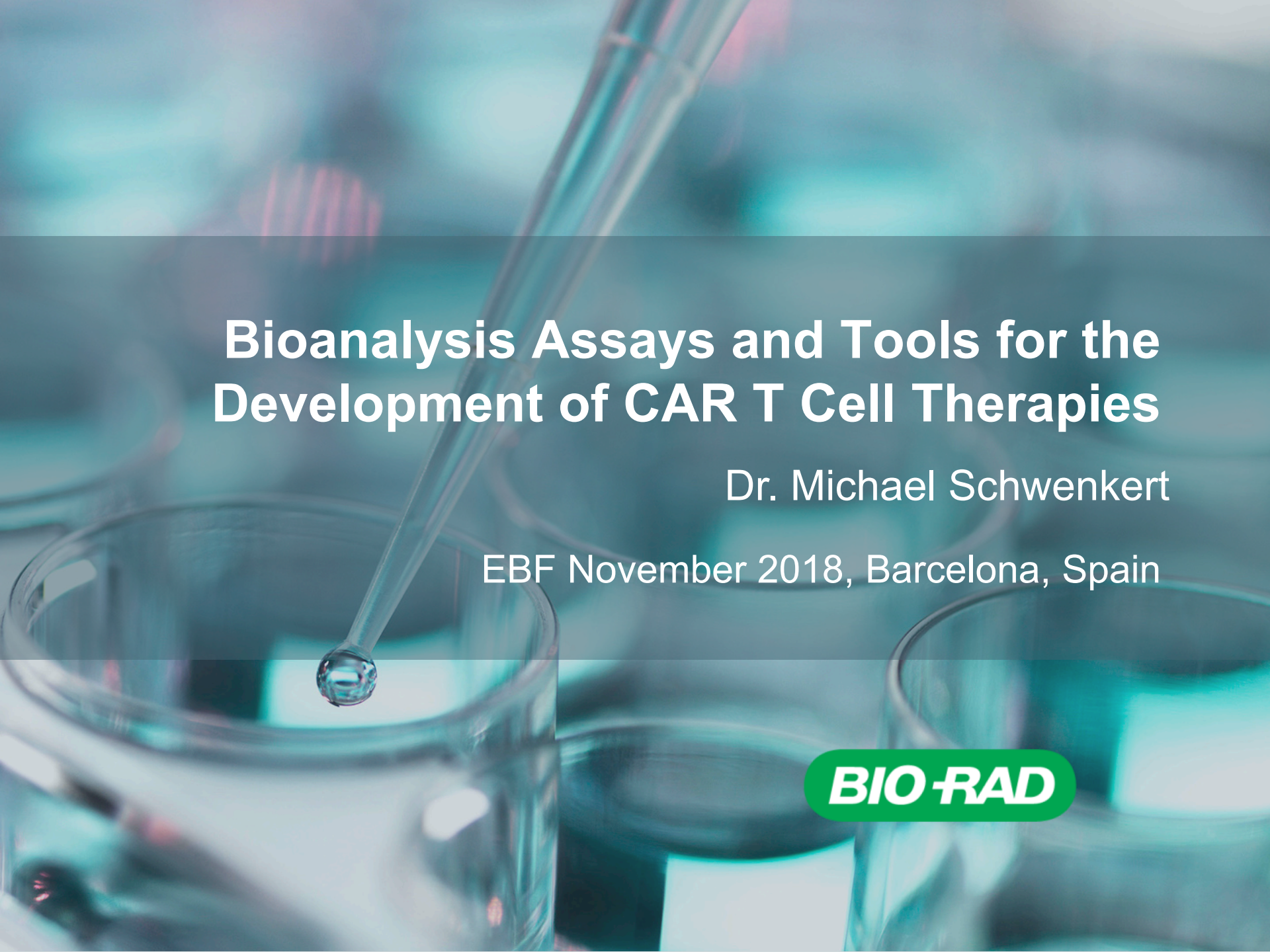

Bioanalysis Assays and Tools for the Development of CAR T Cell Therapies

Dr. Michael Schwenkert

EBF November 2018, Barcelona, Spain

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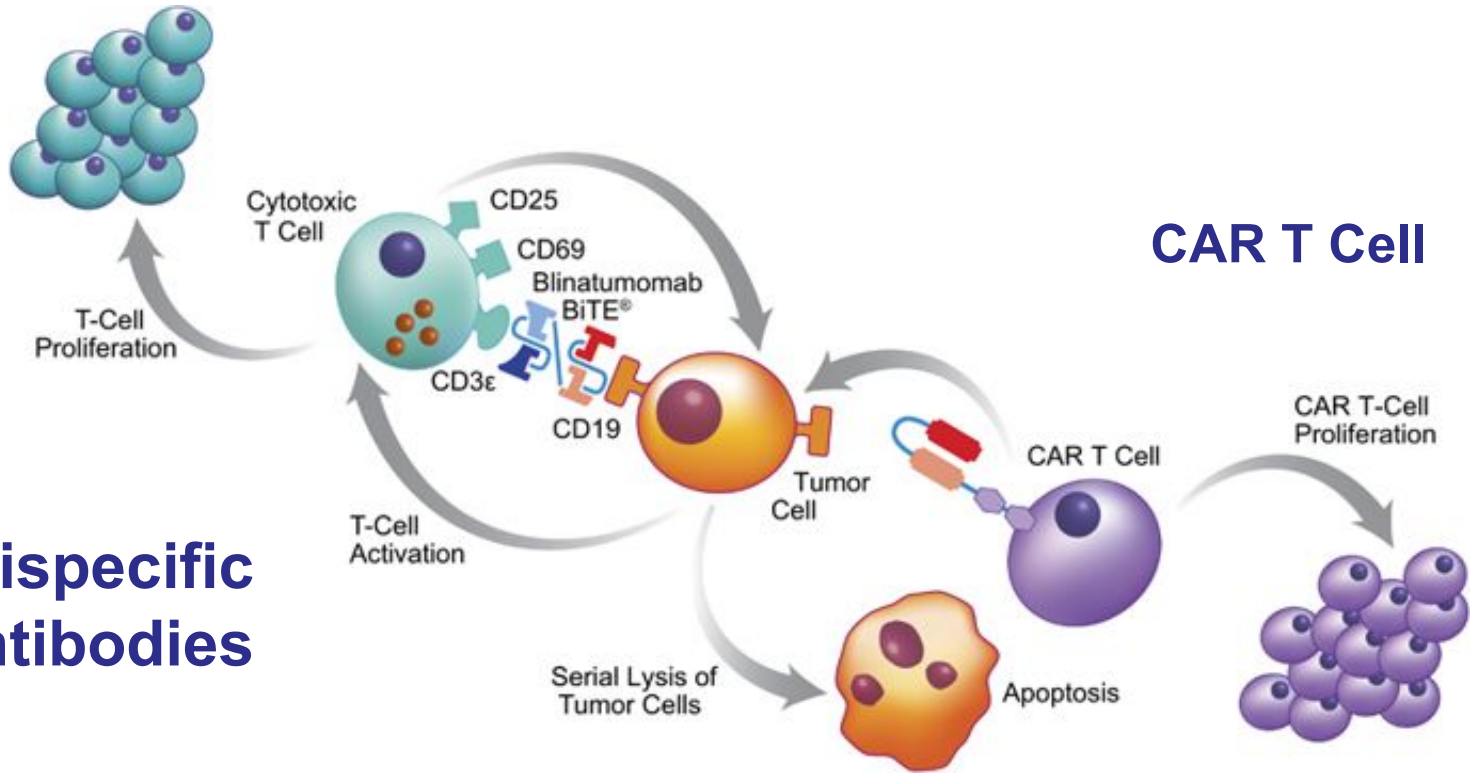
Agenda

- Concept, history & overview of CAR T cell therapy
- Solutions for research in the field of CAR T cell therapy
  - Multiplex immunoassays
  - Digital PCR
  - Flow cytometry
  - CAR T cell specific antibodies
- Summary

Bridging T cells to tumor cells

The power of recombinant antibody technology



CAR T Cell

Bispecific antibodies

Aldoss et al., 2017

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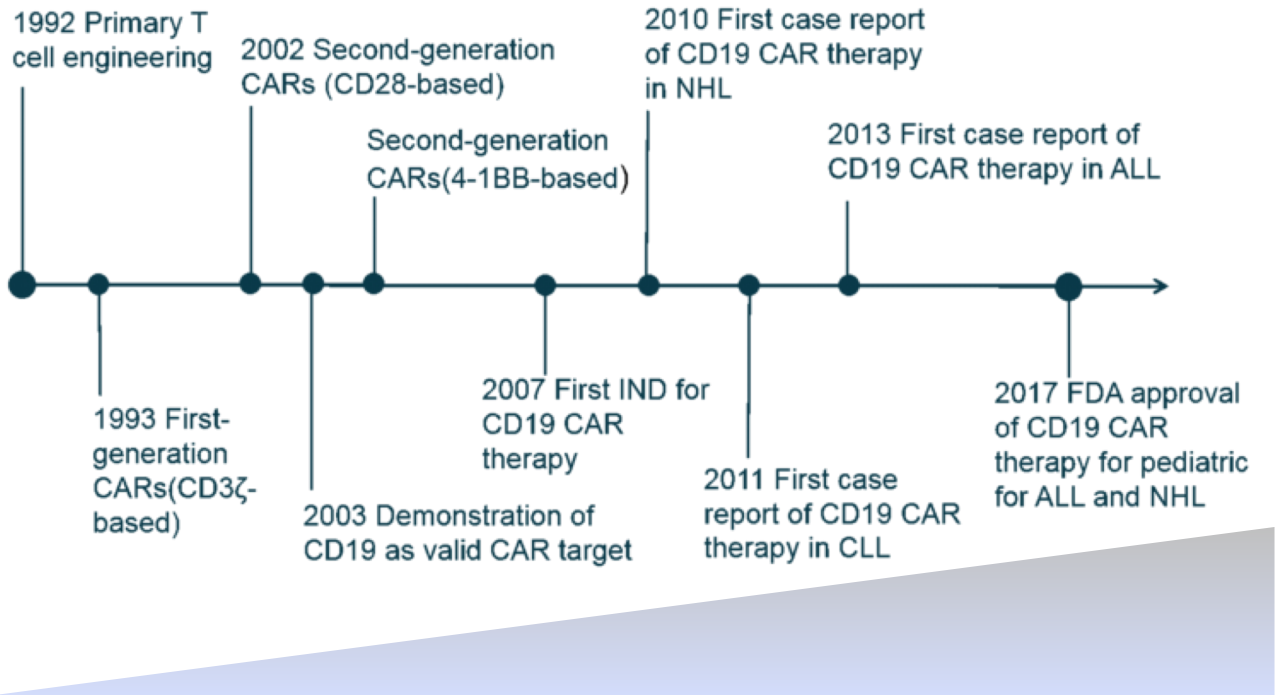
Introduction to CAR T cell research

Chimeric Antigen Receptor T cell (CAR T cell) therapy is a type of adaptive cell transfer (ACT) therapy and a new type of personalized cancer therapy that takes a patient's own immune cells and modifies those cells to specifically eradicate this patient's cancer calls.



Progress and understanding of CAR T accelerating

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CAR T cell development - process overview

- Target discovery of cancer antigen
- Design of the CAR
- Development of the CAR T cell
- Testing of the CAR T cell in preclinical models
- Development of the CAR T cell clinical scale manufacturing
- Testing of the CAR T cell in clinical models
- Regulatory approval for CAR T cell therapy
- Development of the CAR T cell commercial scale manufacturing

Tools for development of CAR T cell therapies

- Sequencing
- Mass spec
- ELISA
- Flow cytometry
- IHC
- Western blotting
- qPCR
- Digital PCR
- In silico design and testing tools
- Label free biosensor assays
- Cell biology tools
- Cell isolation tools
- Viral transfection
- Cell imaging
- CAR T cell specific antibodies

Tools for development of CAR T cell therapies

- Sequencing
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- **ELISA**
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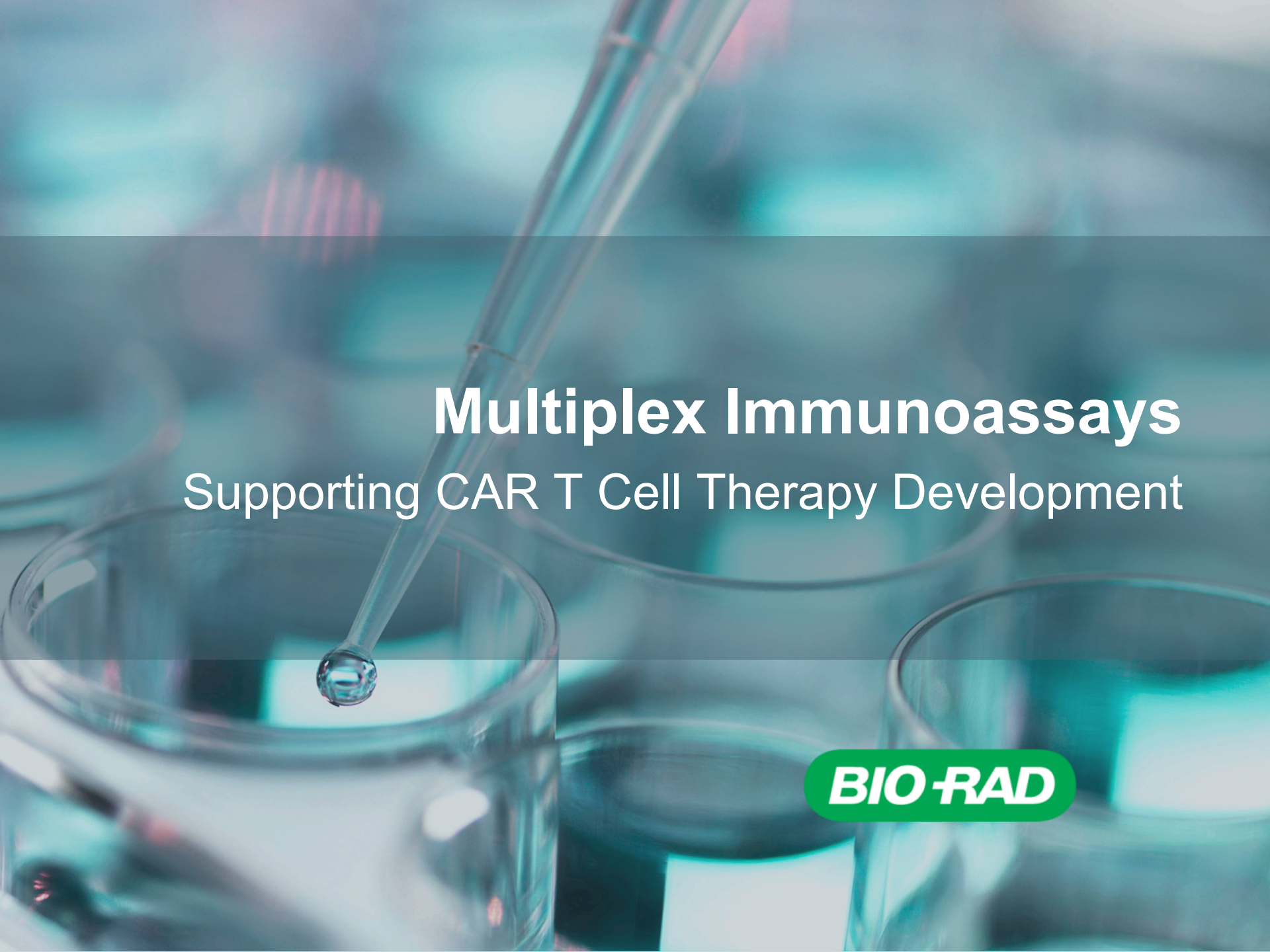
- **CAR T cell specific antibodies**



Multiplex Immunoassays

Supporting CAR T Cell Therapy Development

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Immunoassays used in CAR T cell research

Multiplex immunoassays provide data on more than one target simultaneously, with overall smaller sample volumes and faster speeds than individual assays

Cytokine storm

- Monitoring cytokines is critical for dosage and efficacy studies

Cell trafficking

- Chemokines drive immune cell migration

Combination therapy monitoring

- Pre-conditioning with cytokines, chemotherapy

Model systems

- Complementary multiplex panels in both human and mouse facilitate translation

Key challenges in CAR T approach

- Monitoring serious side effects such as cytokine release syndrome (CRS)
- Localization of CAR T cells
- Target and dosage vs efficacy

Monitoring serious side effects such as CRS

- Treatment with CAR T cells may result in inflammatory response
- Differentiate between different types of response and group accordingly (CRS grade 1 through grade 4)
- The severity can range from mild reactions to a life threating response
- Appropriate treatments must be given in a timely manner

Blood. 2016 Jun 16; 127(24): 2980–2990.
Prepublished online 2016 Apr 26. doi: 10.1182/blood-2015-12-686725
PMCID: PMC4911862
PMID: 27118452

Phase 1 studies of central memory–derived CD19 CAR T–cell therapy following autologous HSCT in patients with B-cell NHL

Xiuli Wang,[1,*] Leslie L. Popplewell,[1,*] Jamie R. Wagner,[1,*] Araceli Naranjo,[1] M. Suzette Blanchard,[2] Michelle R. Mott,[3] Adam P. Norris,[3] ChingLam W. Wong,[1] Ryan Z. Urak,[1] Wen-Chung Chang,[1] Samer K. Khaled,[1] Tanya Siddiqi,[1] Lihua E. Budde,[1] Jingying Xu,[1] Brenda Chang,[1] Nikita Gidwaney,[4] Sandra H. Thomas,[1] Laurence J. N. Cooper,[5] Stanley R. Riddell,[6] Christine E. Brown,[1,†] Michael C. Jensen,[7,†] and Stephen J. Forman[✉1,†]

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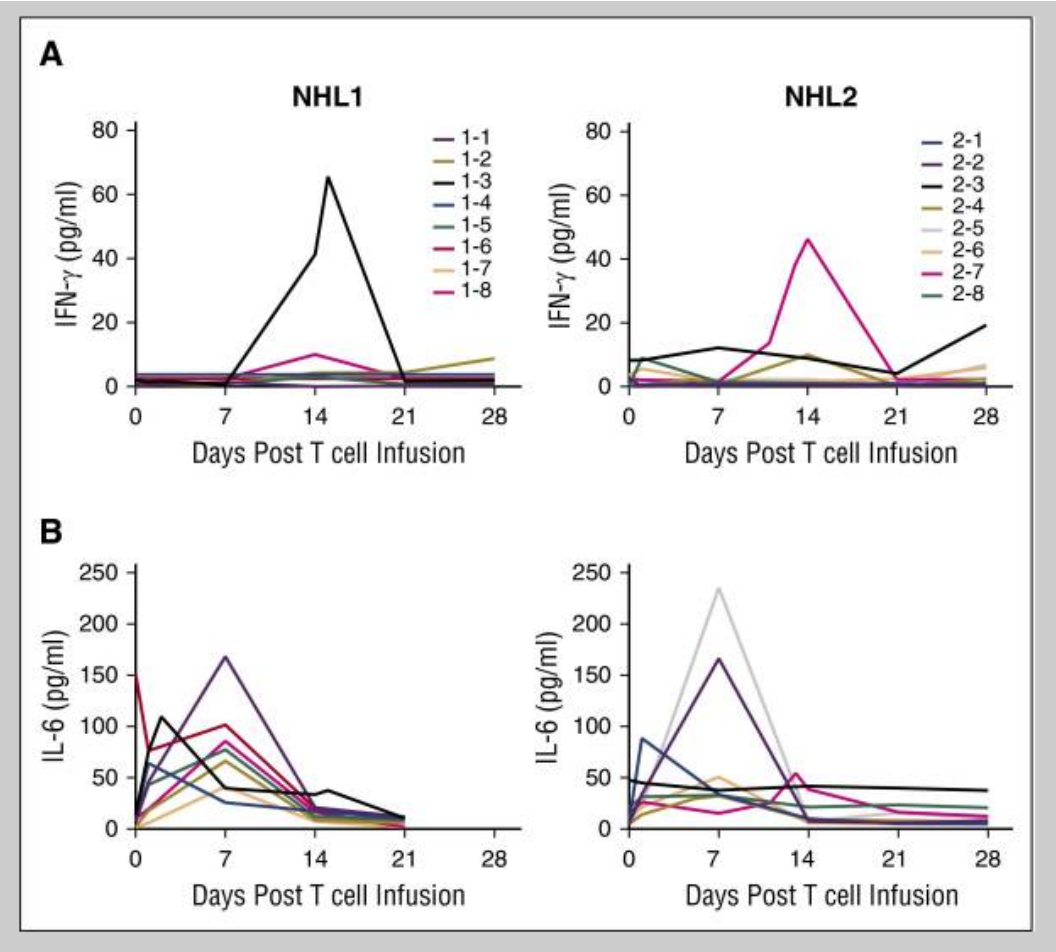
Monitoring serious side effects such as CRS



No CAR T cell infusion-related clinical symptoms were observed.
Serum cytokine levels were measured using a single 30-plex immunoassay and were found to be moderately increased, including IL-6 and IFN-γ, as well as IL-10, TNF-α, GM-CSF, MIP-1 α, and IL-2Rα

Key messages

- Multiplex immunoassay analysis of patient serum allowed clinicians to identify those patients who had severe responses to the CAR T cell treatment
- In this early phase clinical trial case no grade 2 or higher responses were reported
- Highly sensitive multiplex chemokine panels formed a critical part in clinical studies for early assessment of side effects


Digital Droplet PCR
Supporting CAR T Cell Therapy Development
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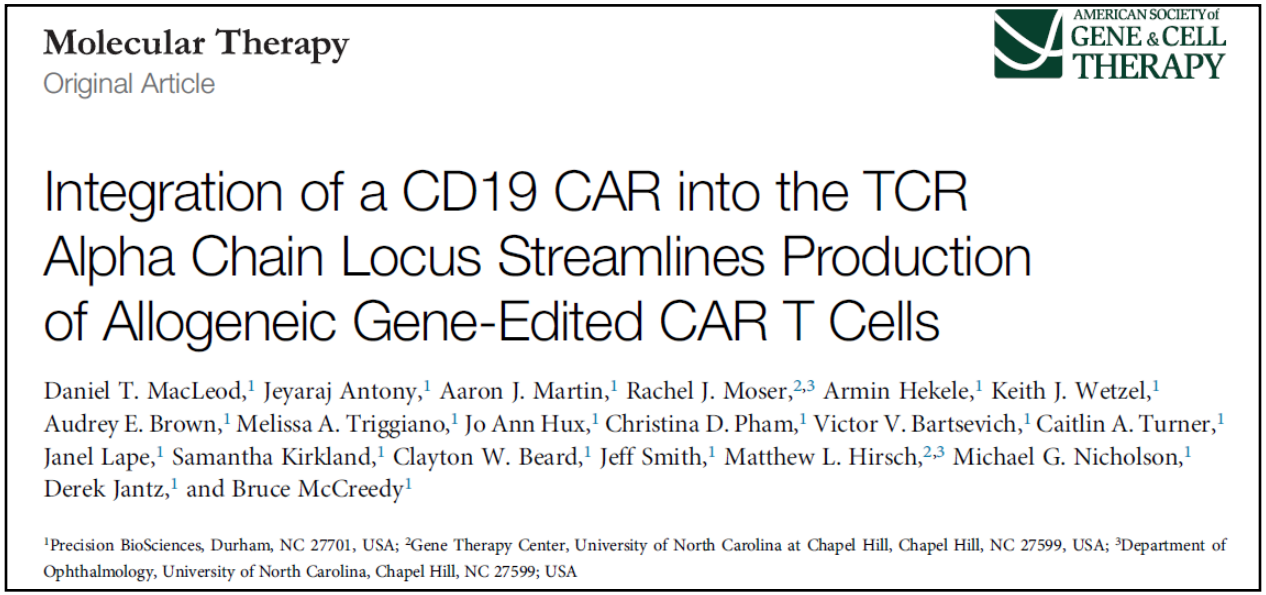
Digital Droplet PCR in CAR T cell R&D

Digital Droplet PCR (ddPCR) is used across many steps in the development of CAR T cell therapy

- Allows absolute quantitation of nucleic acid without need for standard curves and is extremely sensitive
- Used to measure viral copy number as part of the CAR T creation process
- Used to measure the number of copies of the CAR gene once inserted into the T cell
- Used with liquid biopsy to quantify and profile the mutations of individual patients tumors

Development of universal CAR T cells

- Personalized CAR T cells are expensive, are only specific for one patient and take up to 4 weeks to create and administer
- Universal CAR T options (e.g. allogeneic CAR T cells) that are ‘off-the-shelf’ provide a more cost efficient treatment that is readily available to a patient
- Endogenous TCR must be knocked out to avoid GvHD



Molecular Therapy

Original Article

AMERICAN SOCIETY of GENE & CELL THERAPY

Integration of a CD19 CAR into the TCR Alpha Chain Locus Streamlines Production of Allogeneic Gene-Edited CAR T Cells

Daniel T. MacLeod,[1] Jeyaraj Antony,[1] Aaron J. Martin,[1] Rachel J. Moser,[2,3] Armin Hekele,[1] Keith J. Wetzel,[1] Audrey E. Brown,[1] Melissa A. Triggiano,[1] Jo Ann Hux,[1] Christina D. Pham,[1] Victor V. Bartsevich,[1] Caitlin A. Turner,[1] Janel Lape,[1] Samantha Kirkland,[1] Clayton W. Beard,[1] Jeff Smith,[1] Matthew L. Hirsch,[2,3] Michael G. Nicholson,[1] Derek Jantz,[1] and Bruce McCreedy[1]

[1]Precision BioSciences, Durham, NC 27701, USA; [2]Gene Therapy Center, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA; [3]Department of Ophthalmology, University of North Carolina, Chapel Hill, NC 27599; USA

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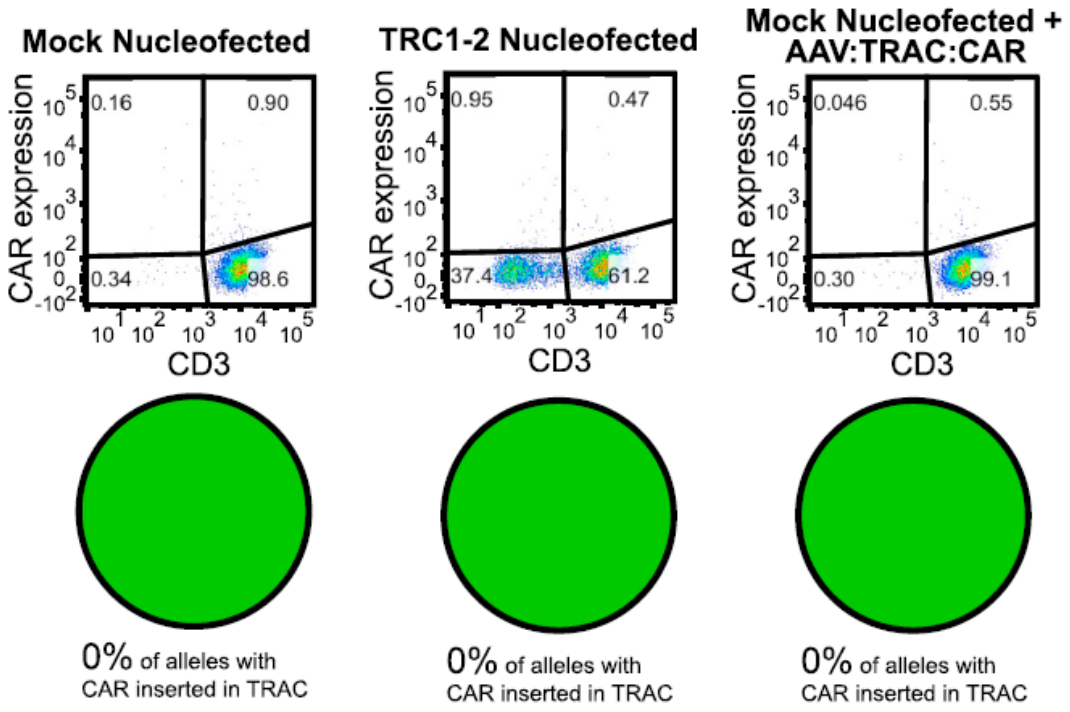
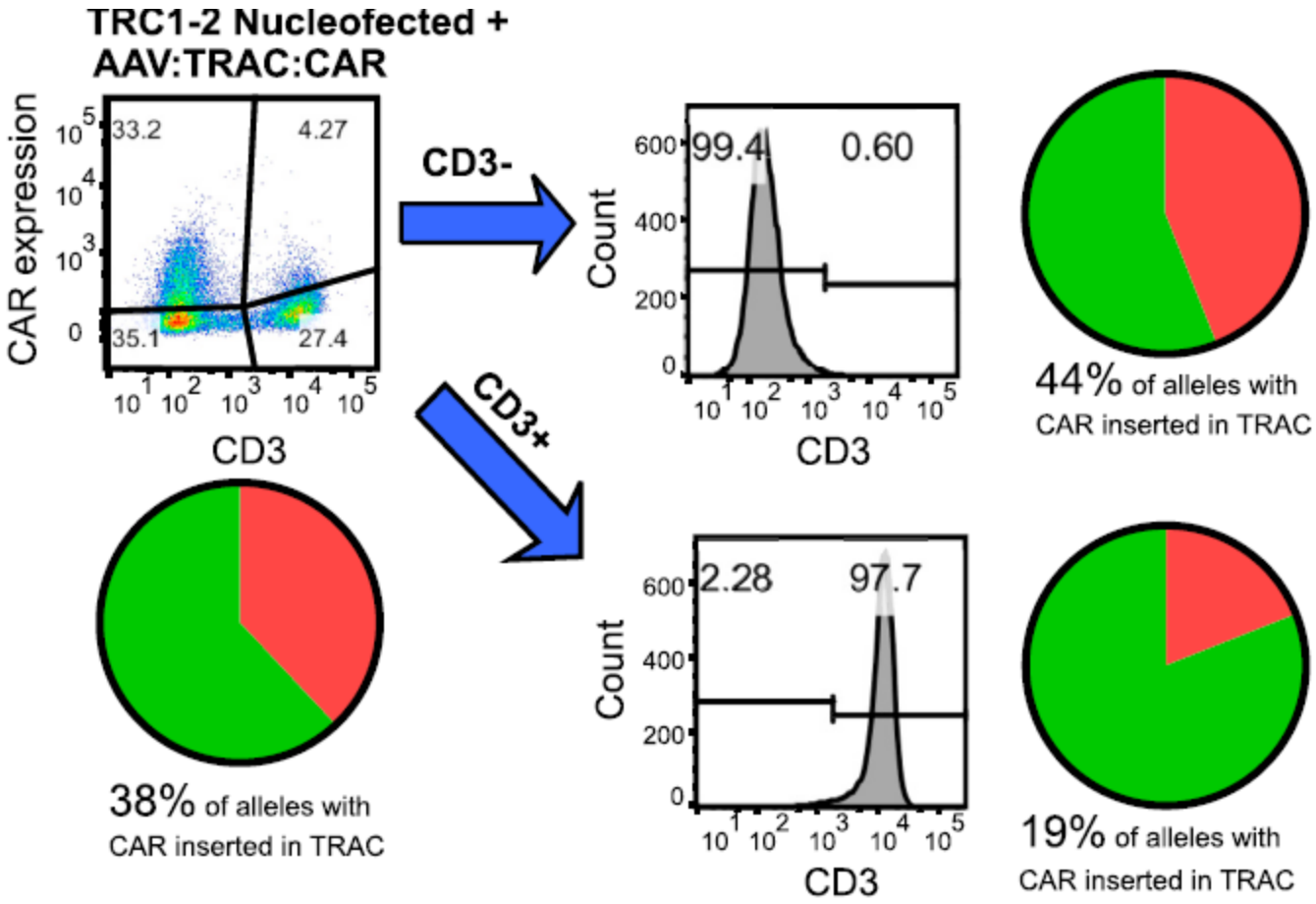
Development of universal CAR T cells

- Which level of vector dose provides the highest amount of edited cells?
- A ddPCR assay was used to measure target integration at the TRAC site using primers specific to the CAR transgene vs reference sequence
- Negative controls showed no expression of CAR gene



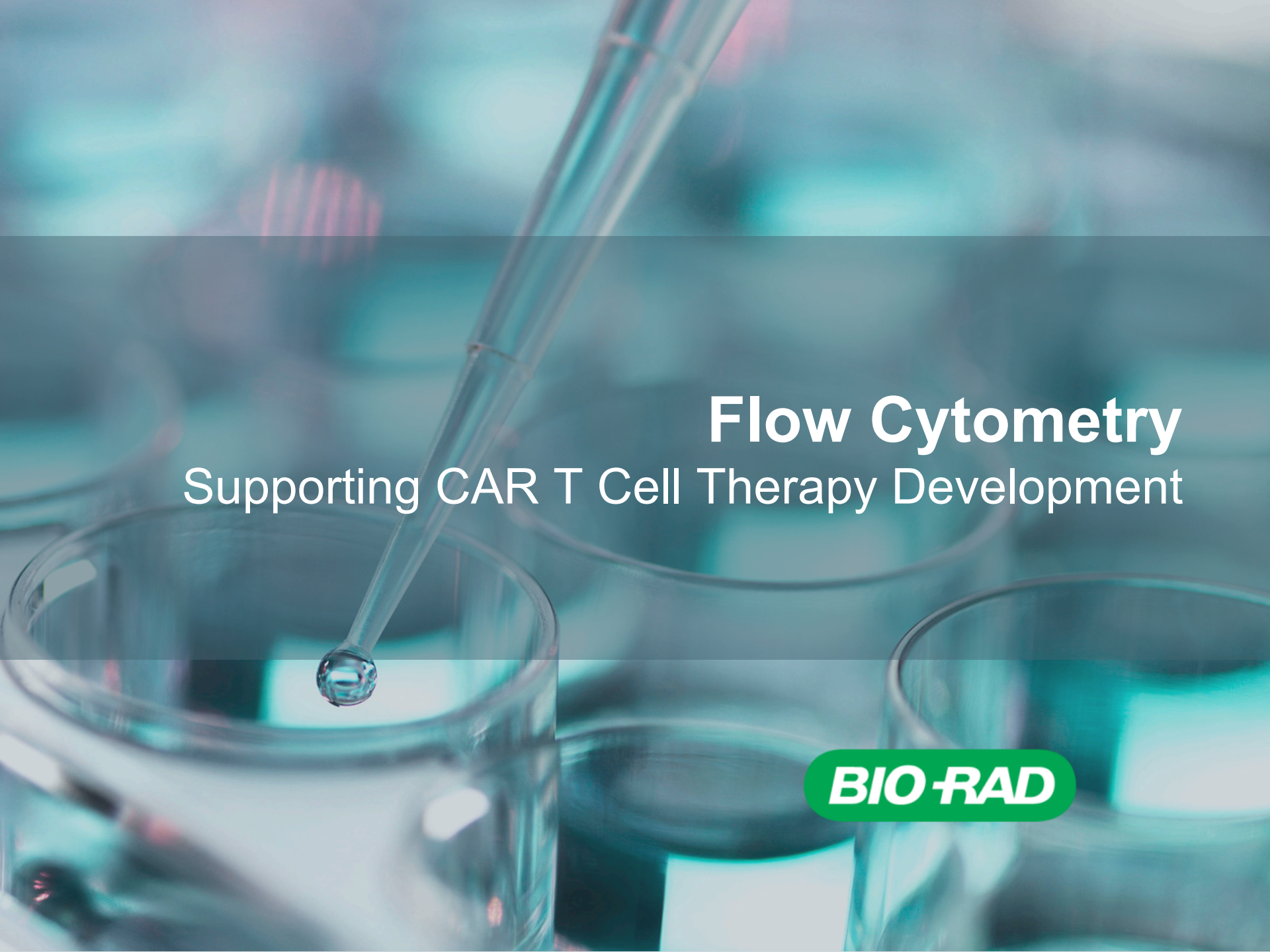
Development of universal CAR T cells

- ddPCR measured 38% targeted gene integration in DNA from cells that were both electroporated and transduced with the CAR vector
- ddPCR measured 44% targeted gene integration in DNA from $CD3^-$ cells vs 19% in $CD3^+$ cells



Key messages

- Knocking down the endogenous TCR was done using an anti-CD19 CAR transgene
- Flow cytometry and ddPCR were used to confirm highly efficient targeted insertion
- Preclinical studies showed that this universal CAR T was effective in mouse models for lymphoma
- ddPCR offers a reliable and highly sensitive method of quantifying genetic alterations in the development of universal CAR T cells



Flow Cytometry

Supporting CAR T Cell Therapy Development

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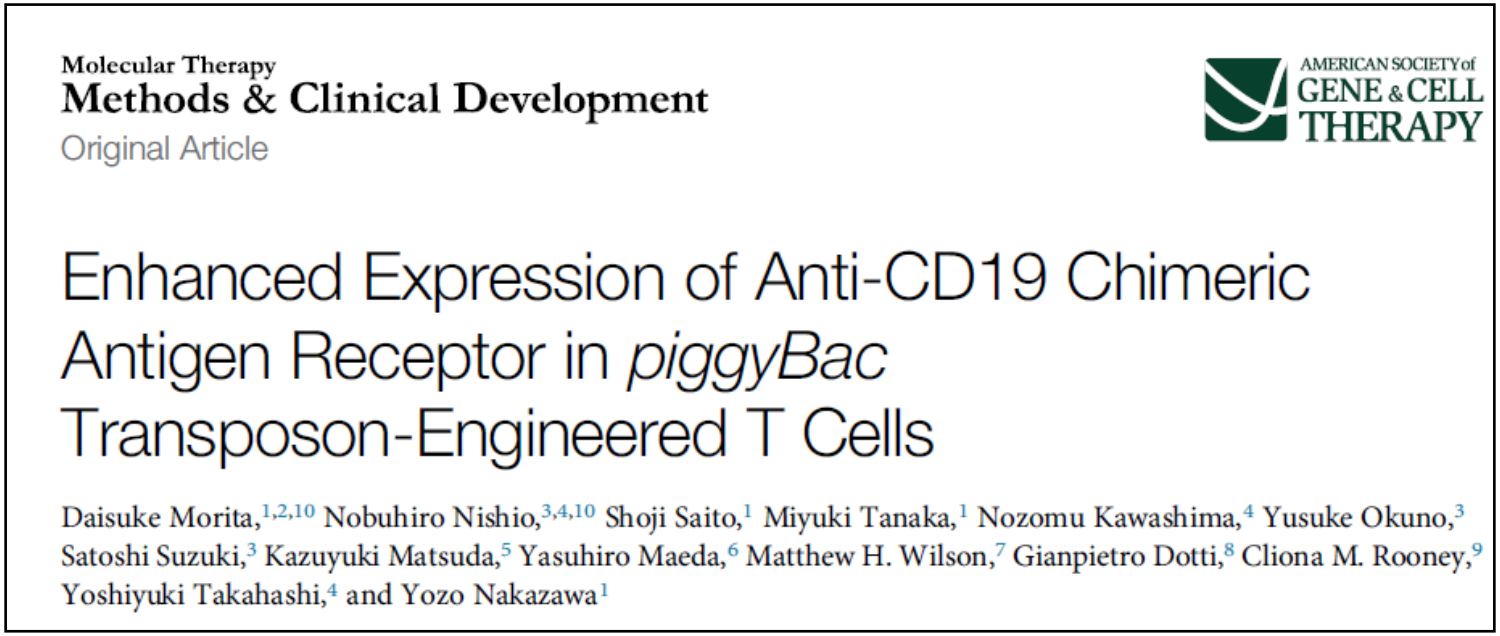
Flow cytometry used in CAR T cell research

- Applicable across many steps in CAR T cell therapy development
- Check binding of CAR T cell to appropriate tumor cell
- Enables understanding of the immune system's response to cancer
- Track success of therapies

Key challenges in CAR T approach

- Reducing cost of manufacturing CAR T cells
- Measuring tumor burden after CAR T infusion



Molecular Therapy
Methods & Clinical Development
Original Article

AMERICAN SOCIETY of GENE & CELL THERAPY

Enhanced Expression of Anti-CD19 Chimeric Antigen Receptor in *piggyBac* Transposon-Engineered T Cells

Daisuke Morita,[1,2,10] Nobuhiro Nishio,[3,4,10] Shoji Saito,[1] Miyuki Tanaka,[1] Nozomu Kawashima,[4] Yusuke Okuno,[3] Satoshi Suzuki,[3] Kazuyuki Matsuda,[5] Yasuhiro Maeda,[6] Matthew H. Wilson,[7] Gianpietro Dotti,[8] Cliona M. Rooney,[9] Yoshiyuki Takahashi,[4] and Yozo Nakazawa[1]

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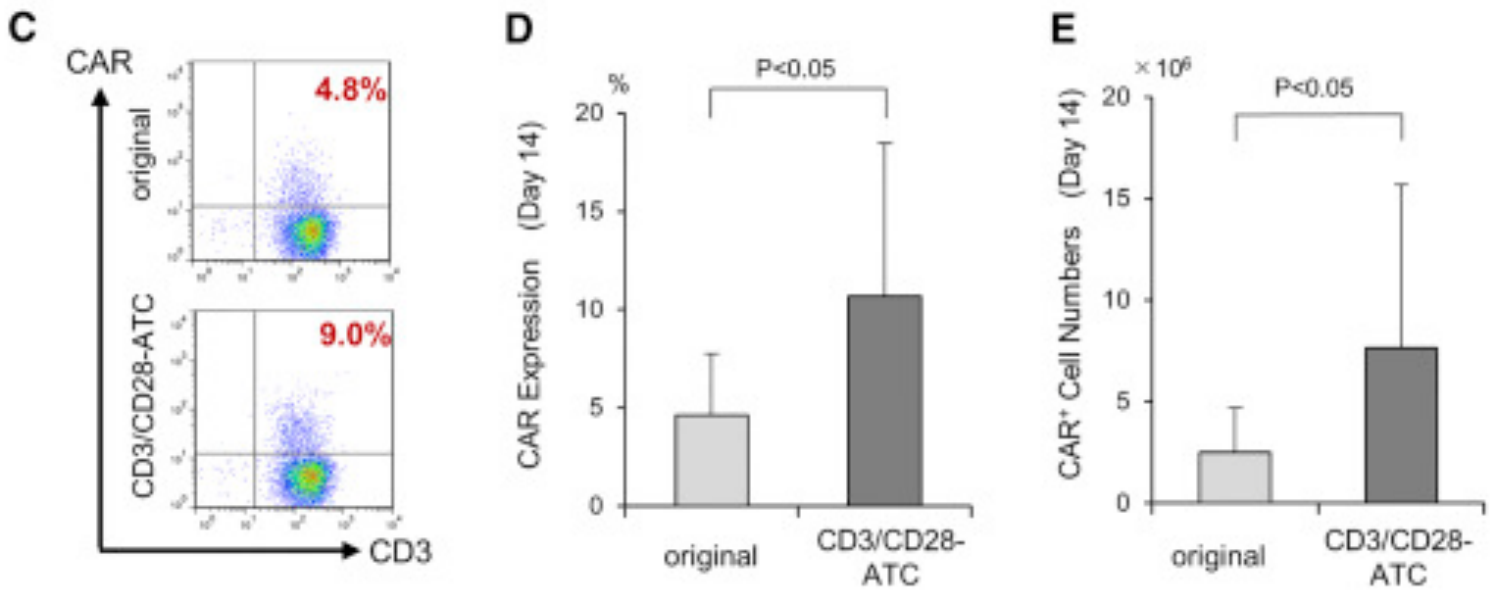
CAR T cells using ATC feeder cells



C - CAR T cells proliferated in presence of the ATC feeder cells result in more CAR expressing cells shown by higher % of total cells – 9% vs 4.8% when examining CAR expression vs $CD3^+$ cells for one patient

D - Higher % of CAR expressing CAR T cells after treatment with the ATC feeder cells

E - Higher absolute numbers of CAR expressing CAR T cells after treatment with the ATC feeder cells

D & E The data are presented as the mean ± SD from nine donors

Performed using 2 laser flow cytometry system with specific anti-idiotypic antibody

Key messages

- Use of ATC feeder cells resulted in increased efficiency and production level of CAR T cells
- Addition of ATC that were pulsed with ACE viral peptides further increased CAR T cell production efficiency

The exploration of additional methods to reduce costs for CAR T production is key to providing this breakthrough in giving personalized therapy to more patients


CAR T Cell Specific Antibodies
Supporting Therapy Development
BIO-RAD

Antibody needs for CAR T cell R&D

- **Bioanalysis in clinical studies using CAR T cells**
  - Specific detection of CAR in the context of patient monitoring (anti-idiotypic antibodies)
  - Controls and calibrators in immunogenicity assessment
- **Enrichment of T cells**
  - Positive selection of T cells
  - Depletion of further blood cells
- **Design and proof of concept of CAR**
  - Target discovery and raising of specific antibodies
  - Subcloning into CAR T and transduction of T cells

Antibody needs for CAR T cell R&D

Bioanalysis in clinical studies using CAR T cells

- Specific detection of CAR in the context of patient monitoring (anti-idiotypic antibodies used in flow cytometry)

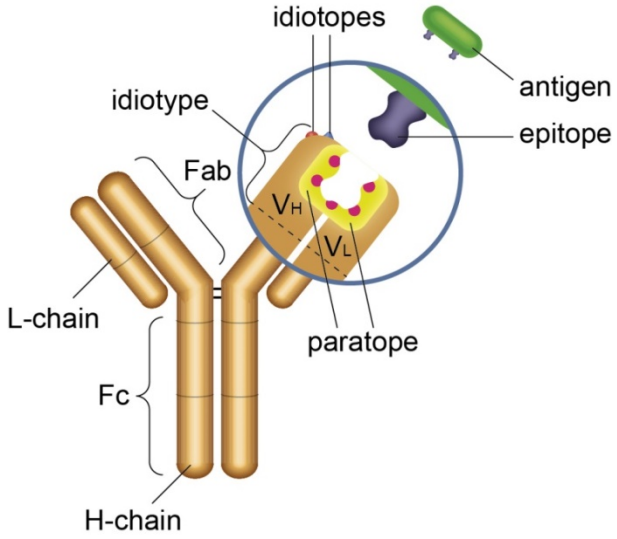
- Controls and calibrators in immunogenicity assessment

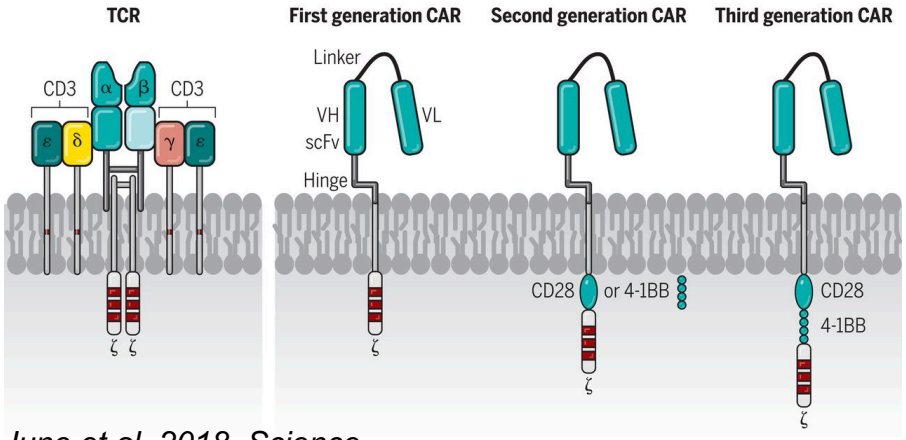
CAR and anti-idiotypic antibodies

Idiotope: unique set of antigenic determinants of the variable portion of an antibody

Anti-idiotypic antibodies:

- Antibodies that are directed against the idiotope
- Specific for one particular antibody or antibody fragment





June et al. 2018, Science

- CARs utilize idiotype (VH and VL fused via a linker; scFv) as extracellular domain
- Anti-idiotypic antibodies act as specific and unique discriminator of CAR T cells

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Key messages

- CAR T cells research and development requires highly specialized antibody reagents
- Antibody tools play a fundamental role in T cell enrichment
- Specialized anti-idiotypic antibodies can be used for bioanalytical assays


Summary
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Supporting CAR T cell therapy development

- Key steps within CAR T cell discovery and development require distinct bioassays
- Multiplex immunoassays, digital PCR technologies and multiplex flow cytometry are all important to researchers developing CAR T cell therapies
- Specialized custom antibody reagents tailored to the needs of CAR T cell research and development further support this promising area of translational research