



CDDF  
MULTI-STAKEHOLDER  
WORKSHOP

29-30 NOVEMBER 2021

# Tumour and immunological environment influences in haematologic and solid tumours: engineering techniques or drug combination?

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University Hospital Fundación Jiménez Díaz

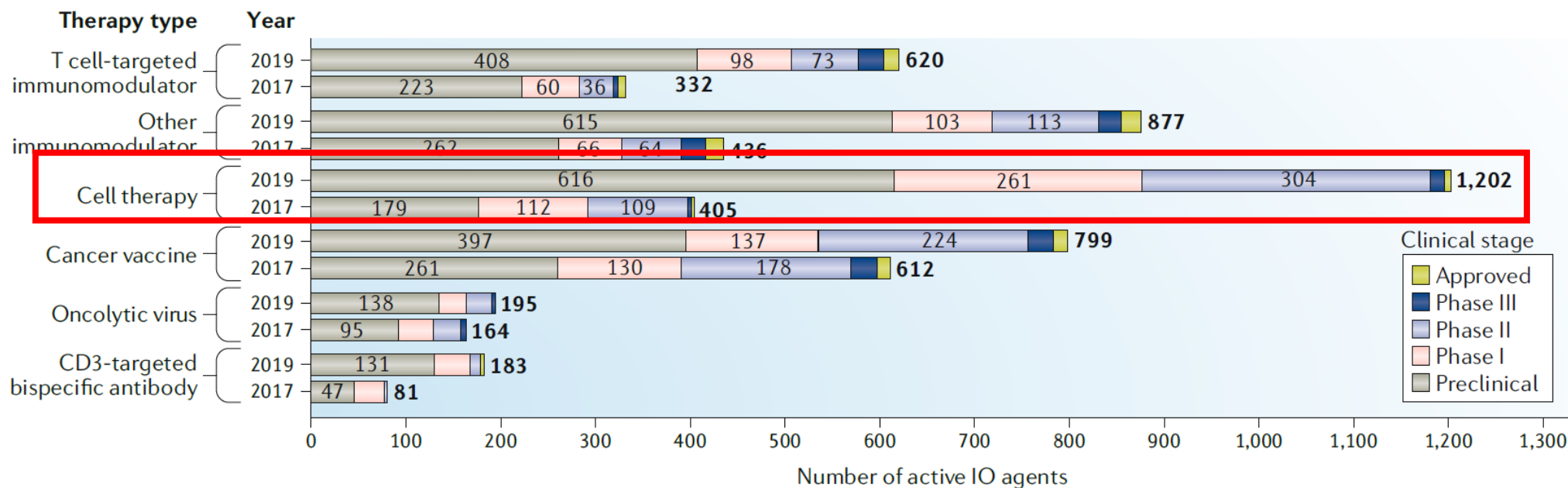


# Disclosures

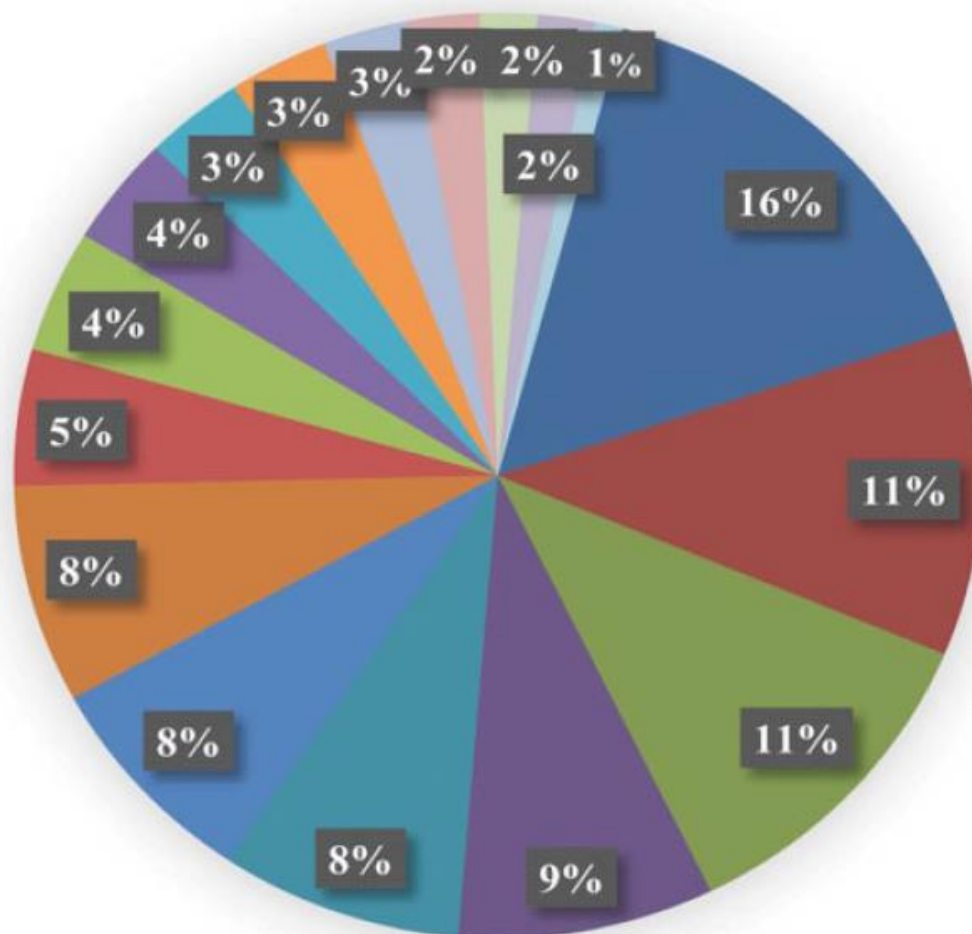
- **Consulting fees from:** Roche, Bayer, BMS, Janssen and Basilea.
- **Principal Investigator – Institutional Funding:** AbbVie, AceaBio, Adaptimmune, ADC Therapeutics, Aduro, Agenus, Amcure, Amgen, Astellas, AstraZeneca, Bayer, Beigene, BioInvent International AB, BMS, Boehringer, Boehringer, Boston, Celgene, Daichi Sankyo, DEBIO PHARM, Eisai, e-Therapeutics, Exelisis, Forma Therapeutics, Genmab, GSK, Harpoon, Hutchison, Immutep, Incyte, Inovio, Iovance, Janssen, Kyowa Kirin, Lilly, Loxo, MedSir, Menarini, Merck, Merus, Millennium, MSD, Nanobiotix, Nektar, Novartis, Odonate Therapeutics, Pfizer, Pharma Mar, PharmaMar, Principia, PsiOxus, Puma, Regeneron, Rigontec, Roche, Sanofi, Sierra Oncology, Sponsor, Synthron, Taiho, Takeda, Tesaro, Transgene, Turning Point Therapeutics, Upshersmith.

# Immuno-oncology Drug Development in 2019

Increase from 2017 to 2019 : 300% in Cell therapy

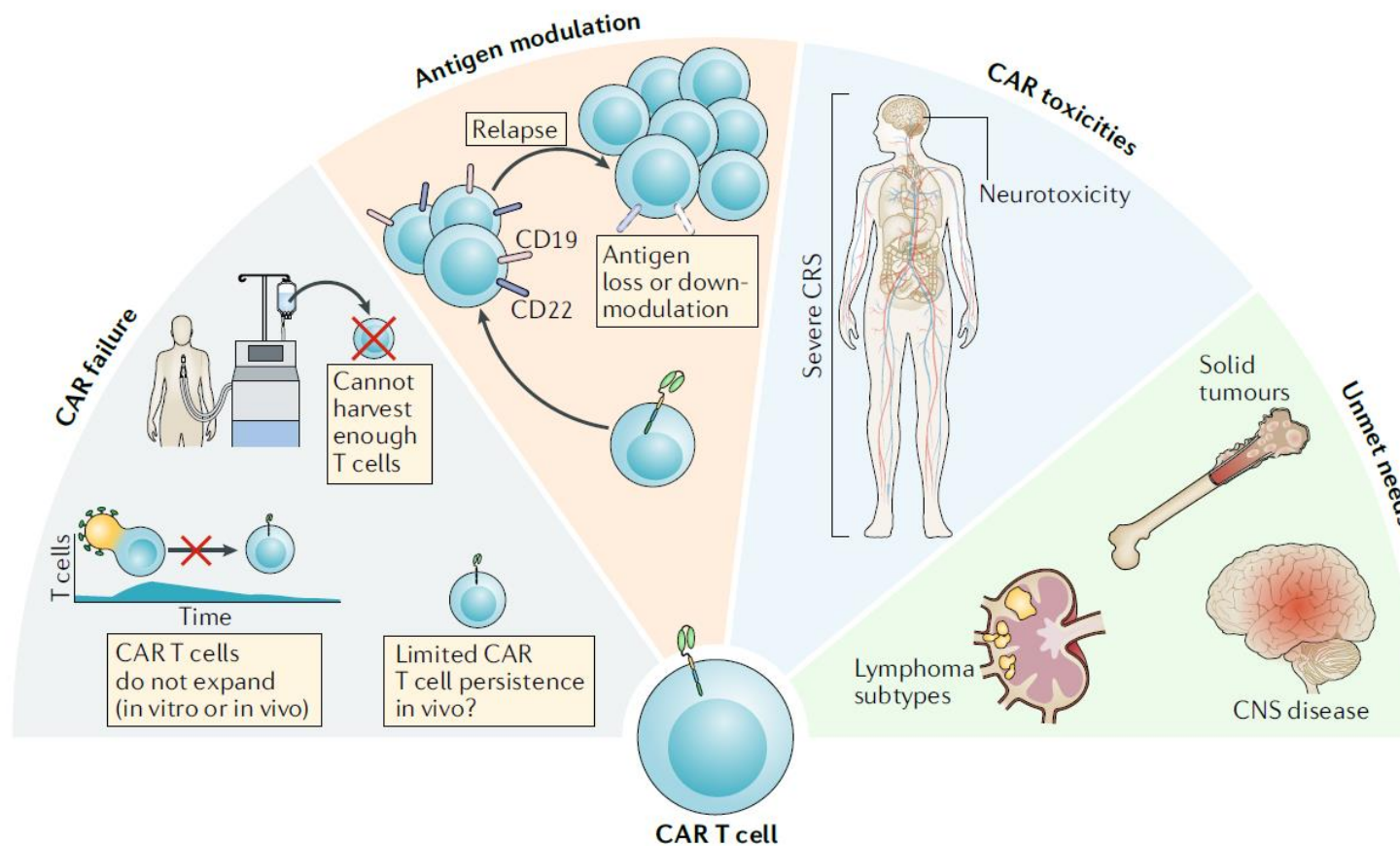


## B Condition Type



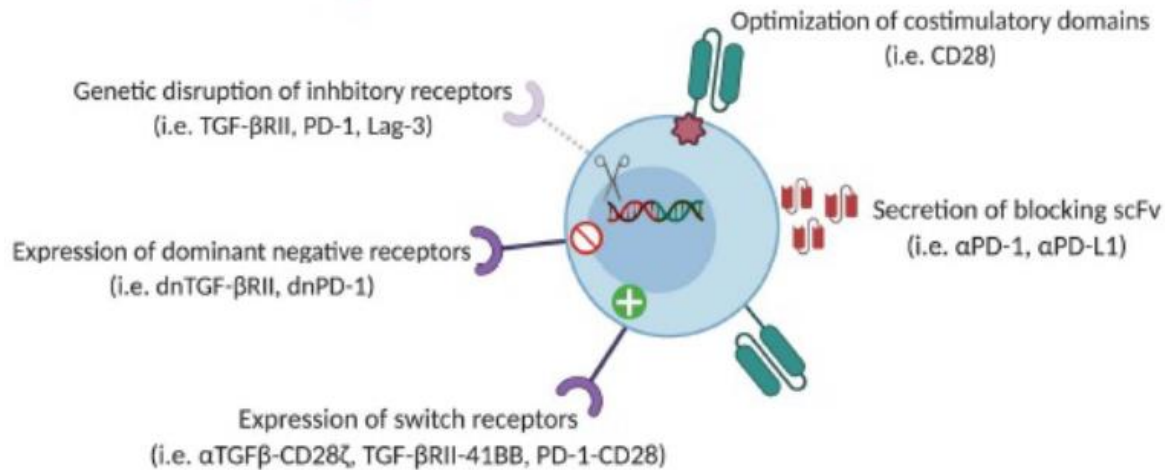
- Melanoma
- Gastrointestinal Cancer
- Leukemia
- Lung Cancer
- Unspecified
- Other
- Sarcoma
- Ovarian, Fallopian Tube, and Peritoneal Cancer
- Neoplasm
- Myeloma
- Bladder Cancer
- Kidney Cancer
- Breast Cancer
- Skin Cancer
- Cervical Cancer
- Mesothelioma
- Brain Cancer

# CAR-T challenges



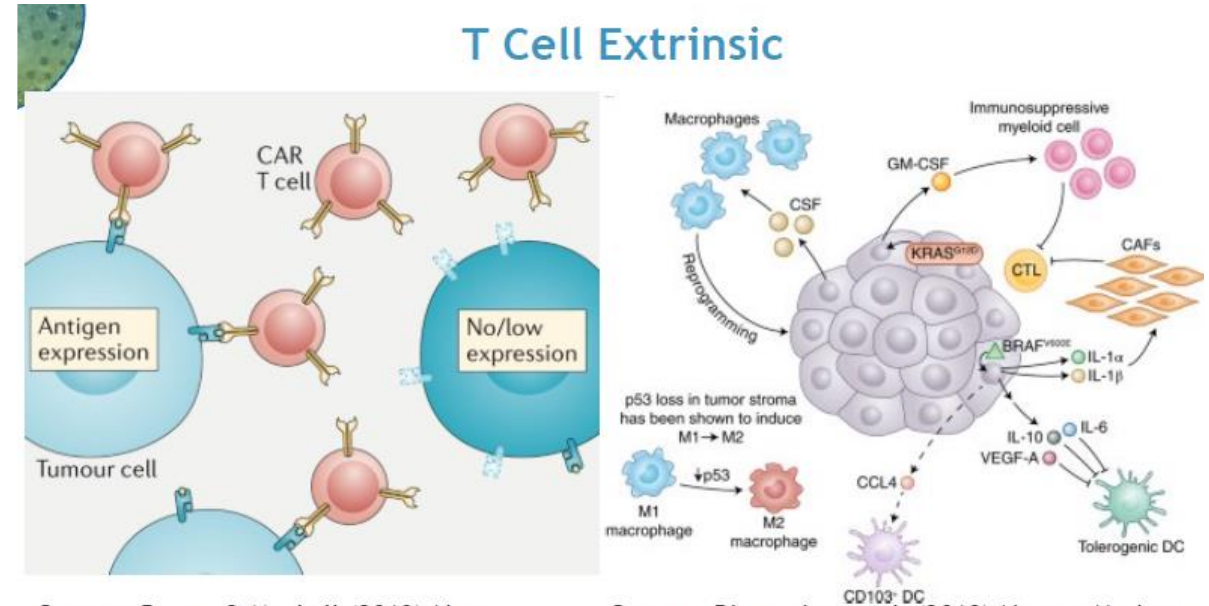
# T Cell Extrinsic and Intrinsic Factors Limit Exposure at the Site of Action and the Ability to Sustain Pharmacological Activity

## T Cell Intrinsic



Source: Rodriguez-Garcia et al. (2020) *Frontiers in Immunology*, 11:1109.

## T Cell Extrinsic



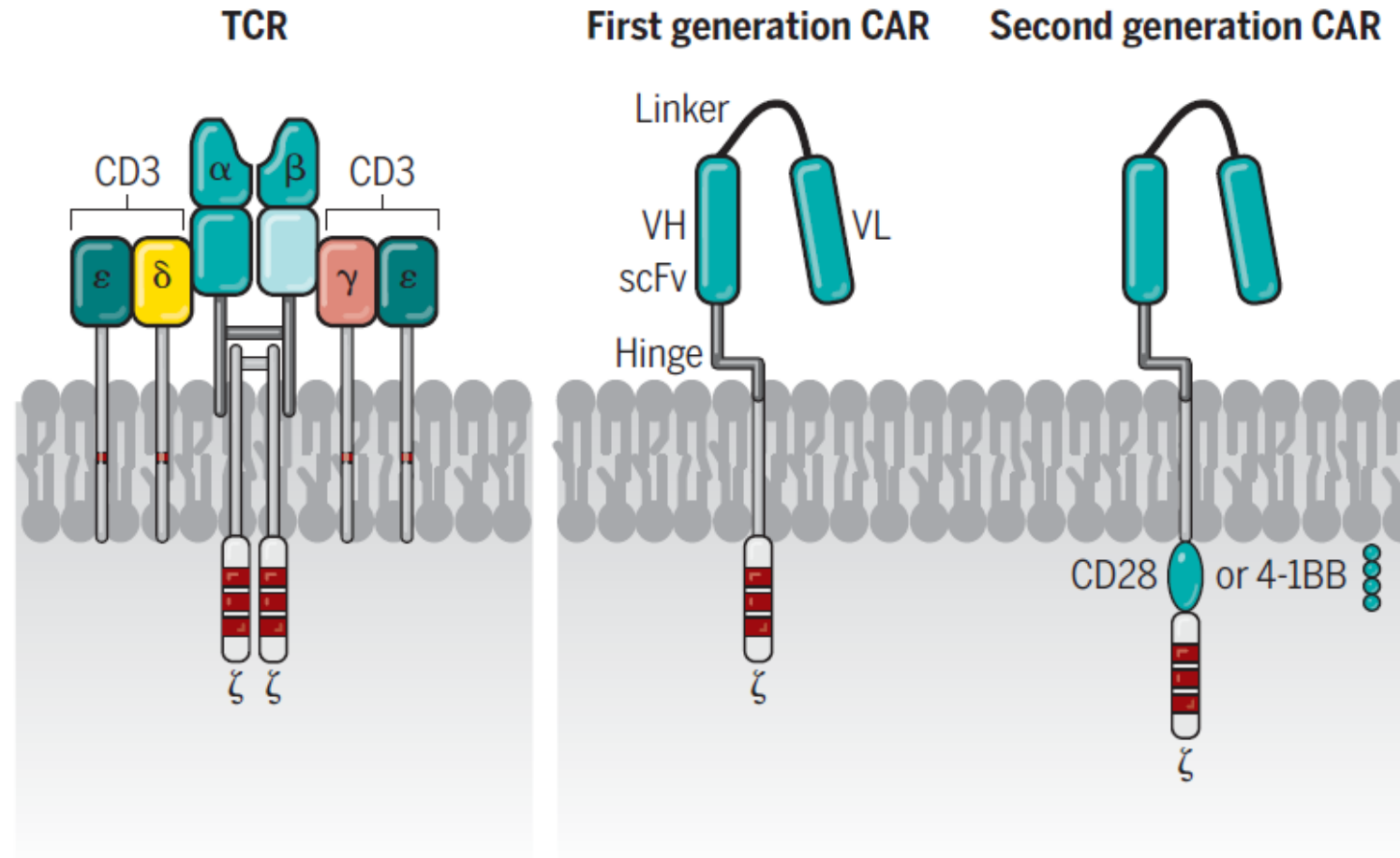
Source: Brown & Mackall (2019) *Nature Reviews Immunology* 19, 73-74

Source: Binnewies et al. (2018) *Nature Medicine* 24, 541-550

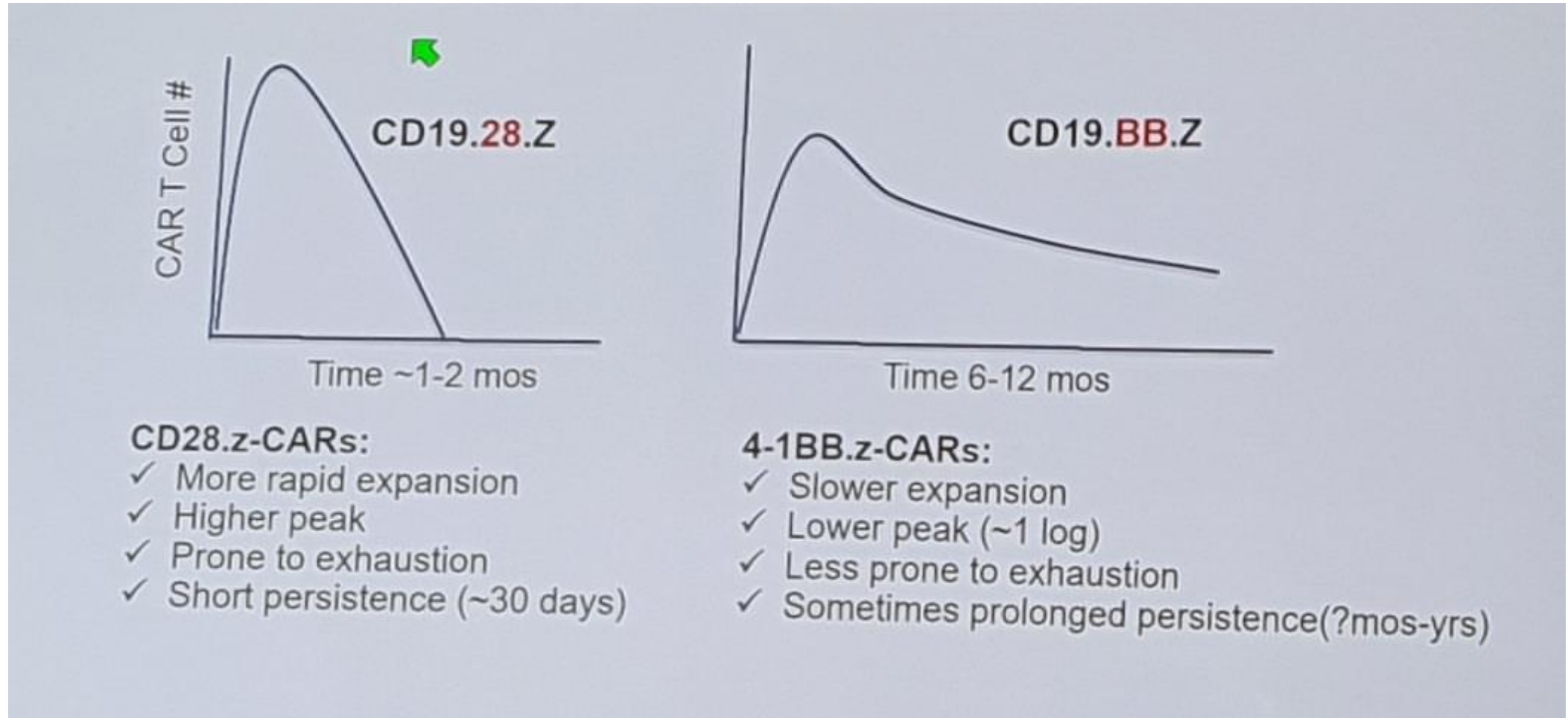
T cell **intrinsic** factors might be best addressed by **optimizing CAR design**, 'armor', and the cell manufacturing process

T cell **extrinsic** factors might be best addressed by combination therapies, or CAR-T cells secreting immuno-modulators

T cell intrinsic

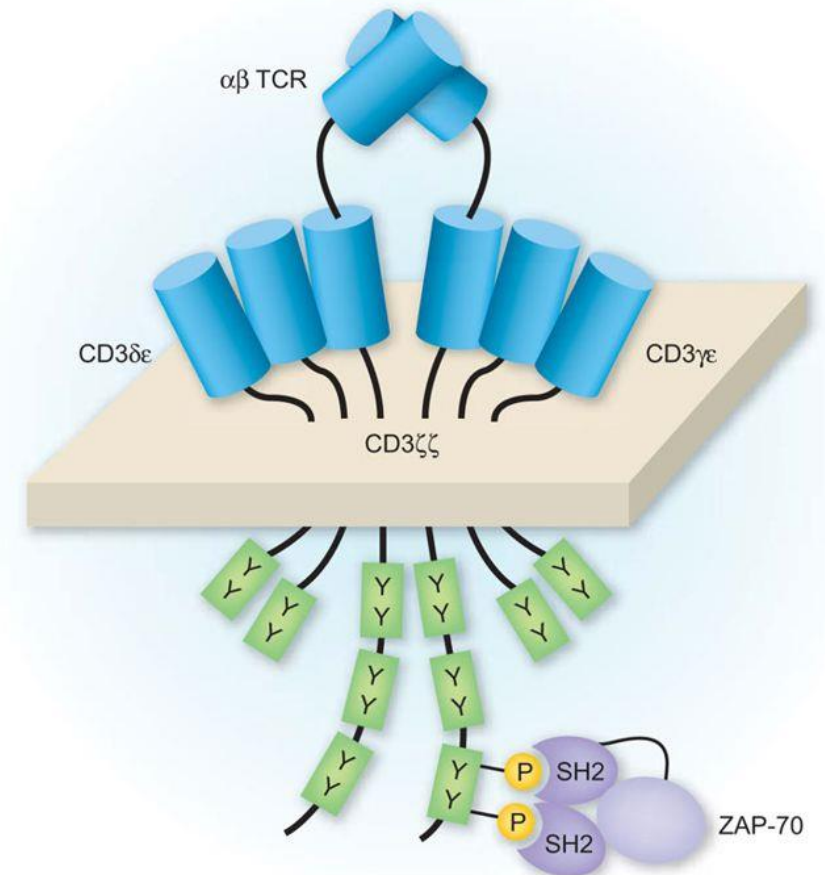
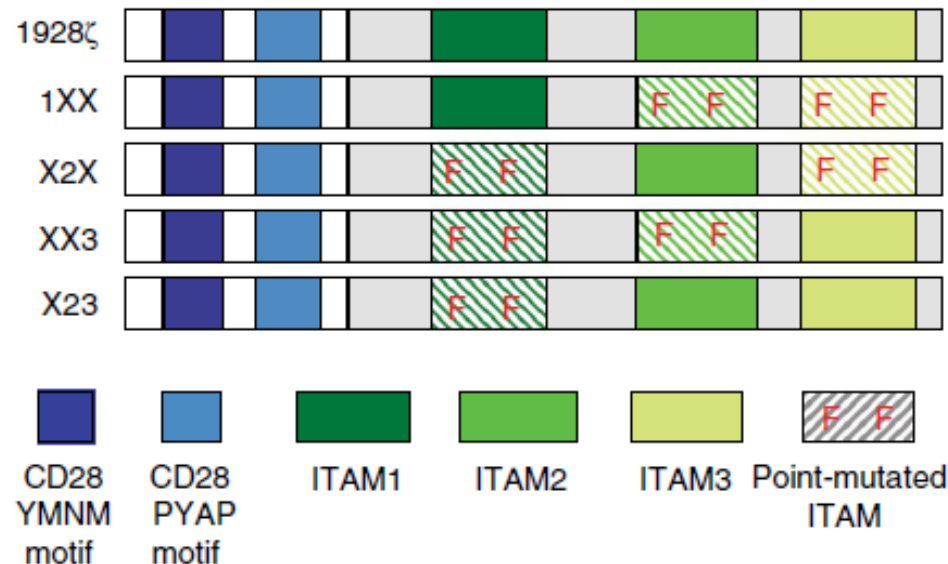


# Distinct CAR immunokinetics Driven by costimulatory domains

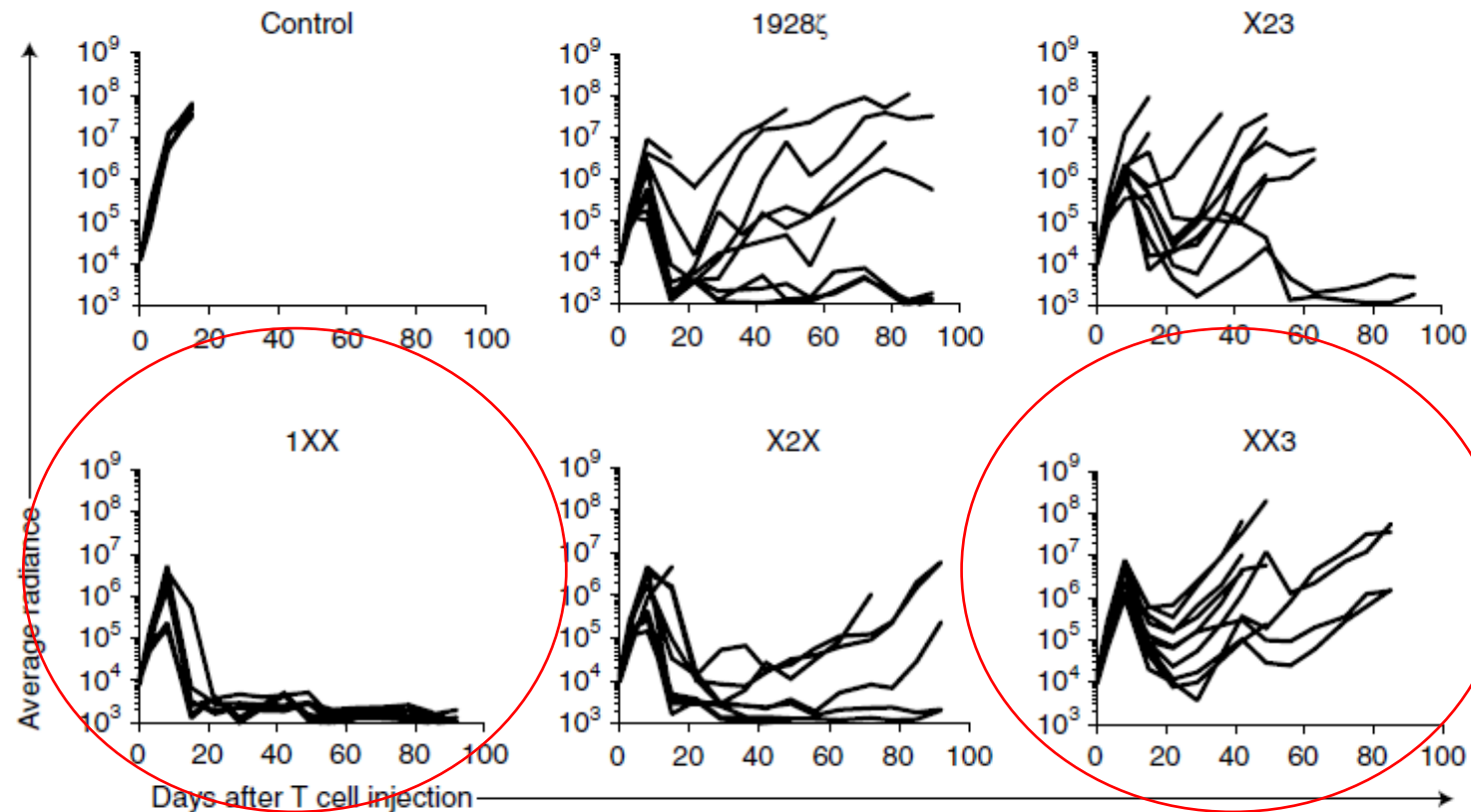


# Calibration of CAR activation potential directs alternative T cell fates and therapeutic potency

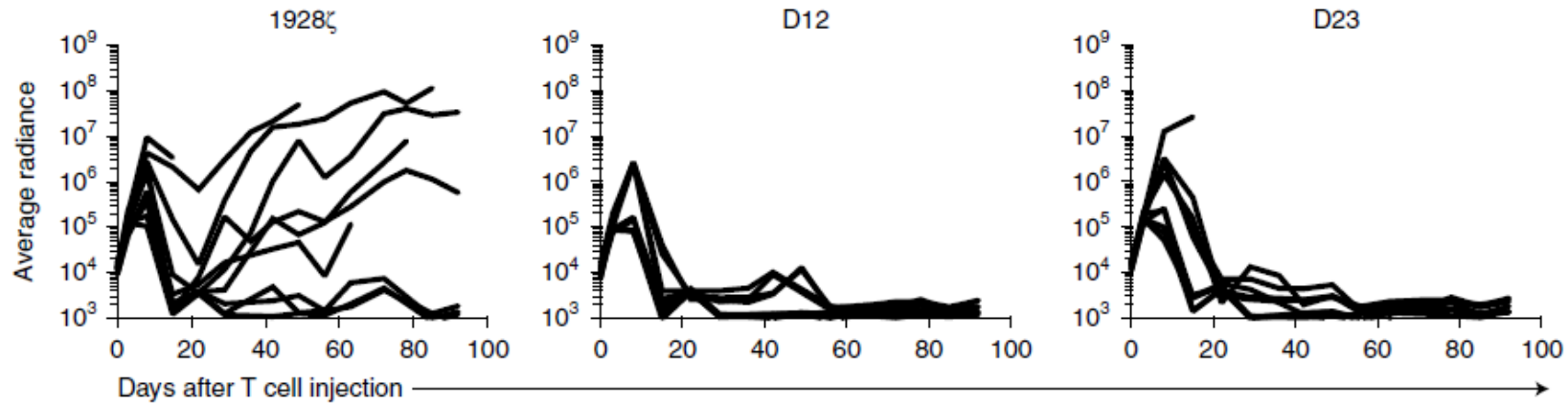
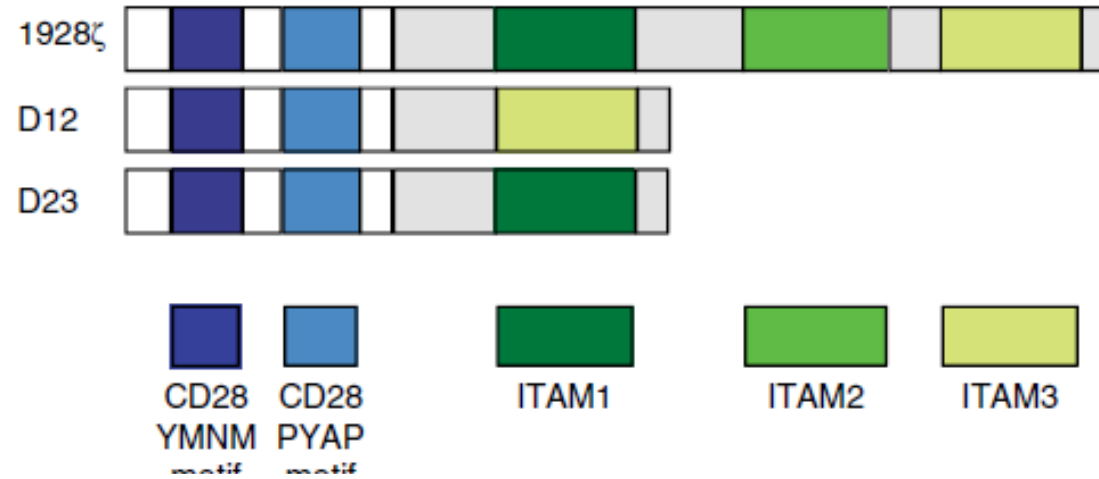
redundancy of CD28 and CD3 $\zeta$  signaling in a chimeric antigen receptor (CAR) design incorporating all three CD3 $\zeta$  immunoreceptor tyrosine-based activation motifs (ITAMs) fosters counterproductive T cell differentiation and exhaustion



## Calibration of CAR activation potential directs alternative T cell fates and therapeutic potency



Diminishes  
exhaustion and  
enhances  
potency



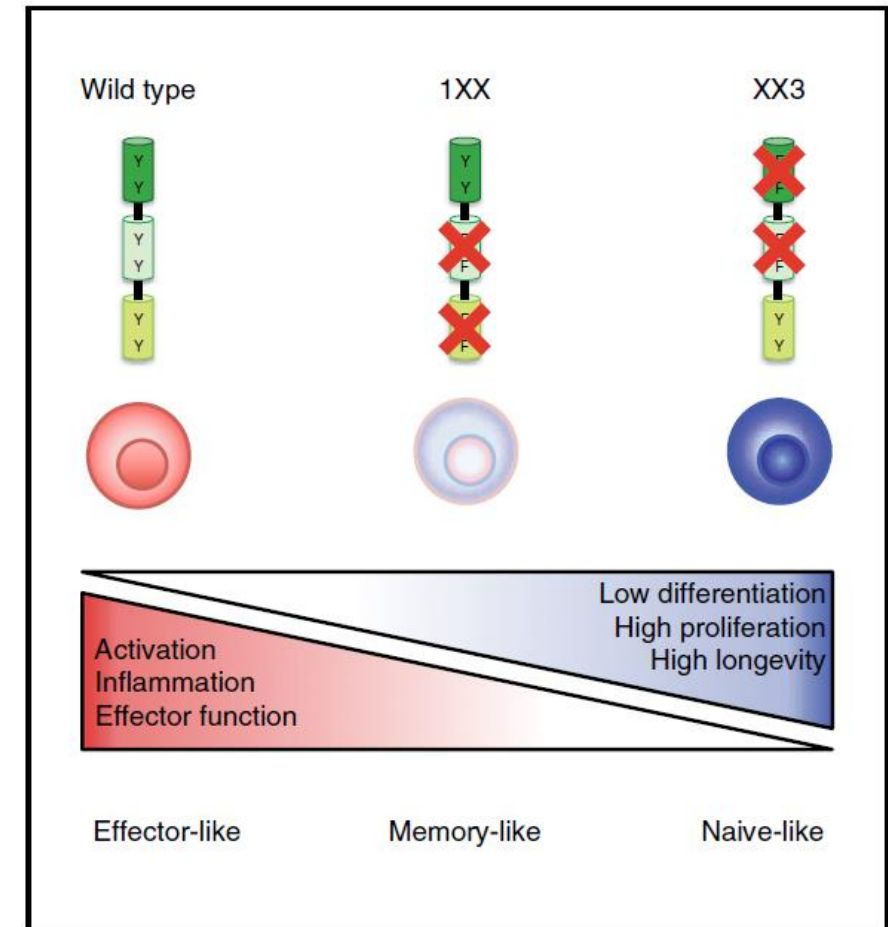
# Calibration of CAR potential through CD3z

1XX induced strong effector functions without shutting off memory programs

Further reducing T cell activation potential through XX3 resulted in highly persisting T cells but mediocre antitumor activity owing to insufficient elicitation of effector functions.

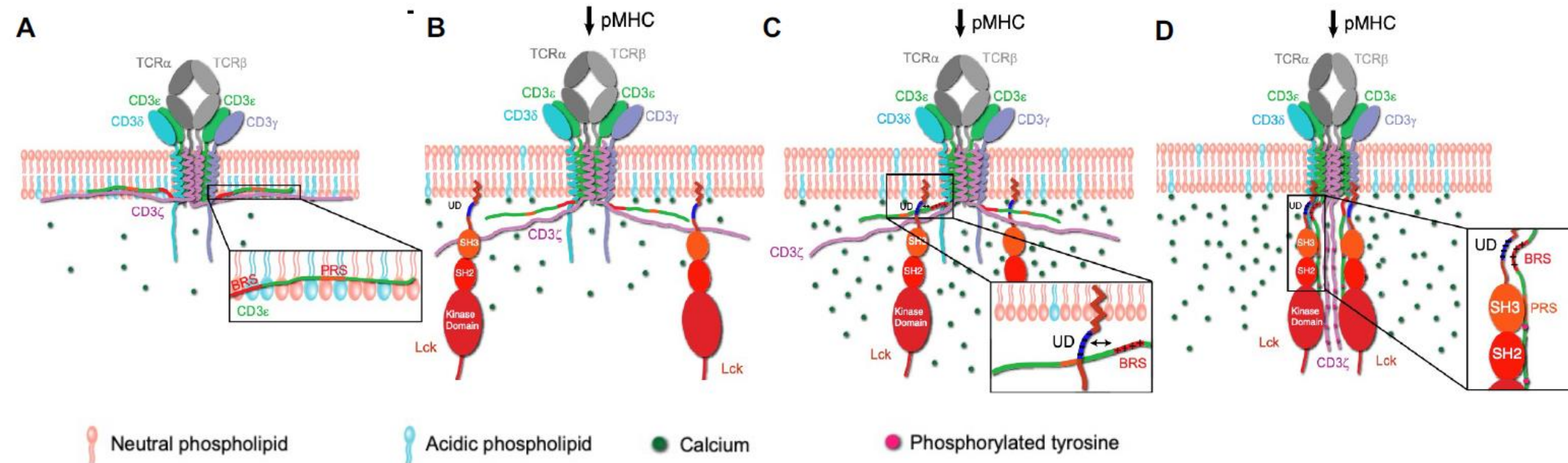
Modified ITAM configuration in 1XX favors persistence of highly functional CARs, balancing the replicative capacity of long-lived memory cells and the acquisition of effective antitumor function.

Preparations for clinical studies that utilize the 1XX CAR design are underway



# 'Signal 1' is Regulated By More Than Simply the ITAMs

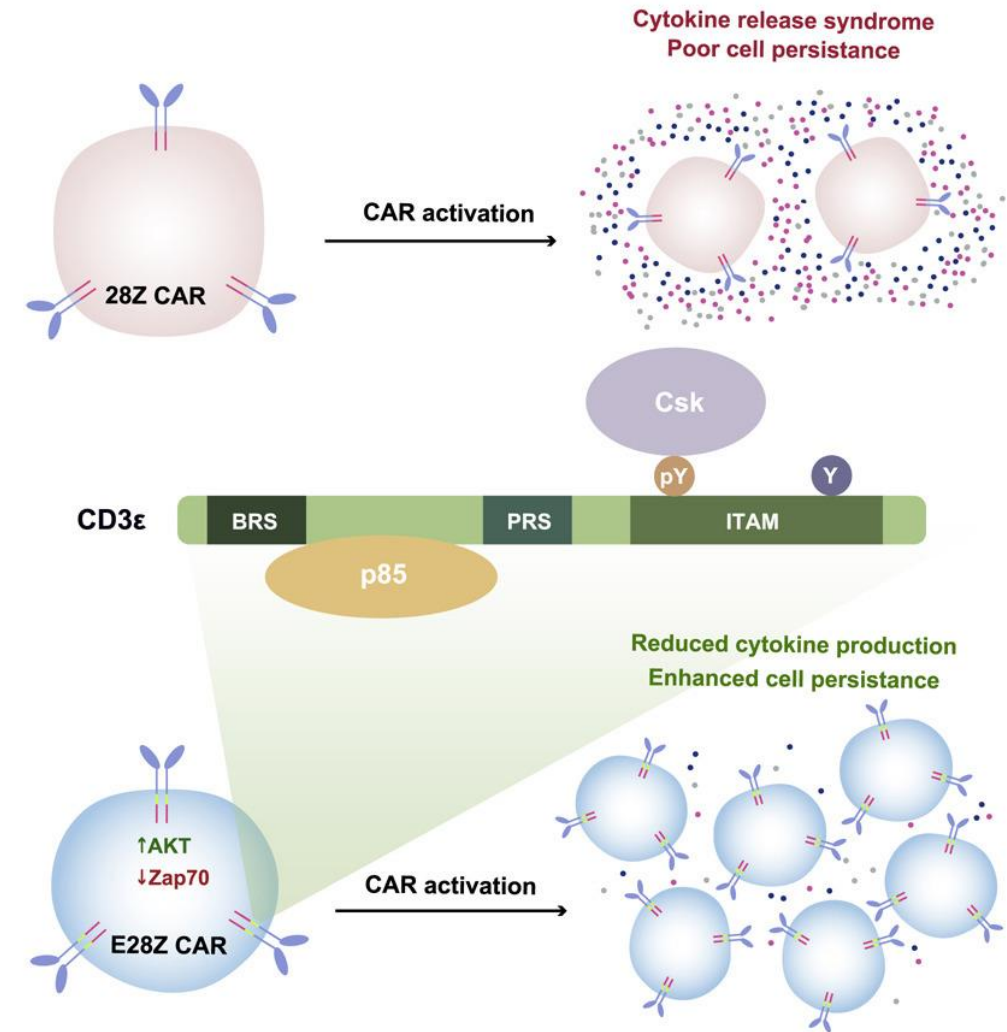
- Basic Residue rich Sequences (BRS) of CD3 affect association with the inner leaflet of the plasma membrane and regulating accessibility and Lck binding



Source: Li *et al.* (2017) *PNAS*, 114(29):E5891-E5899

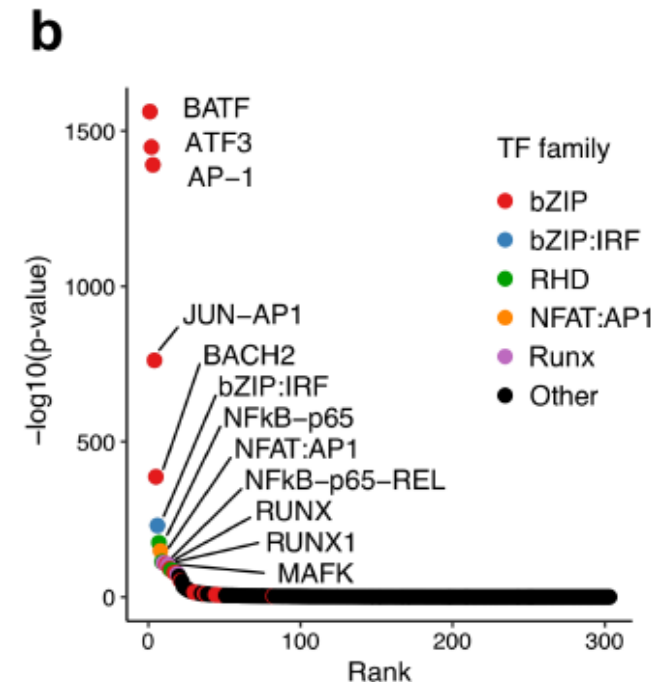
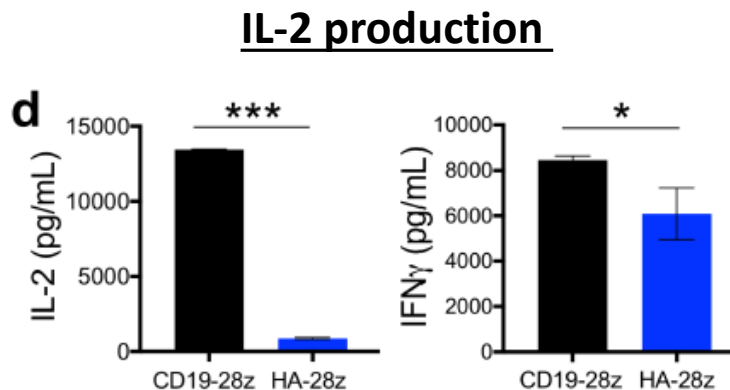
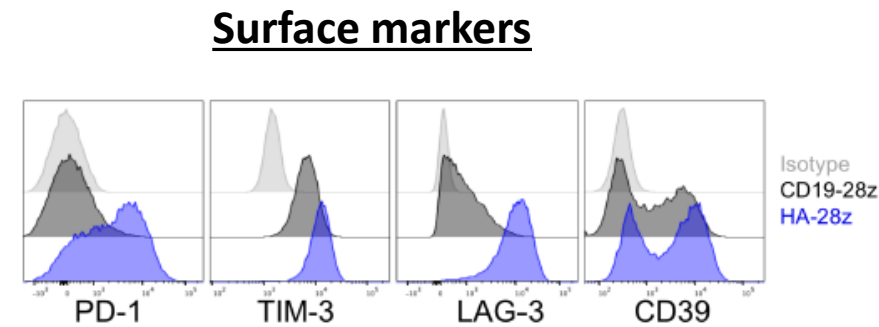
# CD3 $\epsilon$ improves persistence

- Incorporation of the **CD3 $\epsilon$  cytoplasmic domain** into a second-generation chimeric antigen receptor (CAR) improved antitumor activity of CAR-T cells.
- Mechanistically, the Csk-recruiting ITAM of CD3 $\epsilon$  reduced CAR-T cytokine production whereas **the basic residue rich sequence (BRS) of CD3 $\epsilon$  promoted CAR-T persistence via p85 recruitment**



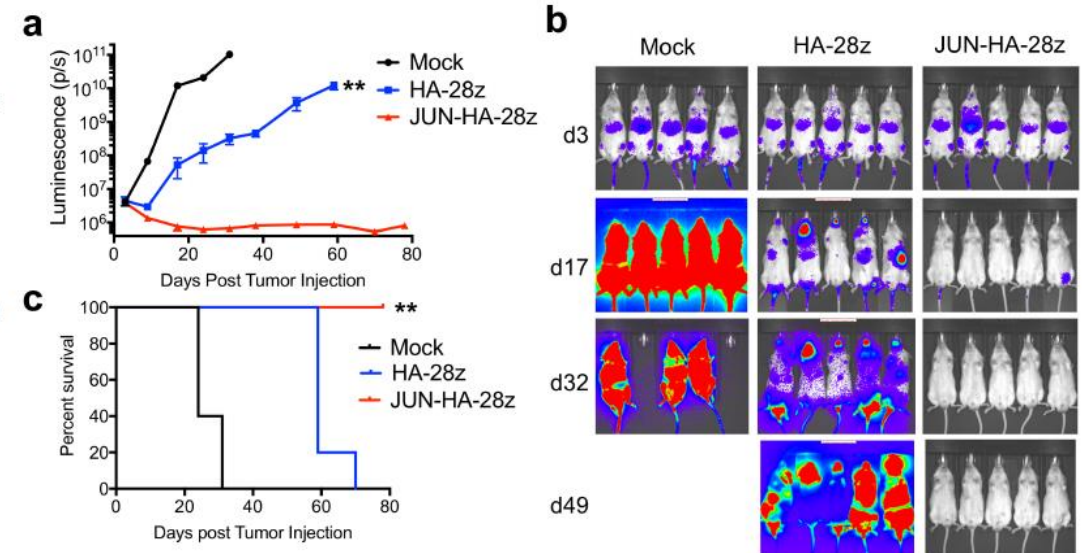
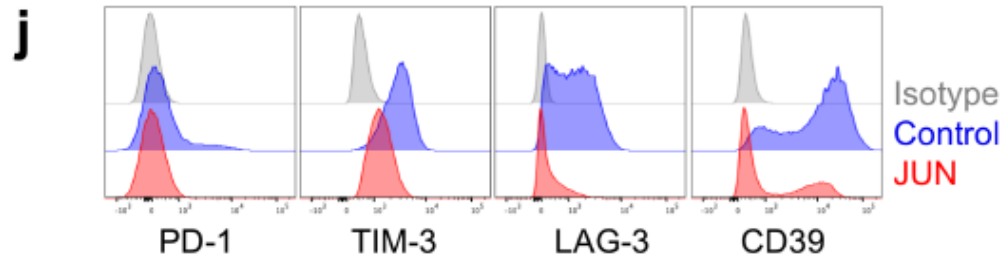
# Overcoming T cell exhaustion

- Exhaustion is associated with a profound defect in IL-2 production alongside increased chromatin accessibility of AP-1 (bZIP) transcription factor motifs.

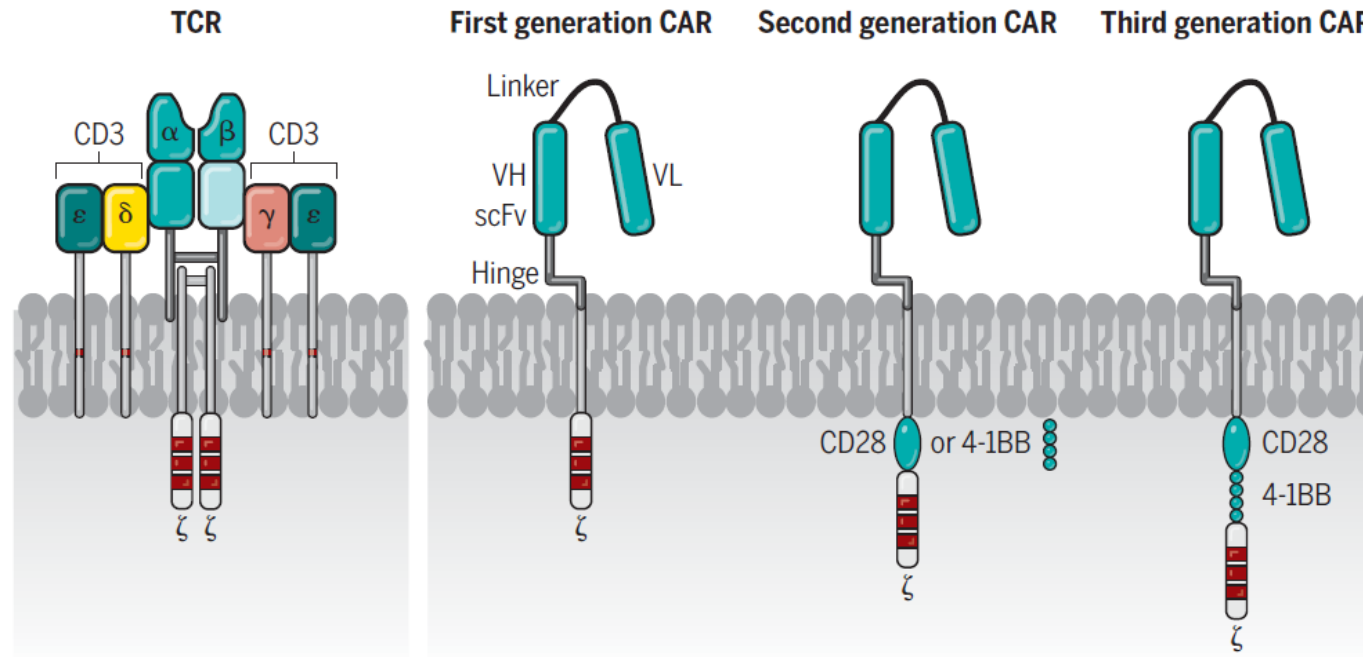


# Overcoming T cell exhaustion

- Engineering CAR T cells to overexpress c-Jun, a canonical AP-1 factor, enhanced expansion potential, increased functional capacity, diminished terminal differentiation and improved antitumor potency in numerous *in vivo* tumor models.



# Third Generation CARs



# Third-Generation CAR T Cells Targeting CD19 Are Associated with an Excellent Safety Profile and Might Improve Persistence of CAR T Cells in Treated Patients

Maria-Luisa Schubert, MD, Anita Schmitt, MD, Brigitte Neuber, Angela Hückelhoven-Krauss, Alexander Kunz, Lei Wang, MD, Ulrike Gern, Birgit Michels, Leopold Sellner, MD, Susanne Hofmann, MD, Carsten Müller-Tidow, MD, Peter Dreger, Michael Schmitt, MD

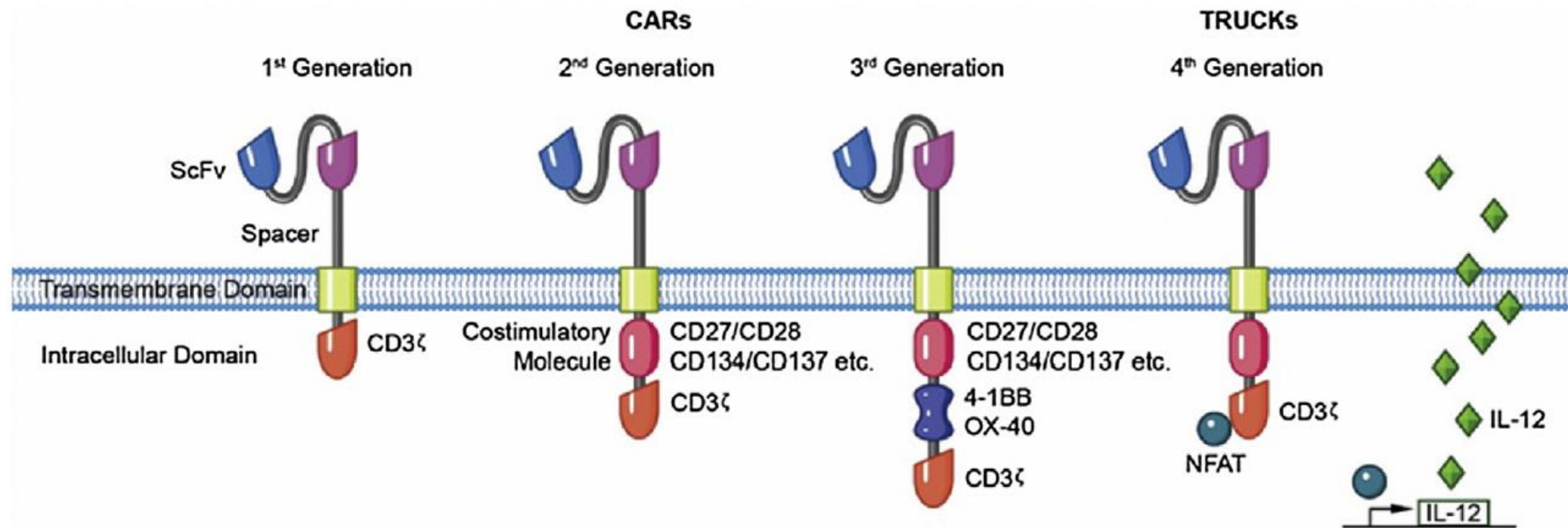
## Methods

Adult and pediatric patients with r/r ALL and patients with r/r chronic lymphocytic leukemia (CLL) or NHL including diffuse large B-cell lymphoma (DLBCL), follicular lymphoma (FL) or mantle cell lymphoma (MCL) are treated with autologous T lymphocytes transduced with a CD19 targeting third-generation CAR retroviral vector (RV-SFG.CD19.CD28.4-1BBzeta). The main purpose of HD-CAR-1 is to evaluate safety and feasibility of escalating third-generation CAR T cell doses ( $1\text{-}20 \times 10^6$  transduced cells/ $\text{m}^2$ ) after lymphodepletion with fludarabine ( $30 \text{ mg}/\text{m}^2/\text{d}$  on days -4 to -2) cyclophosphamide ( $500 \text{ mg}/\text{m}^2/\text{d}$  on days -4 to -2). Patients are monitored for cytokine release syndrome (CRS), immune effector cell-associated neurotoxicity syndrome (ICANS) and/or other toxicities. *In vivo* function, survival and anti-tumor efficacy of CAR T cells are assessed.

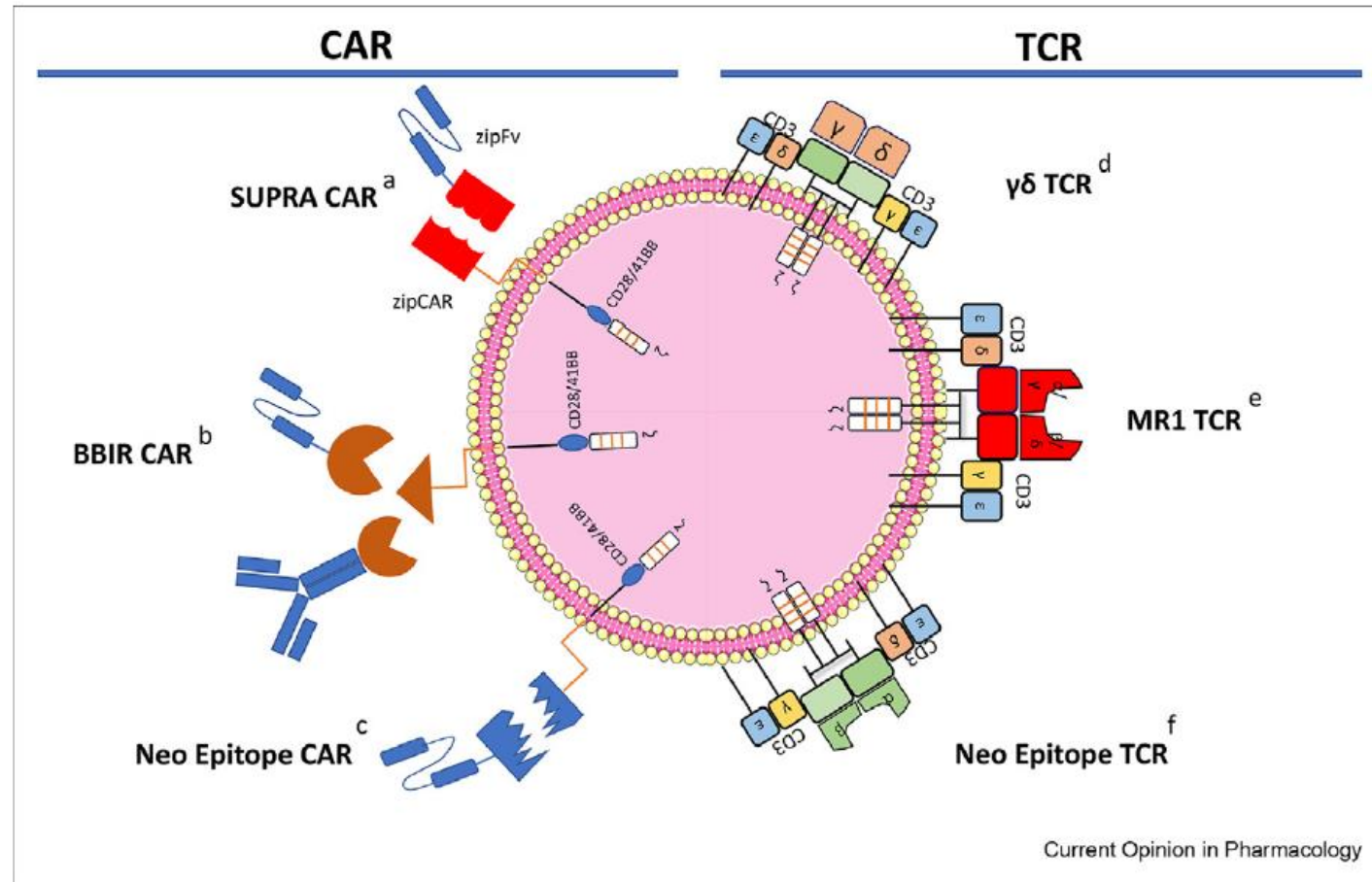
## Results

To date 10 patients (3 adult ALL, 2 CLL, 2 MCL, 2 DLBCL, 1 transformed FL) have been enrolled and subjected to leukapheresis. Transduction efficiency of T lymphocytes ranged between 33%-66% and high numbers of transduced CAR T cells were harvested ( $70\text{-}123 \times 10^6$  CAR T cells). No production failure occurred. CAR T cell products were sterile and free from mycoplasma and endotoxins. The copy number per CAR T cell did not exceed 7. Eight patients (2 adult ALL, 2 CLL, 1 MCL, 2 DLBCL, 1 transformed FL) have received the CAR T cell product (6 patients:  $10^6$  transduced cells/ $\text{m}^2$ ; 2 patients  $5 \times 10^6$  transduced cells/ $\text{m}^2$ ). No signs of CRS or ICANS > grade 2 have been observed. Only one patient required tocilizumab. No neurological side-effects occurred, even not in patients with involvement of the central nervous system (CNS). In quantitative real-time PCR, CAR T cells were detectable in the peripheral blood (PB) in 3 of 4 analyzed patients or the cerebrospinal fluid (CSF) of an ALL patient with CNS involvement. The CAR T cell frequency reached up to 200,000 copies/ $\mu\text{g}$  DNA, in some patients beyond end-of-study at day 90 after CAR T cell administration. Clinical responses to treatment were observed in 6/8 (75%) treated patients so far (2/8 patients have received CAR T cells recently and are not yet evaluable for response).

# T cell redirected for universal cytokine killing (TRUCK)



# Universal cells



T cell extrinsic

# Antigen modulation as resistance

Table 1 | Summary of antigen loss or modulation detected in published clinical trials of CAR T cell therapy

| Target antigen | Publication                 | Number of patients treated | Number of CRs (%) | Number of patients with antigen modulation (%) | Median time to antigen loss or modulation (months) | Comments                                                                                     |
|----------------|-----------------------------|----------------------------|-------------------|------------------------------------------------|----------------------------------------------------|----------------------------------------------------------------------------------------------|
| CD19           | Lee et al. <sup>7</sup>     | 21                         | 14 (67)           | 2 (14)                                         | ~6                                                 | 10 of 12 patients who were MRD-negative after CAR T cell therapy subsequently underwent HSCT |
|                | Maude et al. <sup>6</sup>   | 30                         | 27 (90)           | 4 (15)                                         | ~3                                                 | None                                                                                         |
|                | Gardner et al. <sup>8</sup> | 43                         | 40 (93)           | 7 (18)                                         | ~3                                                 | 11 of 40 subsequently underwent HSCT                                                         |
|                | Park et al. <sup>9</sup>    | 53                         | 44 (83)           | 4 (9)                                          | Unknown                                            | None                                                                                         |
|                | Maude et al. <sup>10</sup>  | 75                         | 61 (81)           | 15 (25)                                        | Unknown                                            | None                                                                                         |
| CD22           | Fry et al. <sup>15</sup>    | 21                         | 12 (57)           | 7 (58)                                         | ~3                                                 | None                                                                                         |

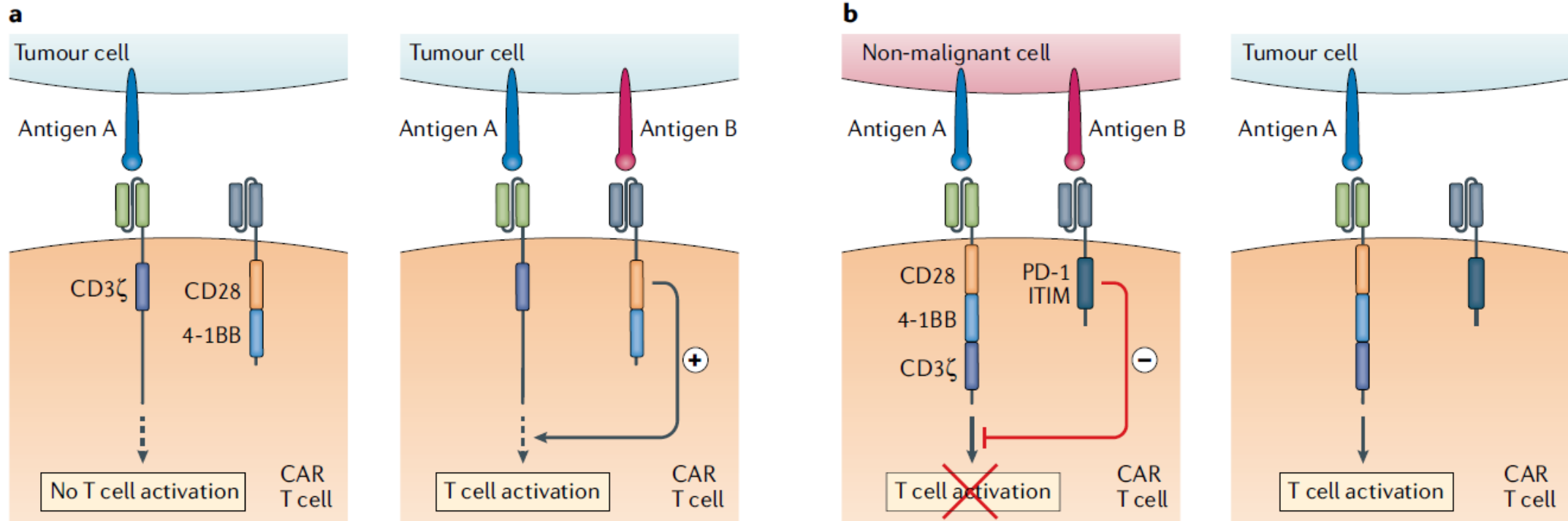
CAR, chimeric antigen receptor; CRs, complete responses; HSCT, haematopoietic stem cell transplantation; MRD, minimal residual disease.

# Targeting multiple antigens

Table 2 | Active clinical trials of multi-antigen CAR T cells in the USA and UK

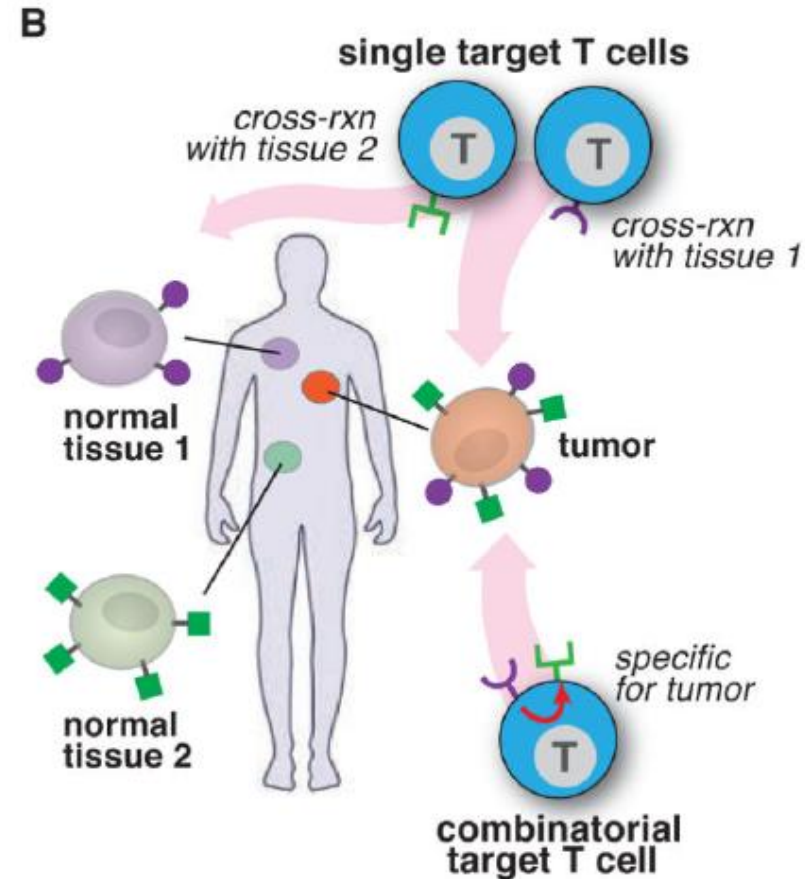
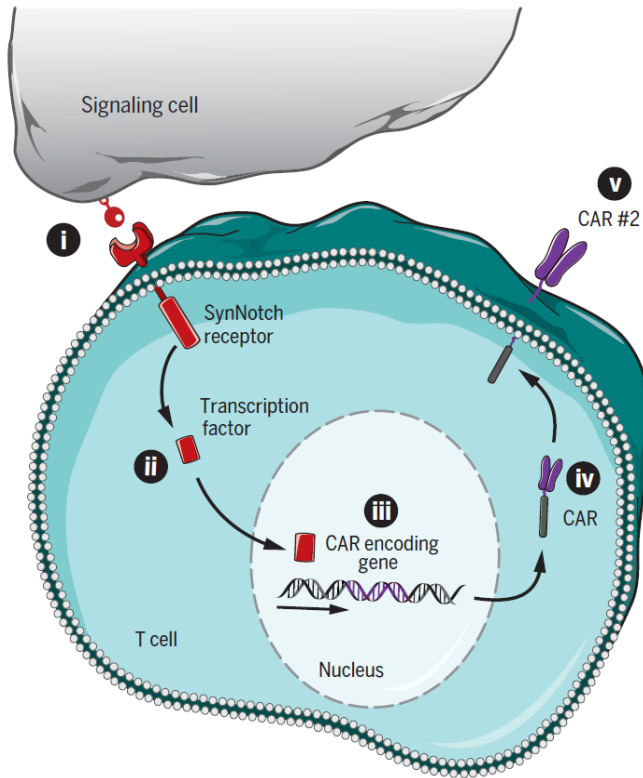
| Target antigens | Disease       | Age group (years) | CAR construct signalling domains                                                                                                            | Treatment centre                                                             | ClinicalTrials.gov reference number |
|-----------------|---------------|-------------------|---------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------|-------------------------------------|
| CD19 and CD22   | ALL and NHL   | 1–26              | CD3ζ–4-1BB (combinatorial approach with anti-CD19 CAR T cells, anti-CD22 CAR T cells and co-transduced anti-CD19 and anti-CD22 CAR T cells) | Seattle Children's Hospital (Seattle, WA, USA)                               | NCT03330691 (PLAT-05)               |
|                 | ALL           | 1–30              | CD3ζ–4-1BB                                                                                                                                  | Lucile Packard Children's Hospital, Stanford University (Palo Alto, CA, USA) | NCT03241940                         |
|                 | ALL and DLBCL | ≥18               | CD3ζ–4-1BB                                                                                                                                  | Stanford University (Palo Alto, CA, USA)                                     | NCT03233854                         |
|                 | ALL and NHL   | 3–30              | CD3ζ–4-1BB                                                                                                                                  | National Cancer Institute (Rockville, MD, USA)                               | NCT03448393                         |
|                 | ALL           | 1–24              | CD3ζ–OX40 (CD19) and CD3ζ–4-1BB (CD22)                                                                                                      | Great Ormond Street Hospital (London, UK)                                    | NCT03289455                         |
| CD19 and CD20   | NHL and CLL   | 18–70             | CD3ζ–4-1BB                                                                                                                                  | Medical College of Wisconsin (Milwaukee, WI, USA)                            | NCT03019055                         |

# Simultaneous Targeting of Two Antigens with CAR-T

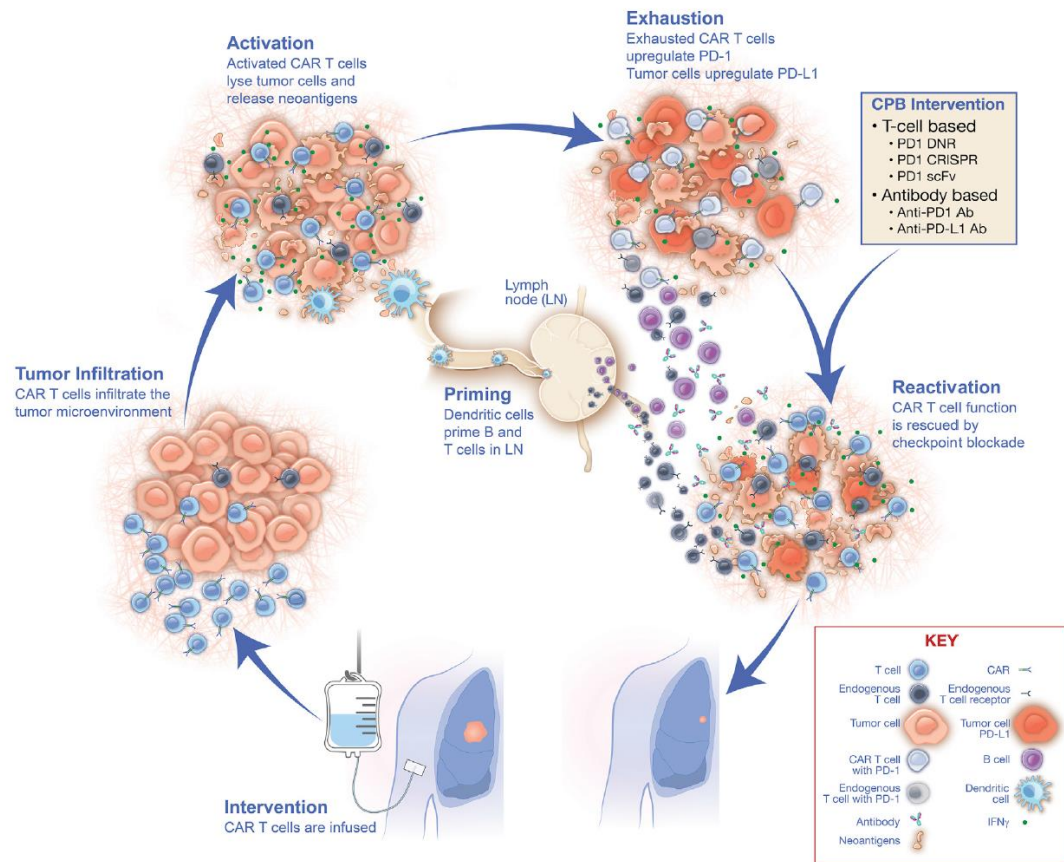


# Conditional CAR expression

conditional CAR expression specific to a second antigen in the presence of the first antigen



# Mechanisms of Rescue of CAR T cell Exhaustion with Checkpoint Blockade

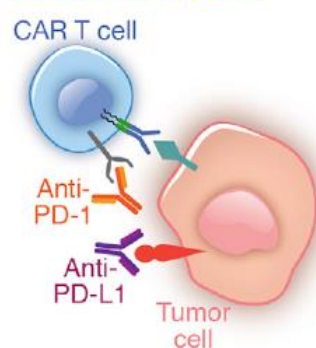


- In response to IFN-g, tumor cells upregulate programmed death-ligand 1 (PD-L1), which interacts with programmed cell death protein 1 (PD-1) on the surface of exhausted CAR T cells.
- CPB can lead to reactivation of the CAR T cell activity.

# Strategies to Combine CAR T Cells and Checkpoint Blockade

## Extrinsic Checkpoint Blockade

### i. Checkpoint blockade antibodies



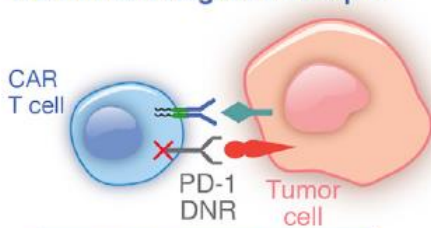
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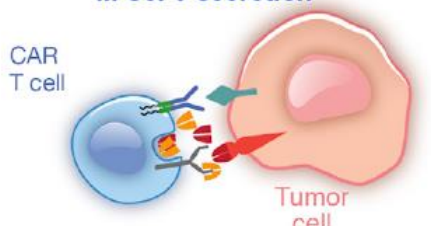
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## Intrinsic Checkpoint Blockade

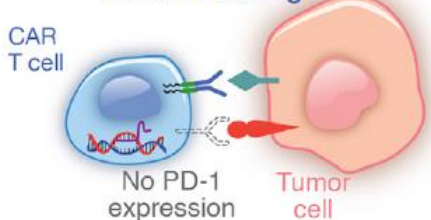
### i. Dominant Negative Receptor



### ii. ScFv secretion



### iii. Gene editing



## IV. Switch Receptor: PD1-CD28

Liu et al. Cancer Res March 15 201

| Characteristic              | PD-1 DNR CAR T Cells | CPB Agents with CAR T Cells    |
|-----------------------------|----------------------|--------------------------------|
| Mechanism                   | cell-intrinsic       | cell-extrinsic                 |
| Potential doses             | single               | multiple doses of CPB          |
| Targeted therapy            | yes                  | no                             |
| Potential toxicity          | localized to tumor   | systemic                       |
| Limits to tumor penetration | unlikely             | limits of antibody penetration |
| Antigen responsiveness      | multiple             | multiple                       |

# Clinical trials with combinations of CAR T and checkpoint blockade

| Trial       | Launch | Phase | Center(s)                                                                                                                     | CPB Agent     | CAR Target/Design                                                                                                                   | Cancer Diagnosis                                                                           |
|-------------|--------|-------|-------------------------------------------------------------------------------------------------------------------------------|---------------|-------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------|
| NCT00586391 | 2009   | I     | Baylor                                                                                                                        | ipilimumab    | CD19/CD19CAR-28-zeta T cells                                                                                                        | B cell lymphoma, chronic lymphocytic leukemia, acute lymphocytic leukemia                  |
| NCT01822652 | 2013   | I     | Baylor                                                                                                                        | pembrolizumab | GD2/iC9-GD2-CD28-OX40 (iC9-GD2) T cells                                                                                             | neuroblastoma                                                                              |
| NCT02650999 | 2016   | I/II  | University of Pennsylvania                                                                                                    | pembrolizumab | anti-CD19 CARs                                                                                                                      | CD19 <sup>+</sup> diffuse large B cell lymphoma, follicular lymphoma, mantle cell lymphoma |
| NCT02706405 | 2016   | I     | Fred Hutchinson                                                                                                               | durvalumab    | autologous anti-CD19CAR-4-1BB-CD3 $\zeta$ -EGFRt-expressing CD4 <sup>+</sup> /CD8 <sup>+</sup> central memory T lymphocytes JCAR014 | diffuse large B cell lymphoma                                                              |
| NCT02926833 | 2016   | I/II  | City of Hope, Stanford, Moffitt, Dana Farber, MD Anderson                                                                     | atezolizumab  | CD19/KTE-C19                                                                                                                        | diffuse large B cell lymphoma                                                              |
| NCT03310619 | 2017   | I/II  | City of Hope, Northwestern University, Massachusetts General, University of Nebraska, University of Pennsylvania, MD Anderson | durvalumab    | JCAR017                                                                                                                             | lymphoma, non-Hodgkin lymphoma, diffuse large B cell lymphoma, follicular lymphoma         |
| NCT03726515 | 2018   | I     | University of Pennsylvania                                                                                                    | pembrolizumab | CART-EGFRvIII T cells                                                                                                               | glioblastoma                                                                               |

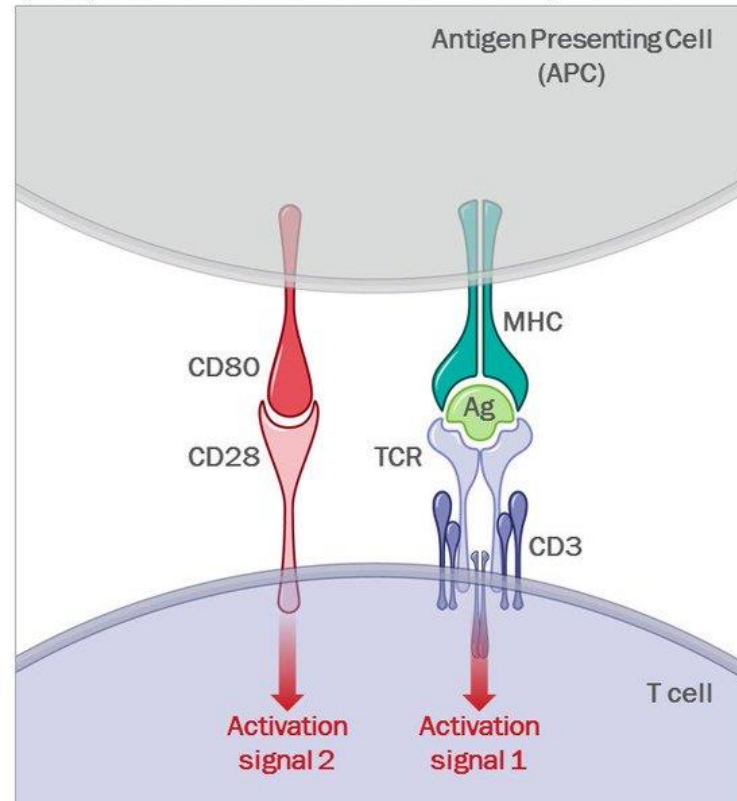
# Ongoing clinical trials with CART for solid tumors

| Ongoing clinical trials with CART for solid tumors. |                                                   |                     |                               |               |             |
|-----------------------------------------------------|---------------------------------------------------|---------------------|-------------------------------|---------------|-------------|
| Antigen                                             | Diagnosis                                         | Signaling domain    | Additional engineering        | Other therapy | NCT no.     |
| B7-H3                                               | Solid tumors                                      | —                   | iC9                           | N             | NCT04432649 |
| B7-H3                                               | CNS                                               | —                   | N                             | N             | NCT04185038 |
| B7-H3                                               | CNS                                               | —                   | N                             | TMZ           | NCT04385173 |
| B7-H3                                               | CNS                                               | —                   | N                             | TMZ           | NCT04077866 |
| CD171                                               | NB                                                | 41BBz or CD28.41BBz | N                             | N             | NCT02311621 |
| CD22                                                | Solid tumors                                      | —                   | Anti-PDL1 armored             | N             | NCT04556669 |
| CD276                                               | Solid tumors                                      | —                   | N                             | N             | NCT04691713 |
| Claudin 18.2                                        | Solid tumors                                      | —                   | N                             | N             | NCT03874897 |
| EGFR                                                | SCCHN                                             | CD28z               | IL-4/IL-2 CCR                 | N             | NCT01818323 |
| EGFR                                                | Solid tumors                                      | —                   | PD-1/CTLA4 expressing         | N             | NCT03182816 |
| EGFR806                                             | Solid tumors                                      | 41BBz               | CD19-CAR                      | N             | NCT03618381 |
| EGFR806                                             | CNS                                               | 41BBz               | N                             | N             | NCT03618381 |
| ERBB2                                               | CNS                                               | CD28z               | N                             | N             | NCT02442297 |
| ERBB2                                               | CNS                                               | 41BBz               | N                             | N             | NCT03500991 |
| ERBB2                                               | Solid tumors                                      | —                   | iRC9 safety switch            | rimiducid     | NCT04650451 |
| GD2                                                 | NB                                                | —                   | iC9, IL15                     | N             | NCT03721068 |
| GD2                                                 | Solid tumors                                      | —                   | iC9                           | N             | NCT03373097 |
| GD2                                                 | Solid tumors                                      | —                   | C7R                           | N             | NCT03635632 |
| GD2                                                 | DIPG, HGG                                         | —                   | C7R                           | N             | NCT04099797 |
| GD2                                                 | NB                                                | —                   | IL-15                         | N             | NCT03294954 |
| GD2                                                 | NB                                                | —                   | N                             | N             | NCT02761915 |
| GD2                                                 | DIPG                                              | 41BBz               | iC9                           | N             | NCT04196413 |
| GP3C                                                | HCC                                               | —                   | N                             | N             | NCT04121273 |
| GPC3                                                | HCC                                               | —                   | TGF- $\beta$ -CAR, IL-7-CCL19 | N             | NCT03198546 |
| GPC3                                                | Solid tumors                                      | 41BBz               | N                             | N             | NCT02932956 |
| GPC3                                                | HCC                                               | 41BBz               | N                             | N             | NCT02905188 |
| GPC3                                                | HCC                                               | —                   | N                             | N             | NCT03884751 |
| HER2                                                | CNS                                               | 41BBz               | N                             | N             | NCT03696030 |
| IL-13Ra2                                            | Melanoma                                          | 41BBz               | N                             | IL-2          | NCT04119024 |
| IL-13Ra2                                            | HGG                                               | 41BBz               | N                             | nivo/ipi      | NCT04003649 |
| IL13R $\alpha$ 2                                    | HGG                                               | 41BBz               | N                             | N             | NCT02208362 |
| Mesothelin                                          | Solid tumors                                      | —                   | PD-1 KO                       | N             | NCT03747965 |
| MSLN                                                | Solid tumors                                      | —                   | $\alpha$ PD1                  | N             | NCT04503980 |
| MSLN                                                | Solid tumors                                      | —                   | N                             | N             | NCT03054298 |
| MUC1                                                | NSCLC                                             | —                   | PD-1 KO                       | IL-2          | NCT03525782 |
| MUC1                                                | Solid tumors                                      | —                   | PD-1/CTLA4 expressing         | N             | NCT03179007 |
| MUC1                                                | Breast                                            | 41BBz               | N                             | N             | NCT04020575 |
| MUC-1                                               | Esophageal                                        | —                   | PD-1 KO                       | IL-2          | NCT03706326 |
| MUC16                                               | Solid tumors                                      | 41BBz               | IL-12                         | N             | NCT02498912 |
| Nectin4/FAP                                         | Solid tumors                                      | —                   | IL7 and CCL19, or IL12        | N             | NCT03932565 |
| PSCA                                                | Prostate                                          | 41BBz               | N                             | N             | NCT03873805 |
| PSCA                                                | Solid tumors                                      | Z                   | iMC                           | N             | NCT02744287 |
| PSMA                                                | Solid tumors                                      | —                   | N                             | N             | NCT04429451 |
| PSMA                                                | Solid tumors                                      | —                   | iC9                           | N             | NCT04249947 |
| PSMA                                                | Prostate                                          | —                   | DNR TGF- $\beta$              | N             | NCT03089203 |
| PSMA                                                | Prostate                                          | —                   | DNR TGF- $\beta$              | N             | NCT04227275 |
| PSMA                                                | Renal, bladder, prostate, lung breast, colorectal | CD28                | UniCAR                        | N             | NCT04633148 |
| ROBO1                                               | Solid tumors                                      | —                   | N                             | N             | NCT03940820 |
| TM4SF1- and EpCAM                                   | Solid tumors                                      | —                   | N                             | N             | NCT04151186 |

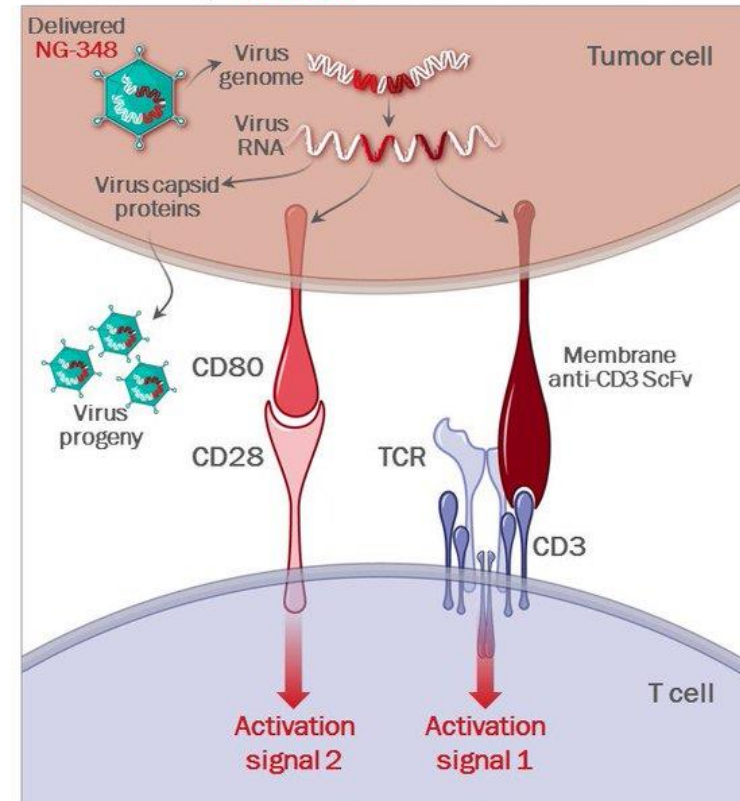
# Oncolytic Adenoviruses

## NG-348 T-cell Activation Mechanism

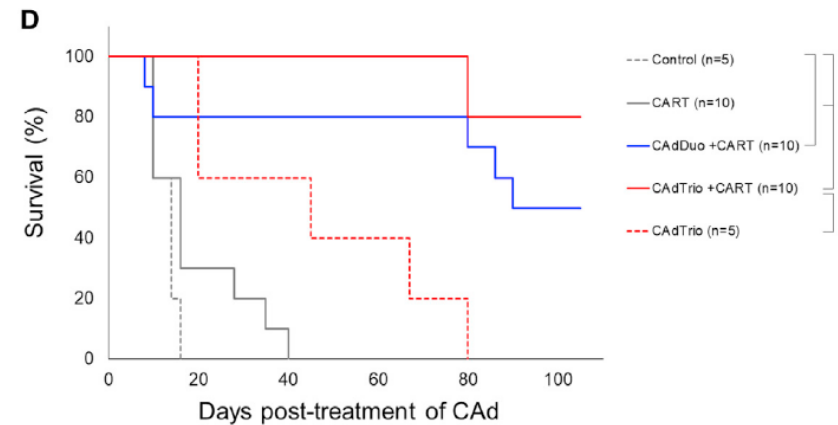
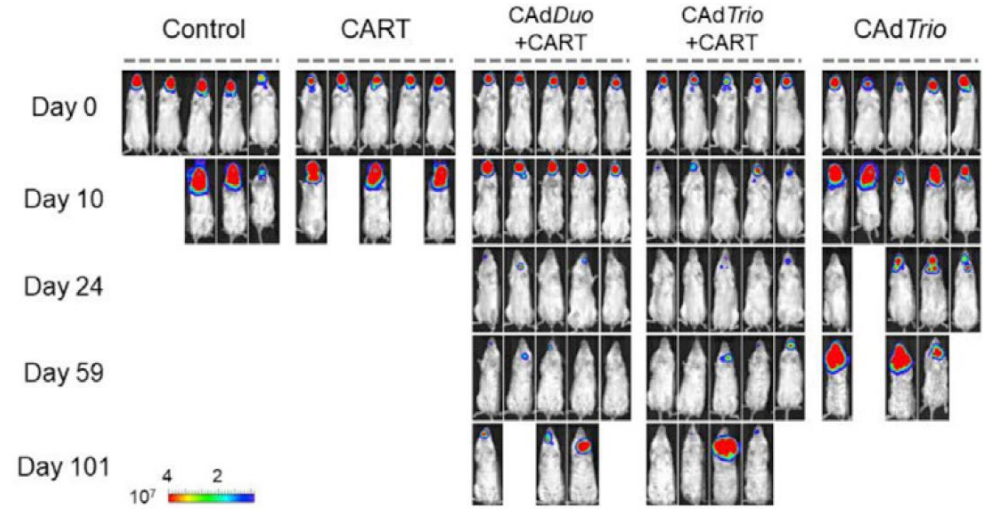
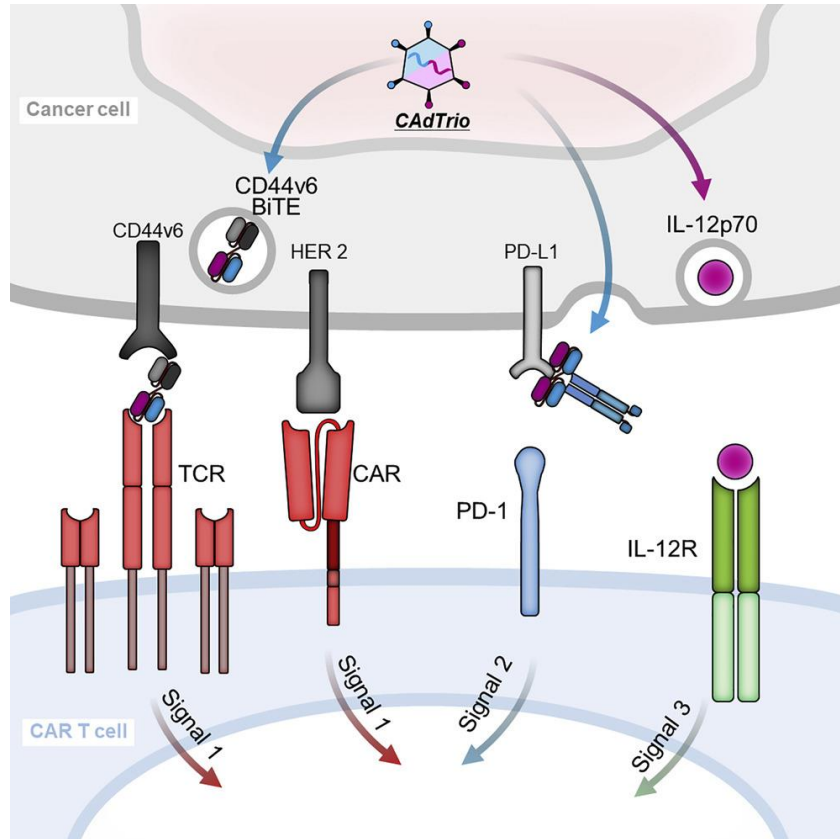
Antigen-dependent T cell receptor (TCR)-mediated T-cell activation by an APC



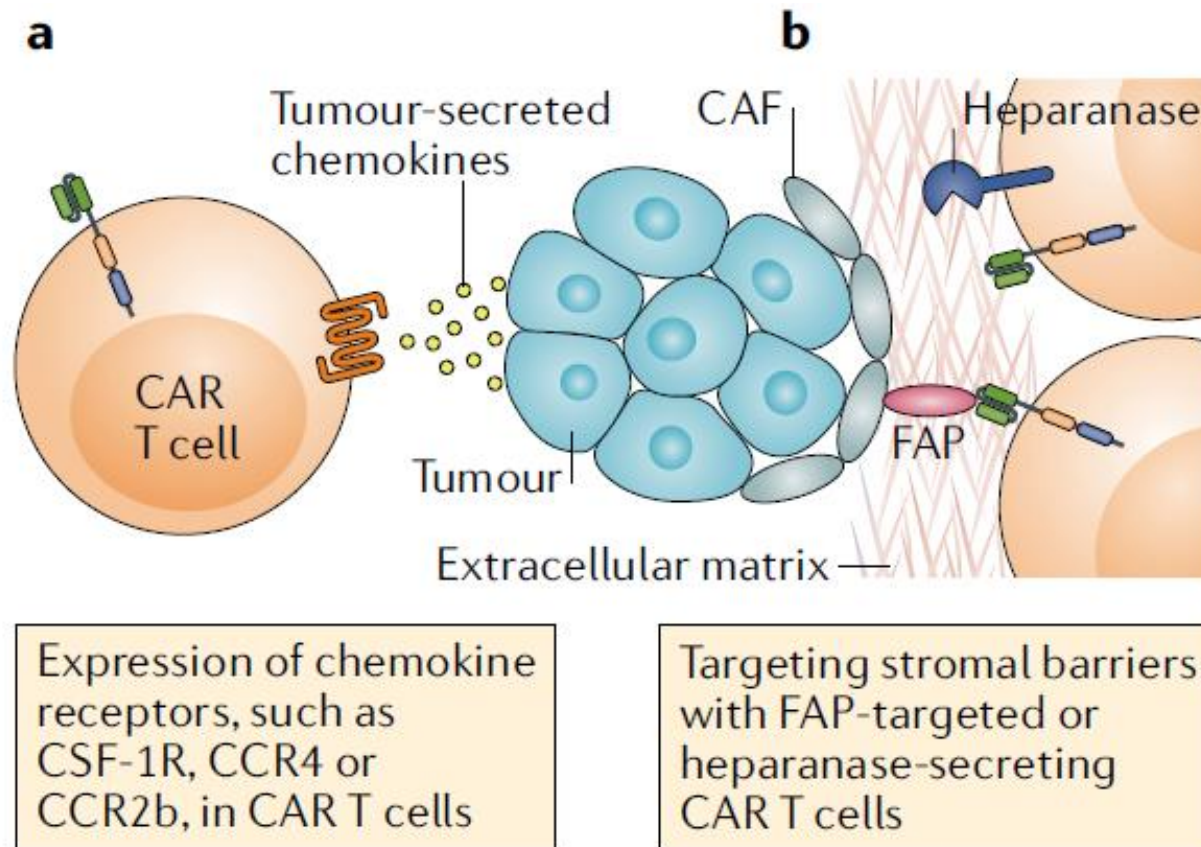
Antigen-independent TCR-mediated T-cell activation by NG-348 infected tumor cells



# Improving CART with Oncolytic Virus



# Increasing tumor homing and penetration



# Conclusion

- Optimizing CAR design is critical to improve CAR T cell intrinsic and extrinsic factors:
  - CAR T potency and persistence
    - ITAMS
    - Basic Residue Rich Sequences
    - Third and Fourth generation CARTs.
  - Exhaustion:
    - Design: c-Jun, Switch molecules
    - Combinatorial treatments
      - Antibodies: PD1, LAG3, TIM3
      - Oncolytic virus

# Thank you



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