Taking CAR T-Cells From The Research Laboratory To The GMP Facility

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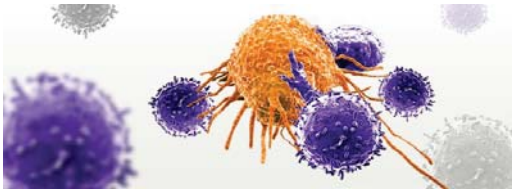
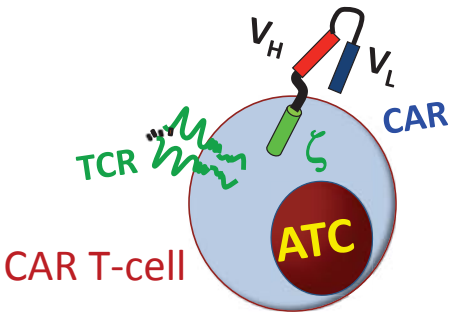


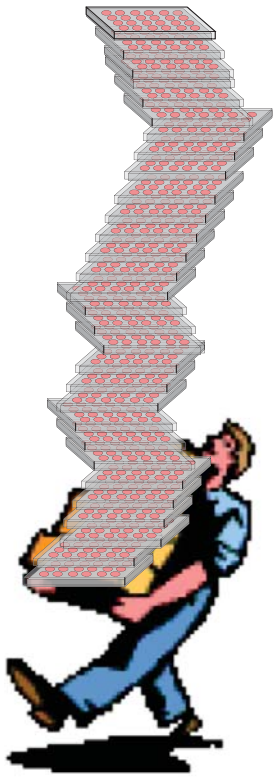
Taking a Manufacturing Strategy from Research Lab to GMP facility

- The Translational Research Laboratories (TRL) of the CAGT
- Role of the Principal Investigator
  - scientist who developed the strategy
- CAR T-cell manufacturing at the CAGT
- Closed systems



Is the Methodology GMP Compliant?

- Are reagents and supplies GMP compliant?
  - Media
  - Other reagents
  - Culture vessels
- Manufacturing strategy
  - Cell selection
  - Transduction
  - Expansion
  - Scale up?
  - Can it be simplified?



Translational Research Laboratories

- One floor above GMP facility (16th floor)
  - GMP staff, QA, QC and a CLIA-certified flow cytometry group
- 17th floor
  - 15 Principal Investigators (PI's) and their labs
  - Research Coordinators and Regulatory Affairs group
  - Facilitates regular exchange of information
- TRL Investigators aware of technologies used in GMP facility
  - Discussion of translation early in project development
  - Reagents and supplies
  - T-Cell culture methodology
  - Transduction
- Facilitates translation of laboratory methods to GMP compliant SOPs

Cell Culture Medium

- TRL Investigators use the T-cell culture medium used in the GMP
  - Selected and reselected for T-cell culture over 30 years

Translational Research Staff

- Trained to work in GMP and in research laboratories
  - Understand both sides
  - Highly versatile
- Work with TRL Investigators to facilitate translation
- Help with
  - Bringing the manufacturing method into GMP compliance
  - Writing SOPs and worksheets/batch records
  - Validating new procedures
  - Equipment qualifications
  - Stability programs

Role of the TRL Investigator

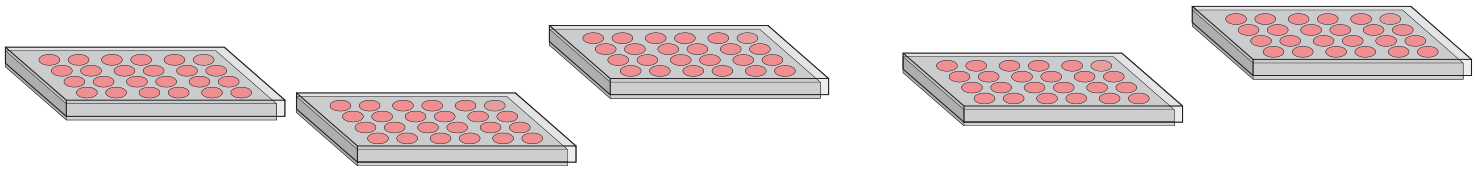
1. Work with translational staff to develop SOPs
   - Start with existing similar SOP
   - Investigator modifies for his/her purpose

CAR T-cell SOPs at CAGT

- 9 current CAR trials
  - 7 different CARs
  - ATCs (CD3/28-activated), VSTs and NKTs
- For ATCs we attempt to use same SOP for all
  - Investigators (or their CARs) can be picky
- Not all CARs are equal
  - CARs affect T-cell growth and differentiation
  - Important factors for clinical outcome

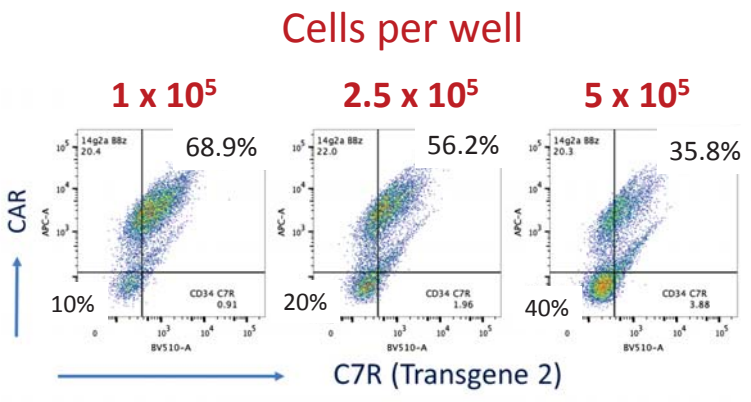
Sometimes we must compromise

- "Do you really need to seed only 1 x 10^5 cells per well for transduction?
  - For 10 x 10^6 transduced T-cells we will need 5 plates!!!"

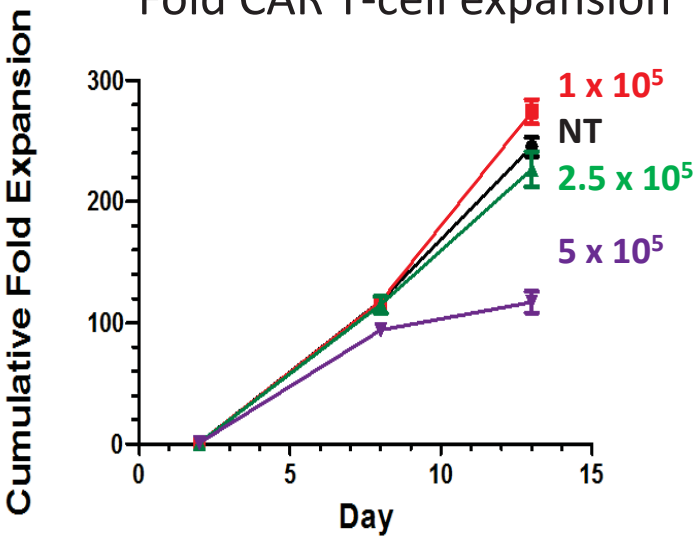


"Do you want to compromise clinical results because of feasibility"?

Cotransduction efficiency



Fold CAR T-cell expansion



Role of the TRL Investigator

1. Work with translational staff to develop SOPs
   - Write the SOP
2. TRL Investigator will train the translational staff on the SOP in TRL laboratory
3. Translational staff train GMP staff in GMP facility

Role of the TRL Investigator

1. Work with translational staff to develop SOPs
   - Write the SOP
2. Train translational staff on SOP in TRL laboratory
3. Translational staff train GMP staff in GMP facility
4. TRL Investigator can maintain involvement with production
   - Scrutinize batch records (not sign off)
     - Help with trouble shooting
   - Perform functional assays on final product
5. Work closely with clinical PI and research co-ordinator

Weekly clinical protocol meetings

- Attended by
  - TRL Investigators, Clinical PI's, Research co-ordinators, GMP staff, QC, QA, GLP staff (for follow up samples)
  - Discuss
    - Patient referrals to protocols
      - Consents and procurement
    - Manufacturing progress and release
    - Infusions
    - Follow up
      - Biological (expansion, persistence…..)
      - Clinical

GMP seminar series

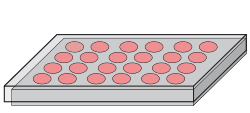
- Investigators present
  - Upcoming clinical protocols
  - Results of ongoing protocols
  - New manufacturing strategies
- Duties of the PI
- Other

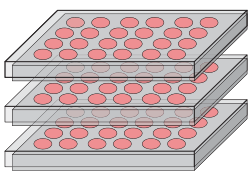
Do we need a closed manufacturing system?

- GRex is good for small-to-large scale T-cell expansion
  - Seed 5 million CART cells in a GRex 10,
  - Harvest 1 to 2 billion from 1 Grex 100
  - Compatible with closed seeding and harvest
- ❖Not suited to retroviral transduction
  - ❖Requires adhesion to retronectin-coated surface

CAR T-cell manufacture using retroviral vectors according to CAGT

Day 1	Day 2/3		Day 5 to 13
T-cell activation on CD3/28-coated NTC plates	Transduction on Retronectin-coated wells	Expansion in GRex	Cryopreservation
24 x 10^6 PBMCs	20 x 10^6 cells		
1 x 10^6 per well	0.375 x 10^6 per well		
1 x 24 well plate	3 x 24 well plates		





What is “in vogue” for a CAR T-cell?

- Minimally differentiated
  - Retains naïve or central memory phenotype
- Short culture period
- High numbers
  - Transduce more T-cells
  - More 24 well plates
- Validation of transduction in T75 flasks

Can we use closed Systems?

- Desirable
- Expensive
  - Hardware, tubing
- Not suited to small scale manufacturing
- Not suited to retroviral transduction
- Not required for phase I/II clinical trials

If Phase I/II is successful

- Worth expense of closing manufacturing and scale up
- Likely supported by industry

Summary

- Close communication is crucial for effective SOP transfer
- The PI can help with troubleshooting in early days
  - Listen to hem/her
- You cannot make T-cells fit your needs and wants
  - They have highly specific growth and media requirements
  - CAR-T-cells grow exponentially, only if those requirements are met
- Can be manufactured from small blood volumes
- The field requires new closed systems for small blood volumes
  - Closed systems not required for phase I/II trials