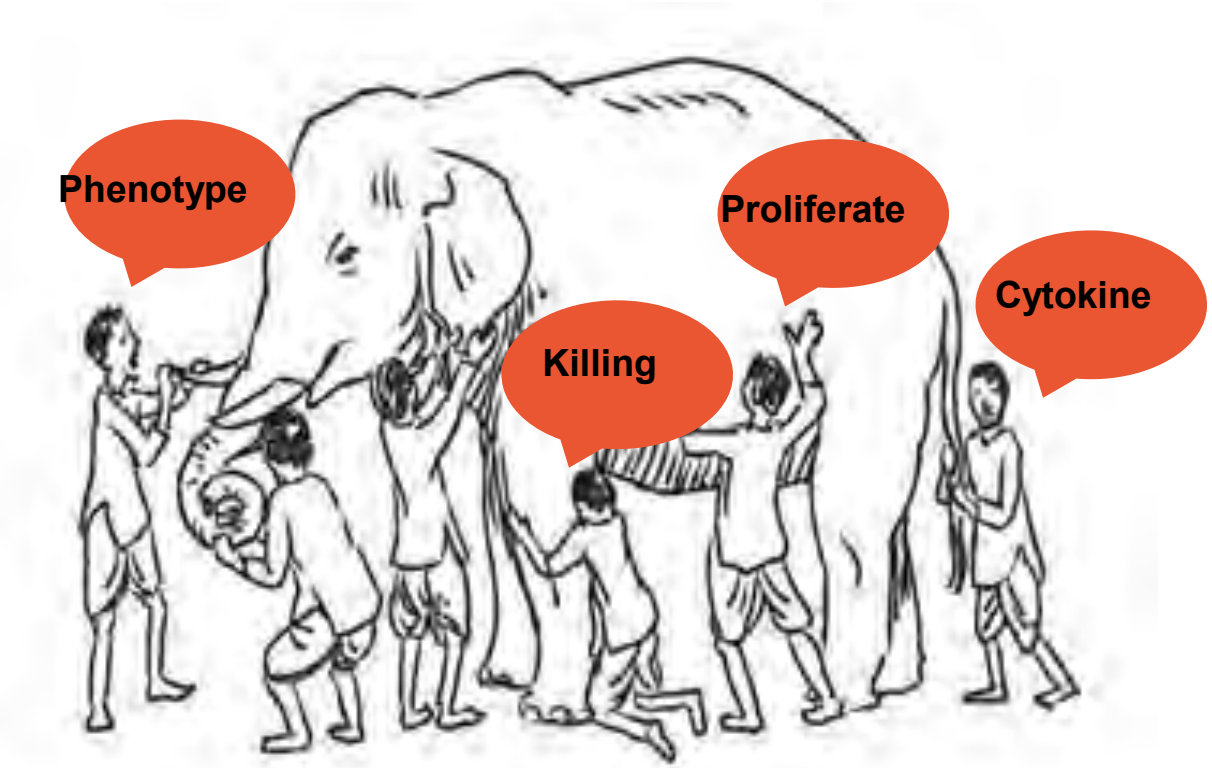
Additional analytical considerations for future CAR T therapies

iPSC:

- iPS cells may carry an inherent risk of tumourigenicity
- The nonclinical qualification of the level of undifferentiated iPS cell impurities need to be addressed
- Reprogramming may induce epigenetic changes in the cells with consequences that are not yet fully understood. A variety of high-throughput methods are available for evaluation of the genetic and epigenetic profiles of the iPS cell lines and their derivatives.

- Guideline on quality, non-clinical and clinical aspects of medicinal products containing genetically modified cells, EMA, July 2018

Comprehensive product characterization is the key to true understanding of CAR T therapies



Modified from www.williepietersen.com

Thank you