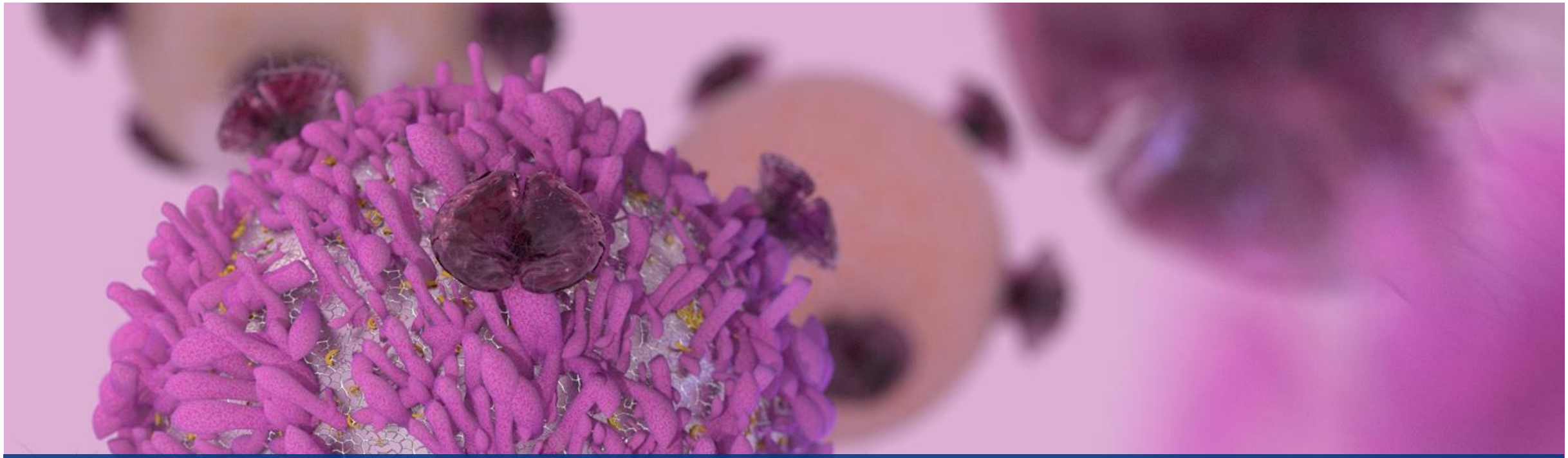


Approaches to Potency Testing for Chimeric Antigen Receptor T Cells

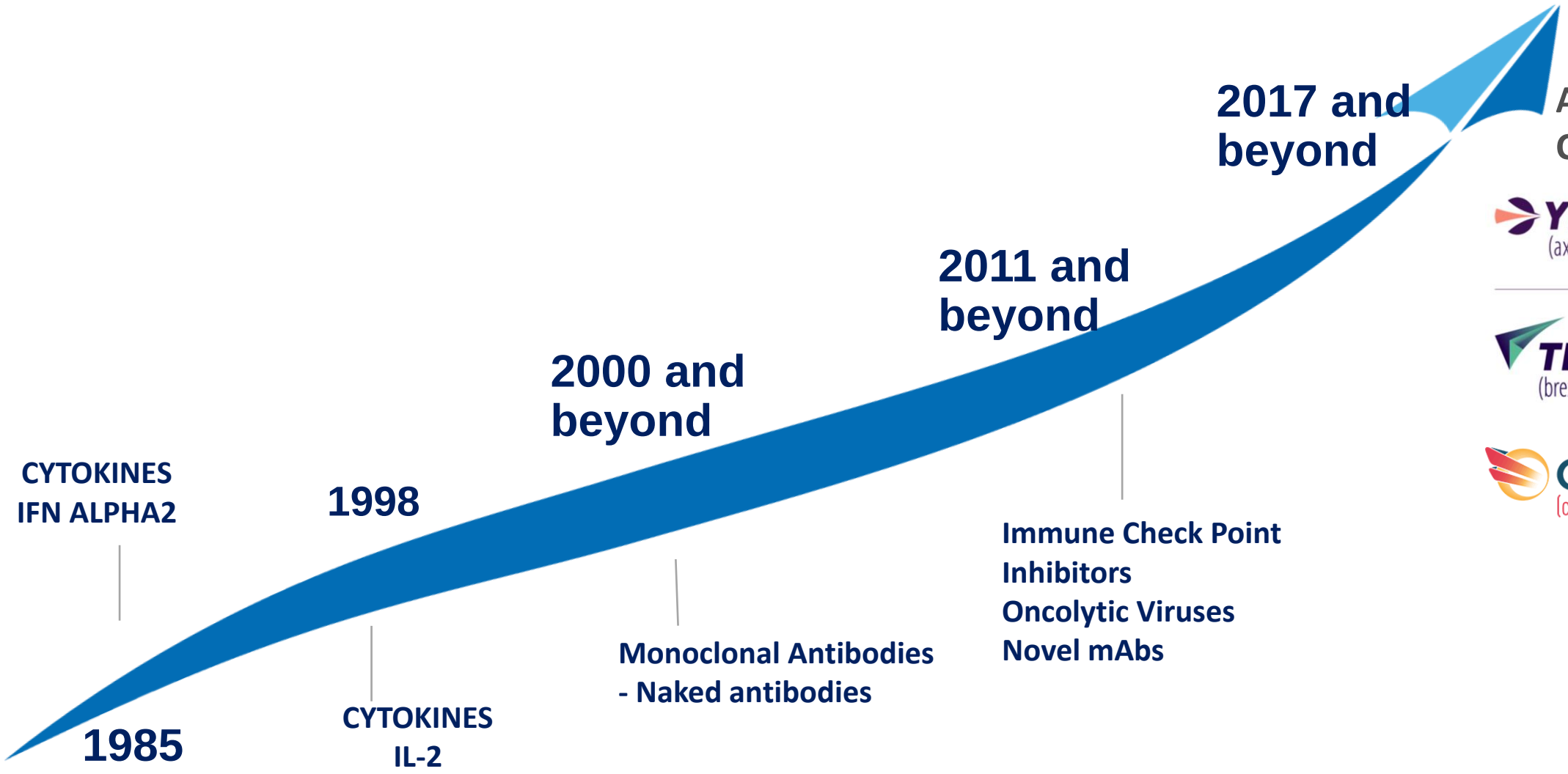
T cell lymphocyte with receptors to kill cancer cell in cancer immunotherapy 3D render

Shree Joshi, Ph.D.
Cell Gene Therapy and Vaccines BioTD Analytical Development

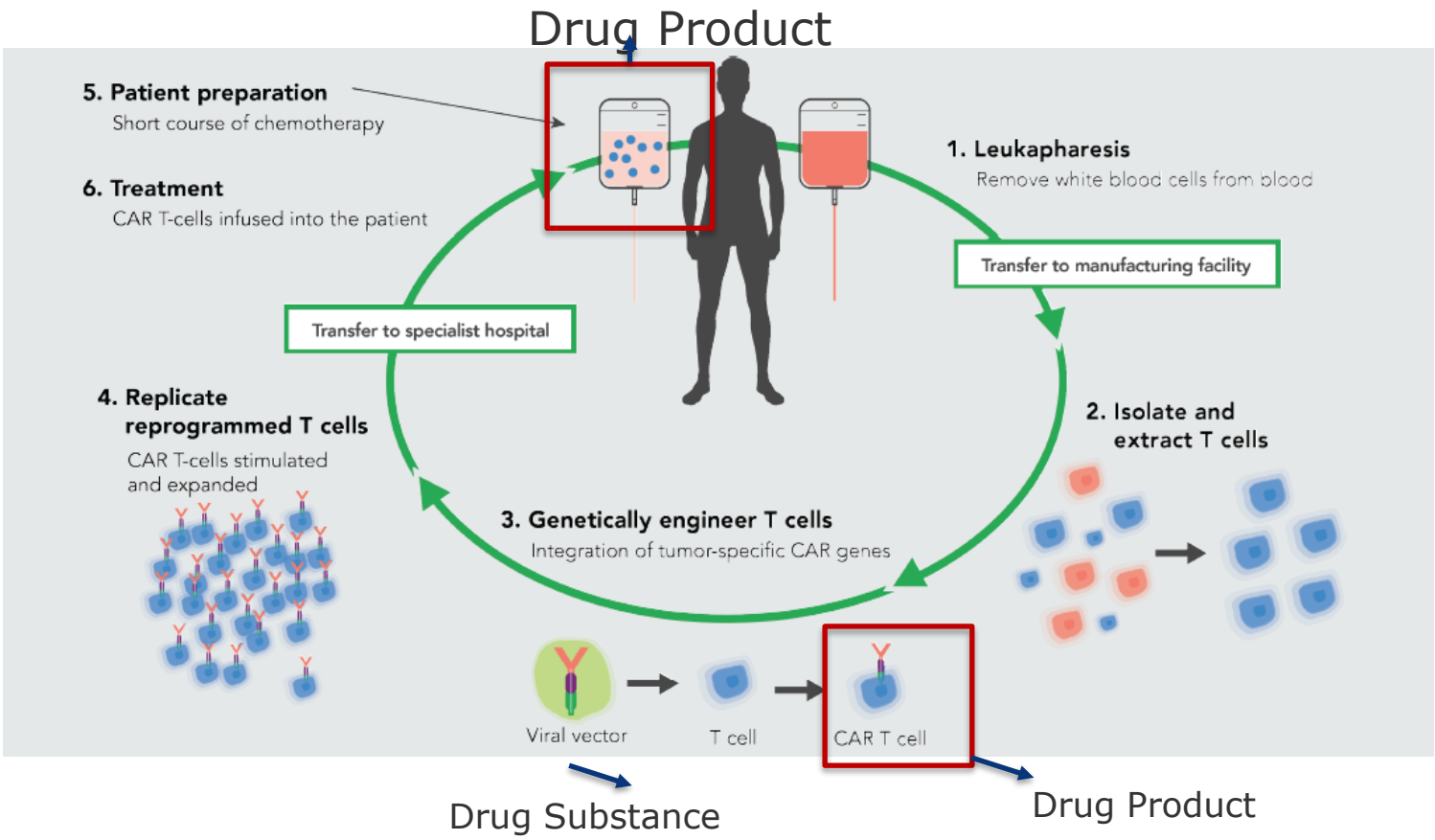
Topics

1. Engineered T Cell Therapy Overview
2. Autologous BCMA CAR-T (CARVYKTI) Manufacturing & function background
3. Evaluation of CAR-T drug product potency assays
4. Conclusion/Next Steps

A Cancer Immunotherapy Journey:
To Engineered T-Cell Therapy



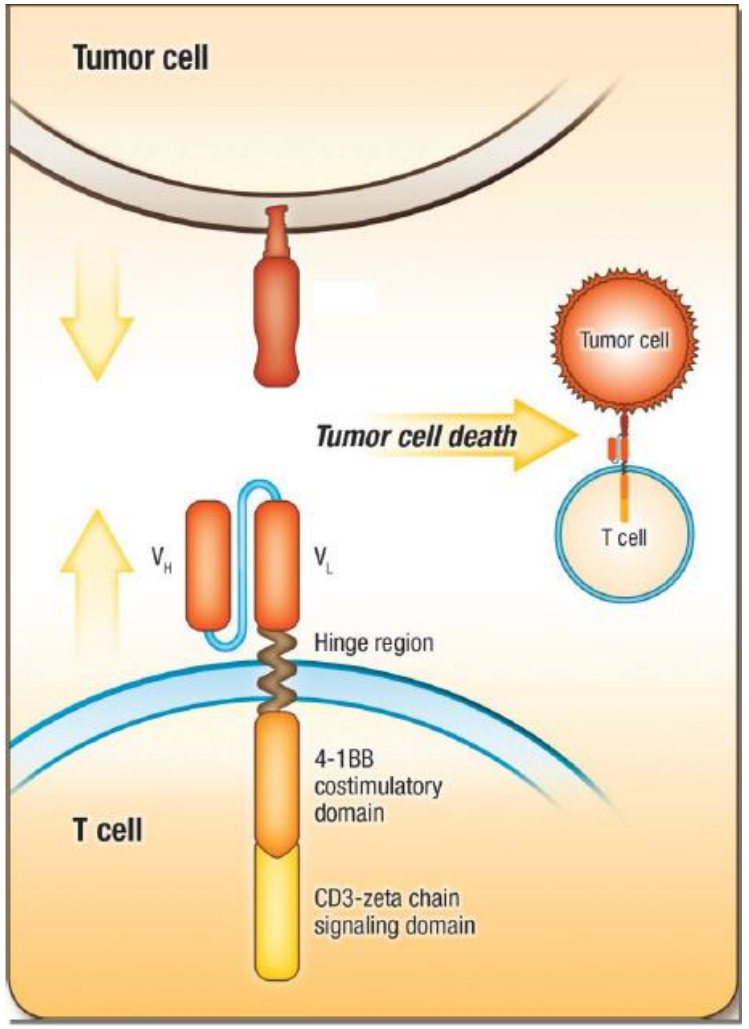
Overview CAR-T Autologous Manufacturing



Harnessing the Power of Patients' Own T-Cells

CARVYKTI is a living drug designed to target BCMA+ Myeloma cells

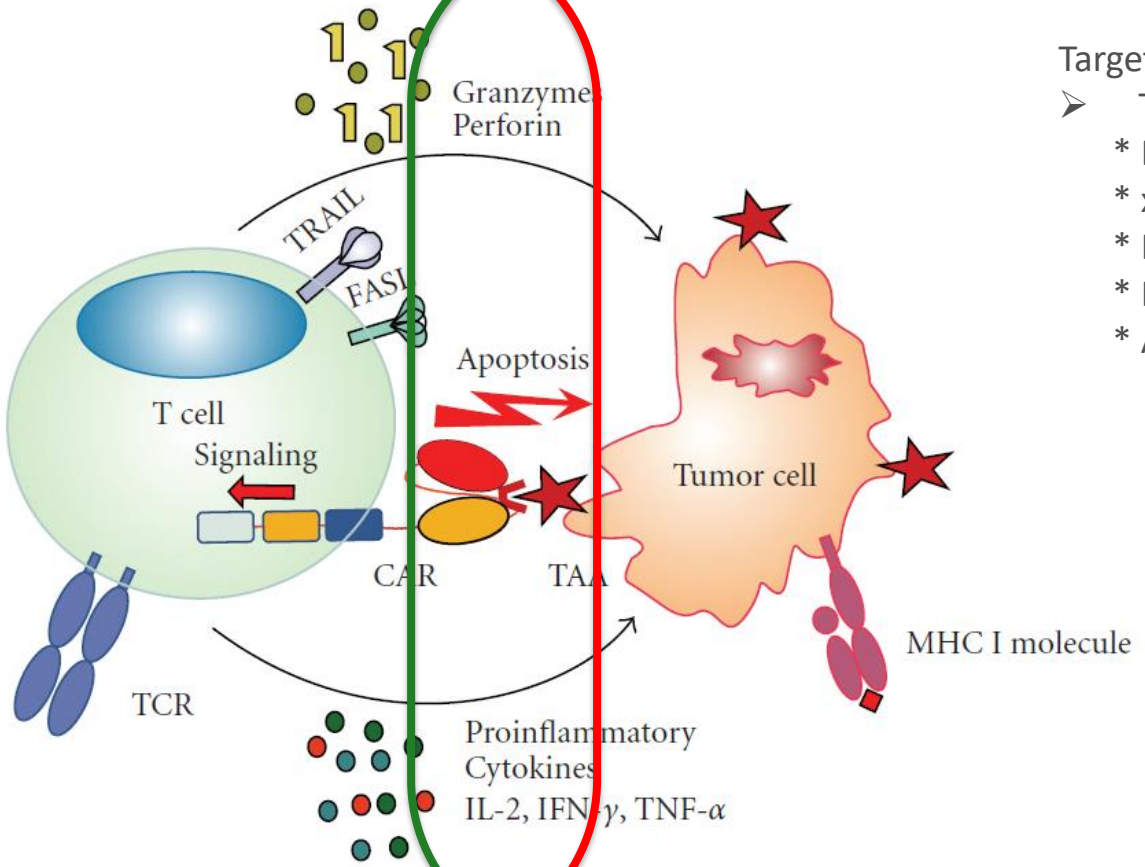
- Anti-BCMA antibody fragments can bind to BCMA antigen on myeloma cells, leading to:
  - CAR-T cell activation and proliferation
  - CAR-T cells directly kill myeloma cells
  - Activation of other immune cells



CAR-T Cell Mode Of Action Assessments

Effector T cell side:

- Cell Proliferation: Flow cytometry based or other platforms.
- Production of cytotoxic molecules: By flow cytometry or by MSD or ELISA.
  * Perforin
  * Granzyme B
  * IFNγ, IL-2 and TNFα
- T cell degranulation: By flow cytometry using CD107a staining
- T cell activation markers: CD69, CD25 and more.



Target cell side:

- Tumor killing assays:
  * Flow cytometry based.
  * xCelligence.
  * Incucyte
  * Reporter cell lines
  * Apoptosis assays.

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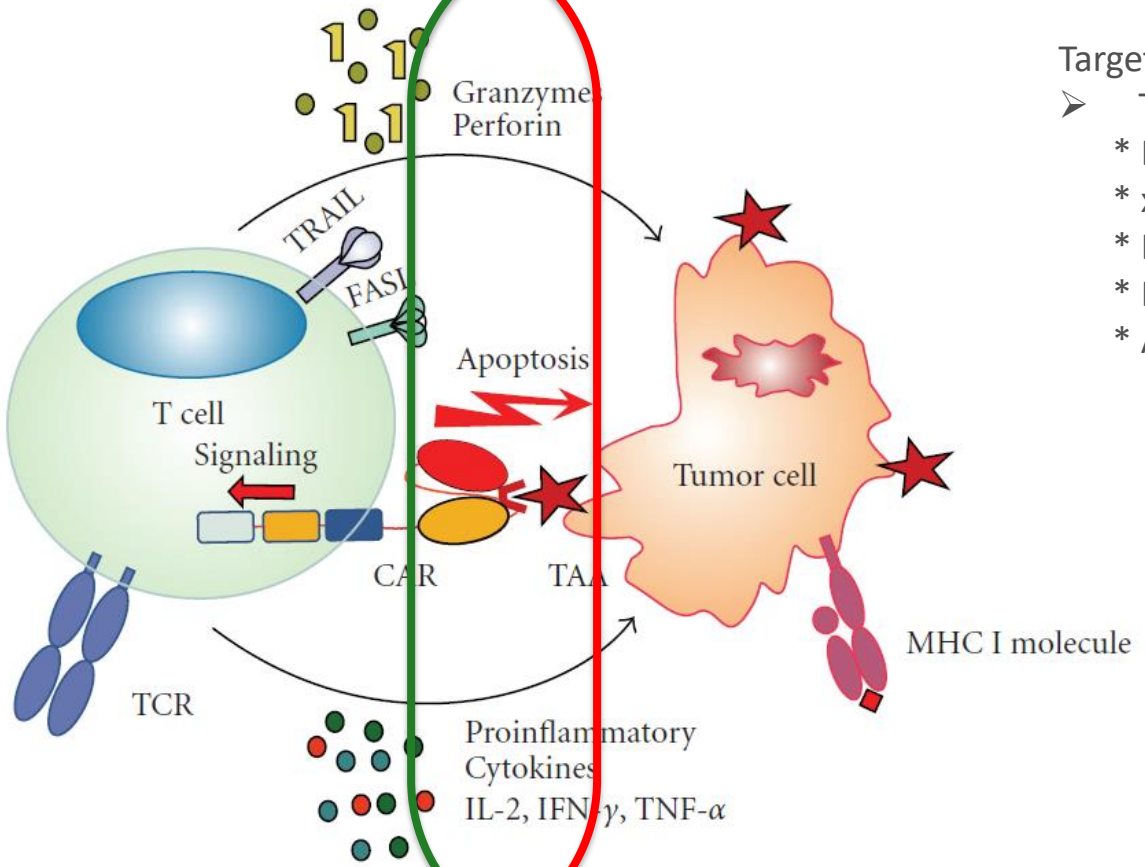

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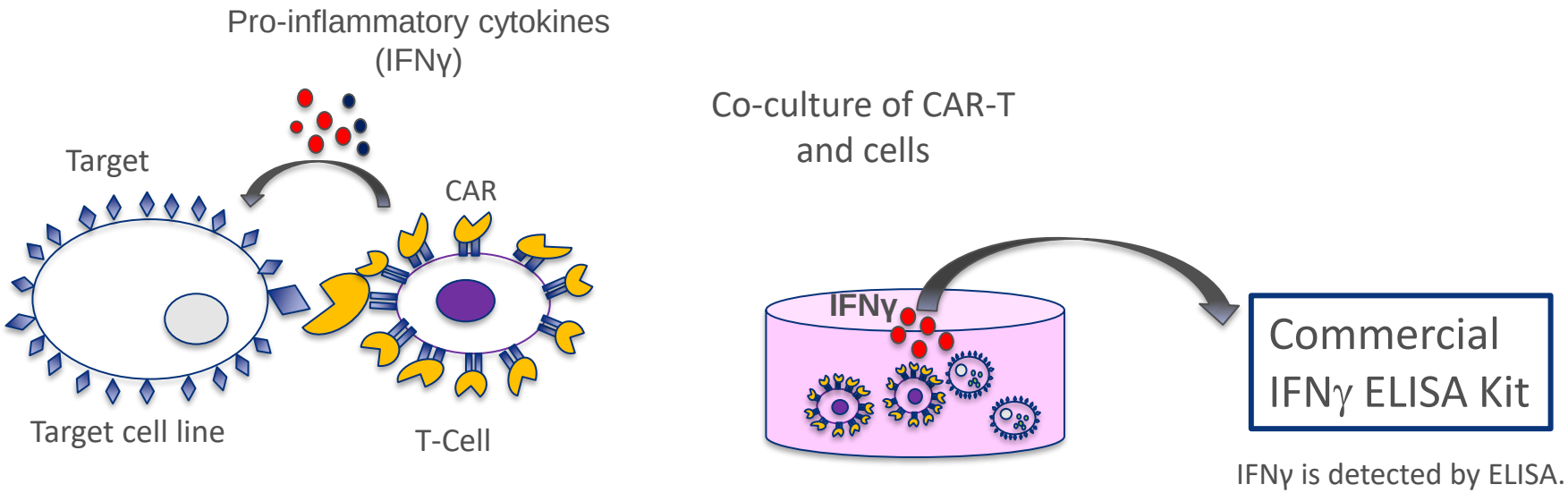
Criteria for Potency Release Assay

- Potency assays…

- Should be QC Friendly
- Should be able to reflect potential Mode of Action (MoA)
- Can be validated according to GMP
- Can be transferable to CMO

Principle of IFNγ cytokine release assay as measure for potency of CAR-T Drug product.

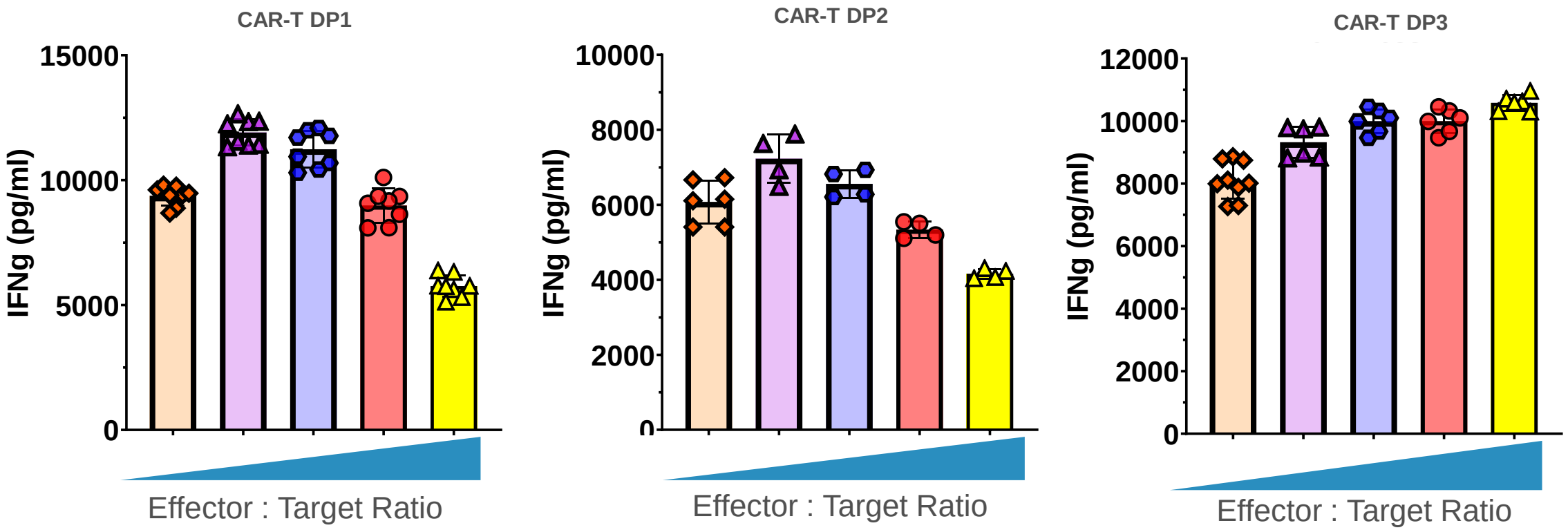


- IFNγ is one of the most abundantly induced cytokines upon T-cell activation and plays an important role in enhancing T cell motility and cytotoxicity.
- The concentration of secreted IFNγ is an indicator of anti-tumor activity and can be used as a potency determination of CAR transduced autologous T cells.

Key Assay Optimization Parameters

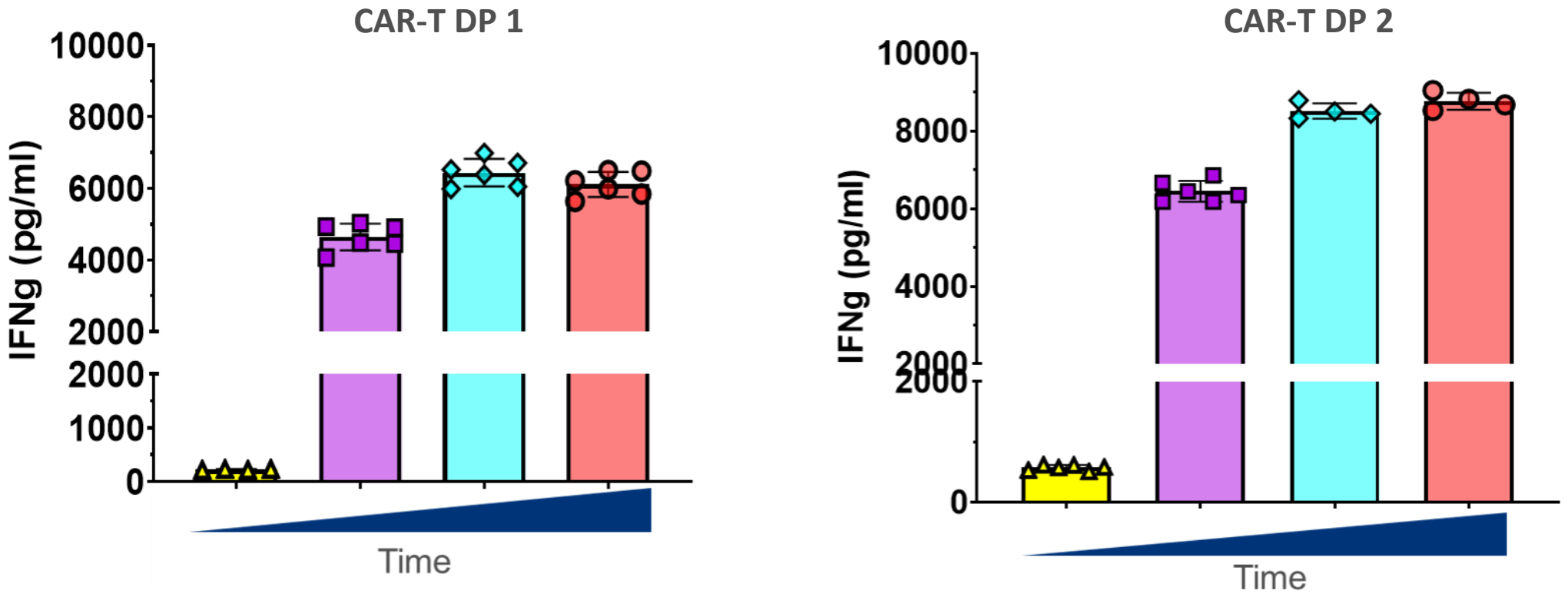
- Effector and target cell density evaluation per culture well surface area.
- Co-culture size setup: seeding density, specificity.
- Effector to target (E:T) ratio.
- Co-culture incubation time course to determine time length.
- Assay specificity was also tested during development and validation of the IFNγ ELISA method.

Effector to Target Ratio selection



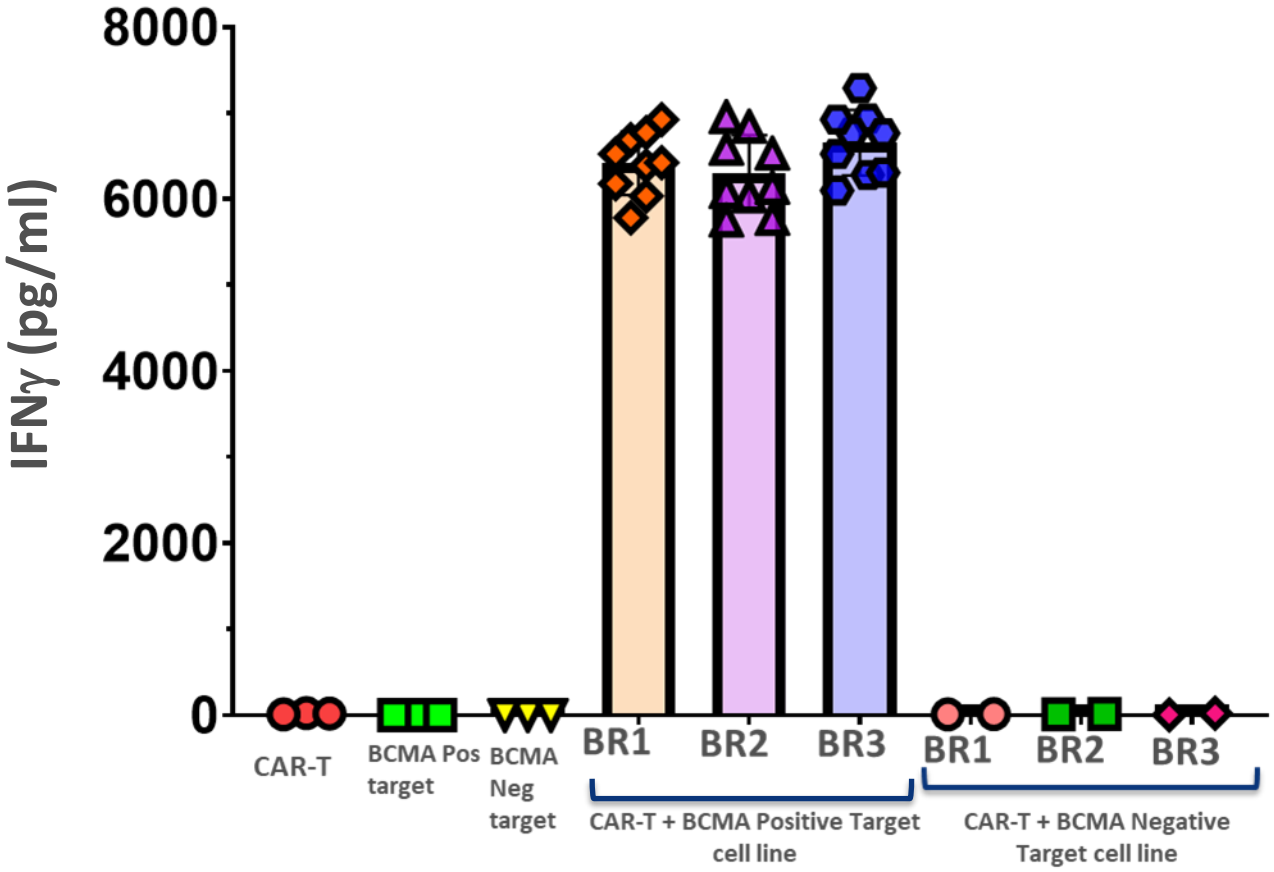
Effector to target ratio selection was performed by testing multiple different CART DP with varying CAR expression and considering appropriate culture conditions.

Co-culture incubation time course



Two separate lots of CAR-T DP that had varying levels of CAR expression were tested at 4 different time points. Selection was based on assay being accurate and reproducible as well as QC friendly time point.

High secretion of IFNg when CAR-T are stimulated with BCMA positive, but not with BCMA negative cell line, demonstrating specificity.



BR=Biological replicate

Validated Assay based on ICH guidelines

Key analytical characteristics considered for assay validation:

- **Linearity:** (cell free coculture supernatant dilutional linearity)
- **Accuracy:** closeness of agreement between value which is accepted, and the value found.
- **Precision :** closeness of agreement between series of measurements from multiple tests of same sample.
- **Precision: Repeatability** – intra-assay precision.
- **Precision: Intermediate Precision-** inter- assay variation between multiple assays over multiple days.
- **Range:** lowest and highest concentrations that produce acceptable accuracy, intermediate precision and linearity.
- **Specificity:**
  1. detection of analyte in the presence of matrix, impurities etc.
  2. Specificity of target cell-induced IFNγ secretion by CAR-T DP shown by using Antigen negative target cell line in coculture in parallel to coculture with antigen positive target cell line
- **Robustness:** experimental variables systematically tested.

Reference: ICH (Q2) R1, Validation of Analytical Procedures: Text and Methodology

Characterization is key
Future developments for Potency and Characterization

Other Potential Potency assay in development:

- Flow based assays for tumor killing and CAR-T cell activation
- Real time imaging-based potency assay.

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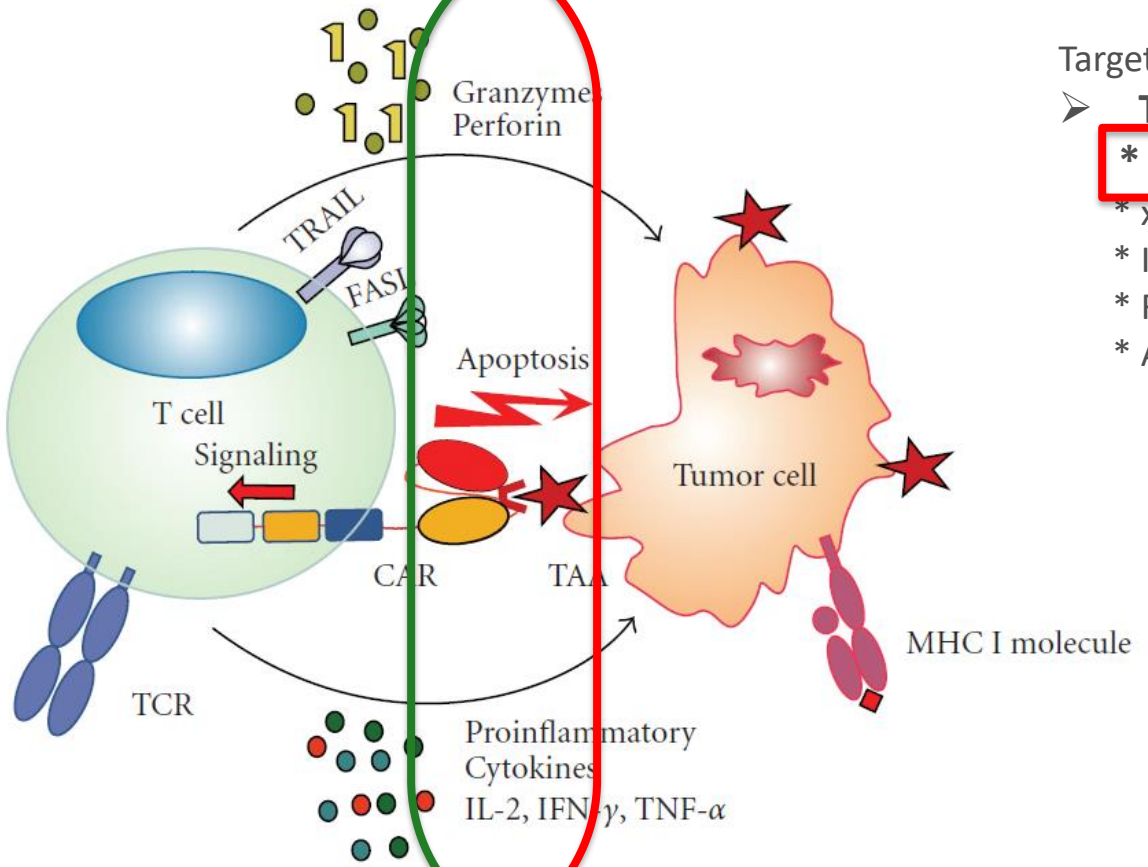

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Tumor killing and Activation markers by Flow

➢ Assay setup:

Day one

CAR-T DP Labeling with CFSE and tumor cells with CTV

-CAR-T alone

-CAR-T + target cell

Co culture

Day two

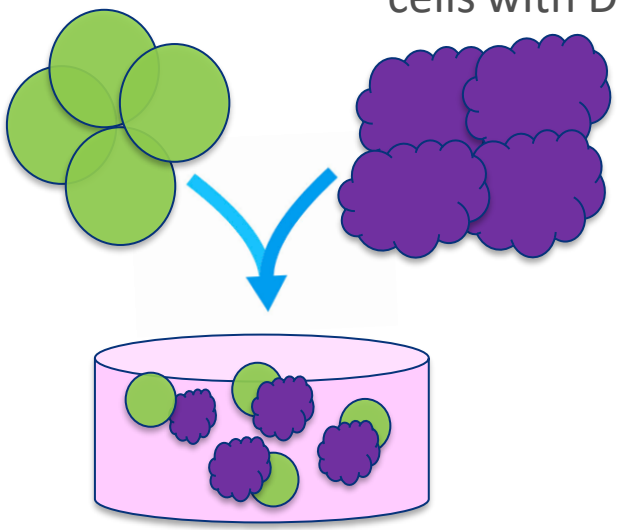
Harvest, stain with cell markers and viability dye and data acquisition

Harvested coculture cells are acquired after staining with T cell subsets and activation markers as well as live/dead stain.

T cell activation is measured as well as resultant tumor killing.

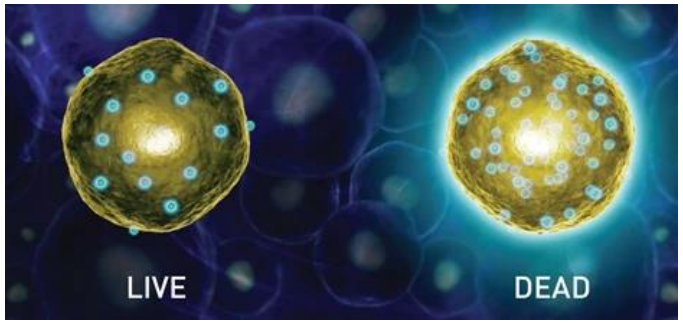
Label CAR-T with Dye 1

Label target tumor cells with Dye 2



incubation

Co-culture at diff. E:T ratios



Principle of the LIVE/DEAD Fixable Dead Cell Stains. The cell-impermeant, amine-reactive dye only binds to the surface of the live cell, resulting in very dim fluorescence. The dye can penetrate the cell membrane in dead cells and will bind to internal proteins, resulting in very bright fluorescence. *Ref: Thermofisher website

Reagent information reference: https://www.thermofisher.com/

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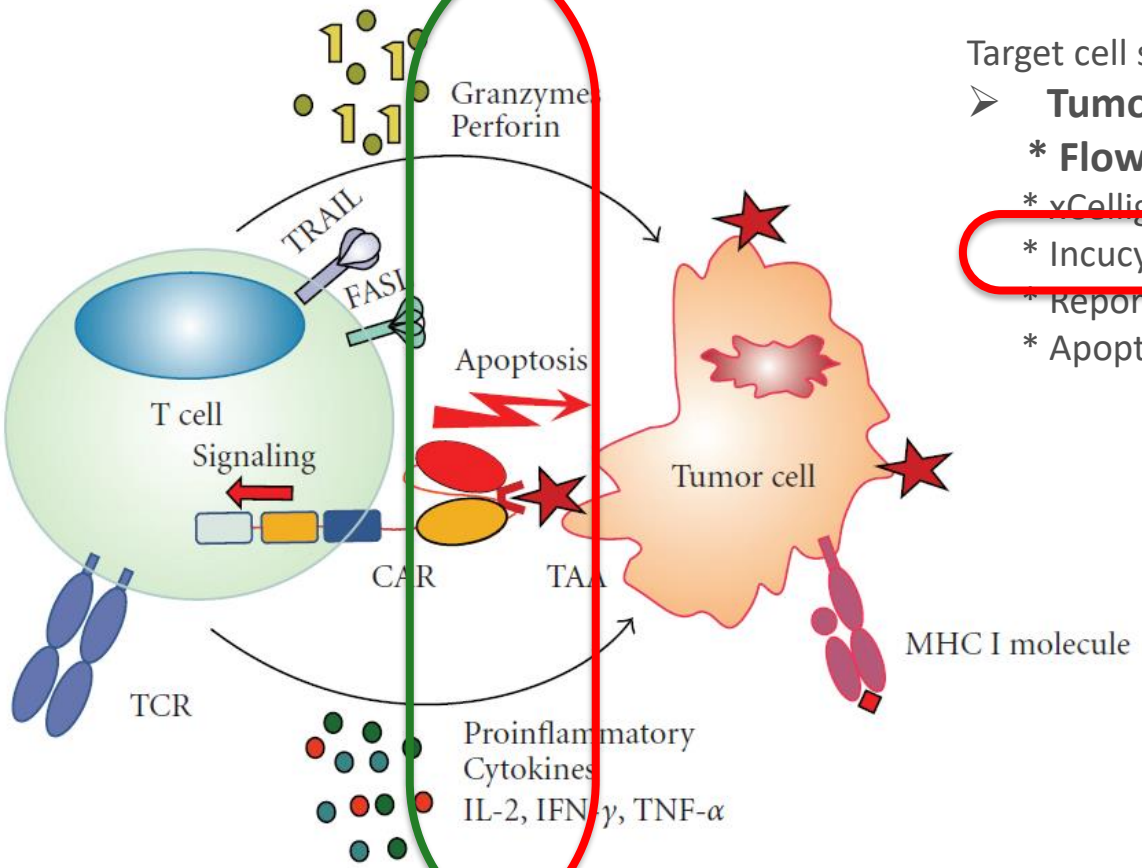

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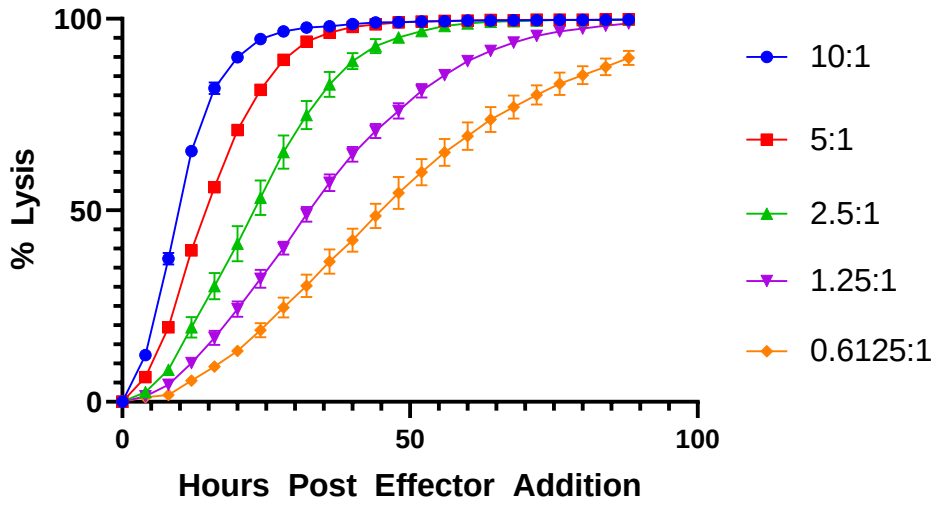
Incucyte Cytotoxicity for Tumor killing



- Real time imaging of cells in culture and measurement of labelled target cells lysis.
- Direct target cell killing can be measured.
- Cells and supernatant can be used for additional assays, e.g. Cytokine productions from supernatant and phenotyping of CART cells post co-culture.

Incucyte® Systems for Live-Cell Imaging and Analysis (essenbioscience.com)

Conclusion:

Selection of IFNγ Assay as a current Potency method.

- IFNγ is produced abundantly upon specific T cell activation and released in co-culture supernatant.
- Surrogate potency assay for T cell activation and target cell killing.
- Reflects potential Mode of Action (MoA)
- QC Friendly
- Can be validated according to GMP
- Can be transferable to CMO
- In future, alternate assays measuring target cell killing can be developed to assess CAR-T DP potency.

Thank you

Questions?