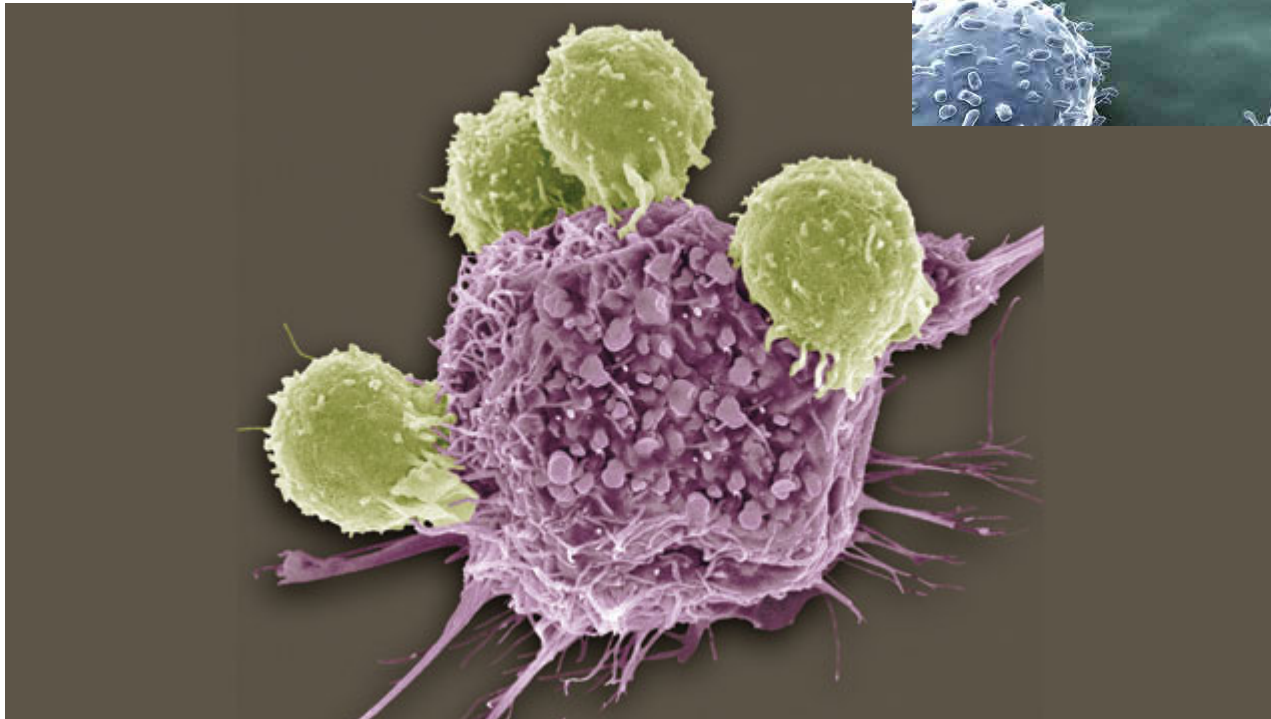
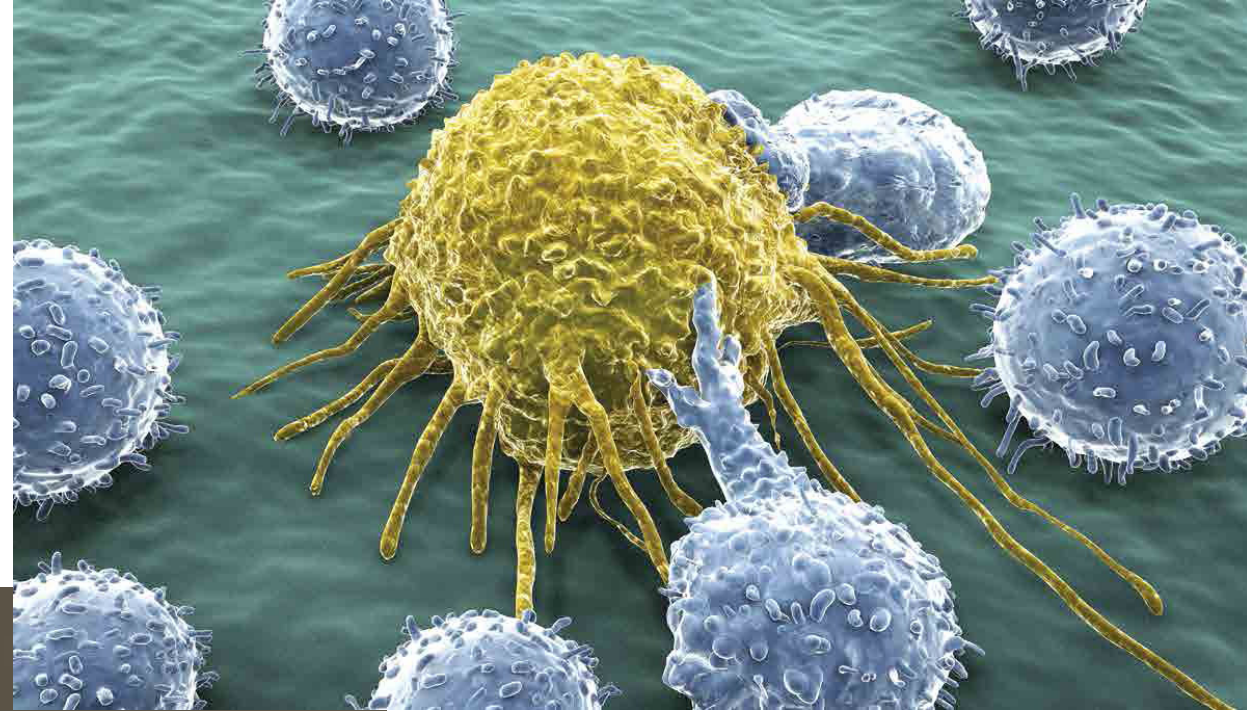


# ***Cell Therapy Production at the Biopharmaceutical Development Program, FNLCR***



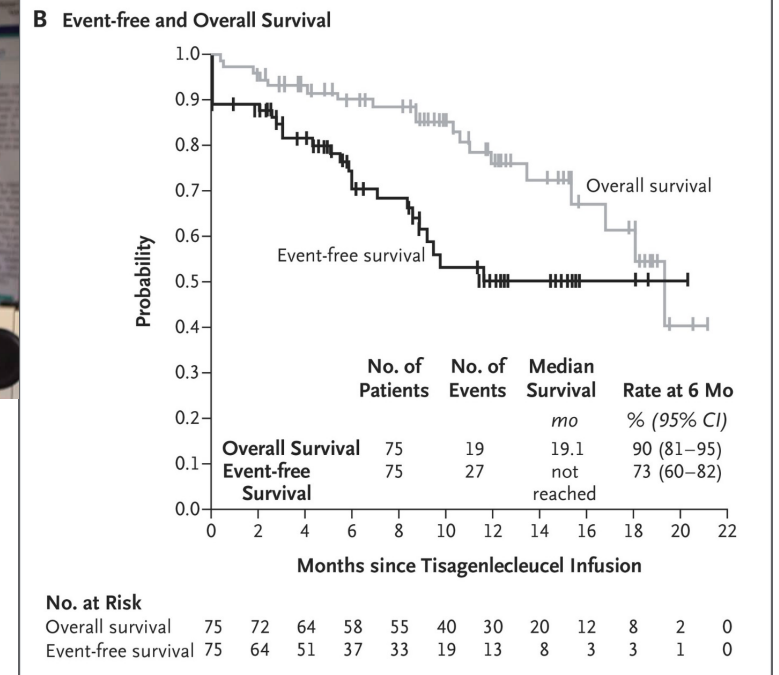
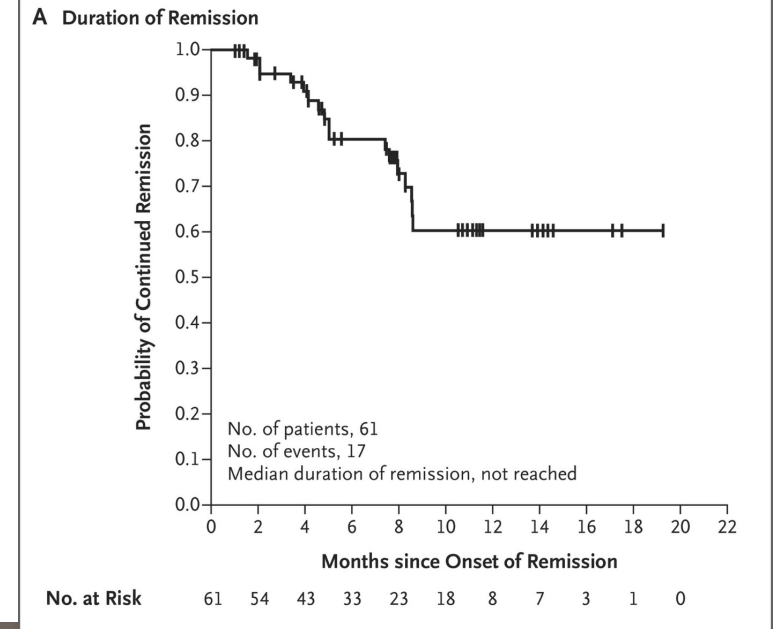
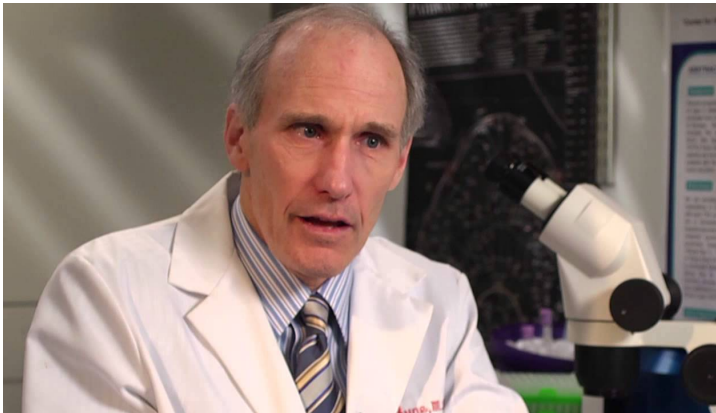


## FDA approval brings first gene therapy to the United States

CAR T-cell therapy approved to treat certain children and young adults with B-cell acute

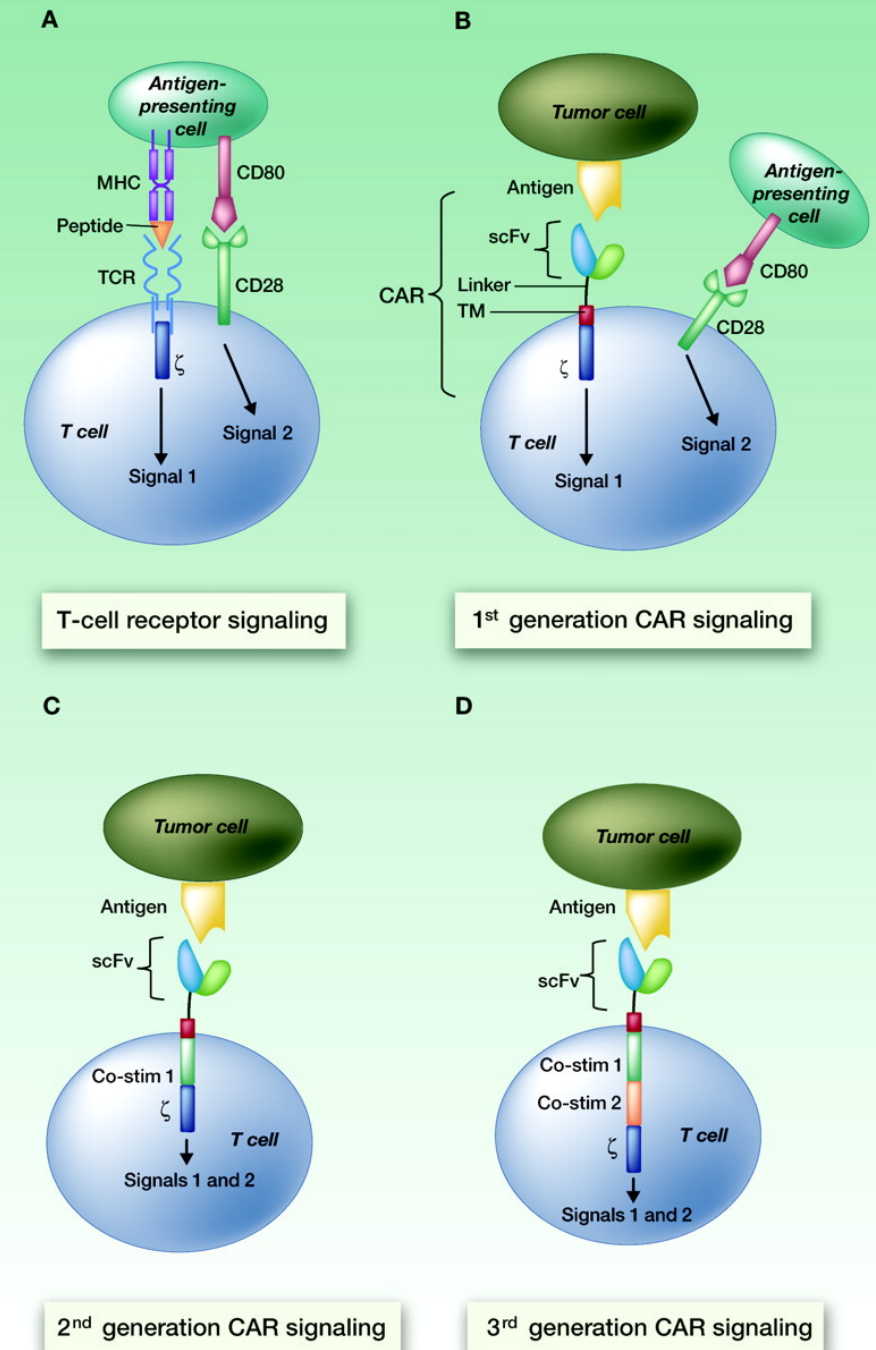
## FDA approves CAR-T cell therapy to treat adults with certain types of large B-cell lymphoma

Yescarta is the second gene therapy product approved in the U.S.



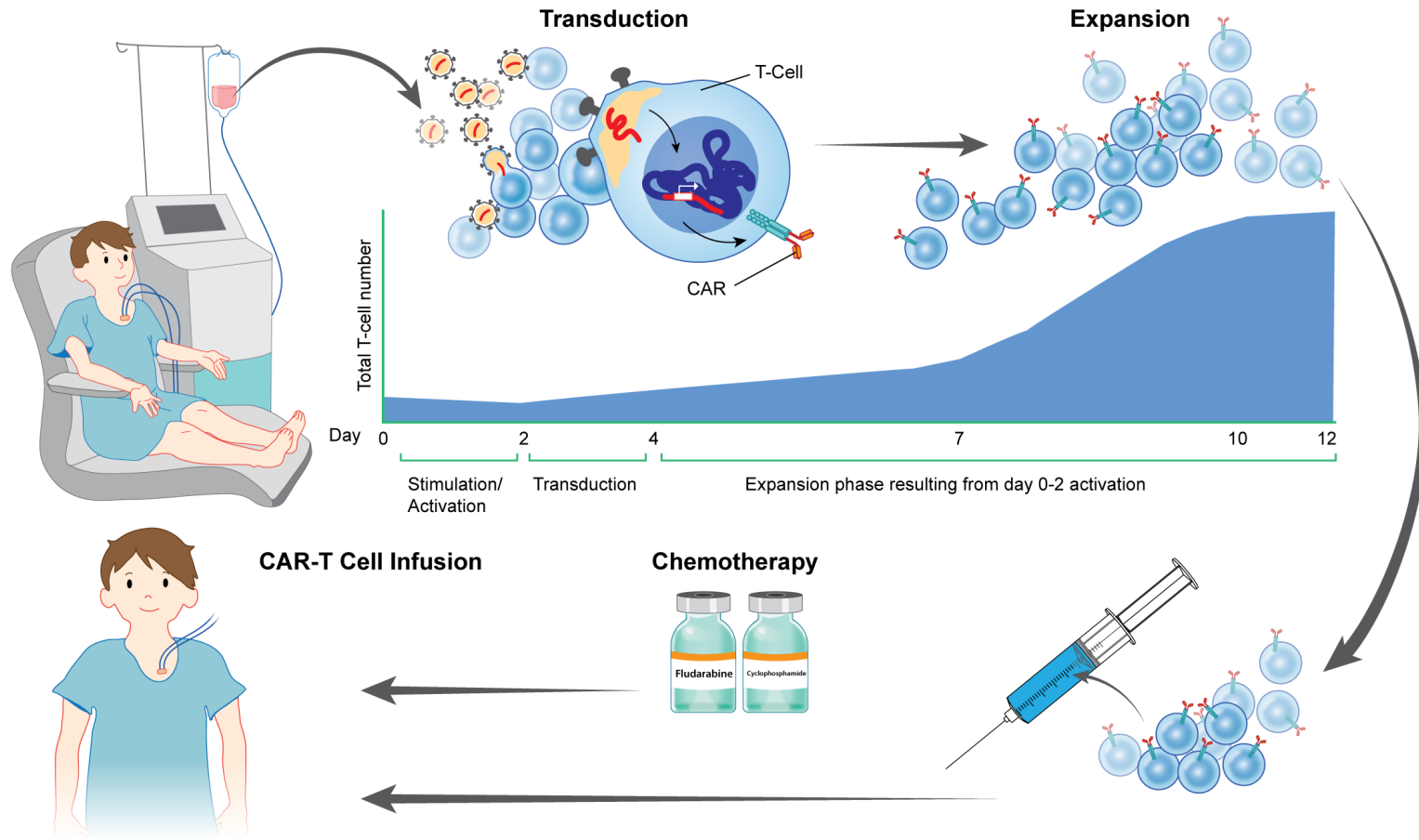
# CAR-T Cell Therapy

- What is a CAR
  - Chimeric Antigen Receptor
- Retains the functionality of a T-cell with the antigen recognition properties of antibody
- Customized receptor
  - Extracellular antigen-binding domain
  - Intracellular signaling domain of T cells





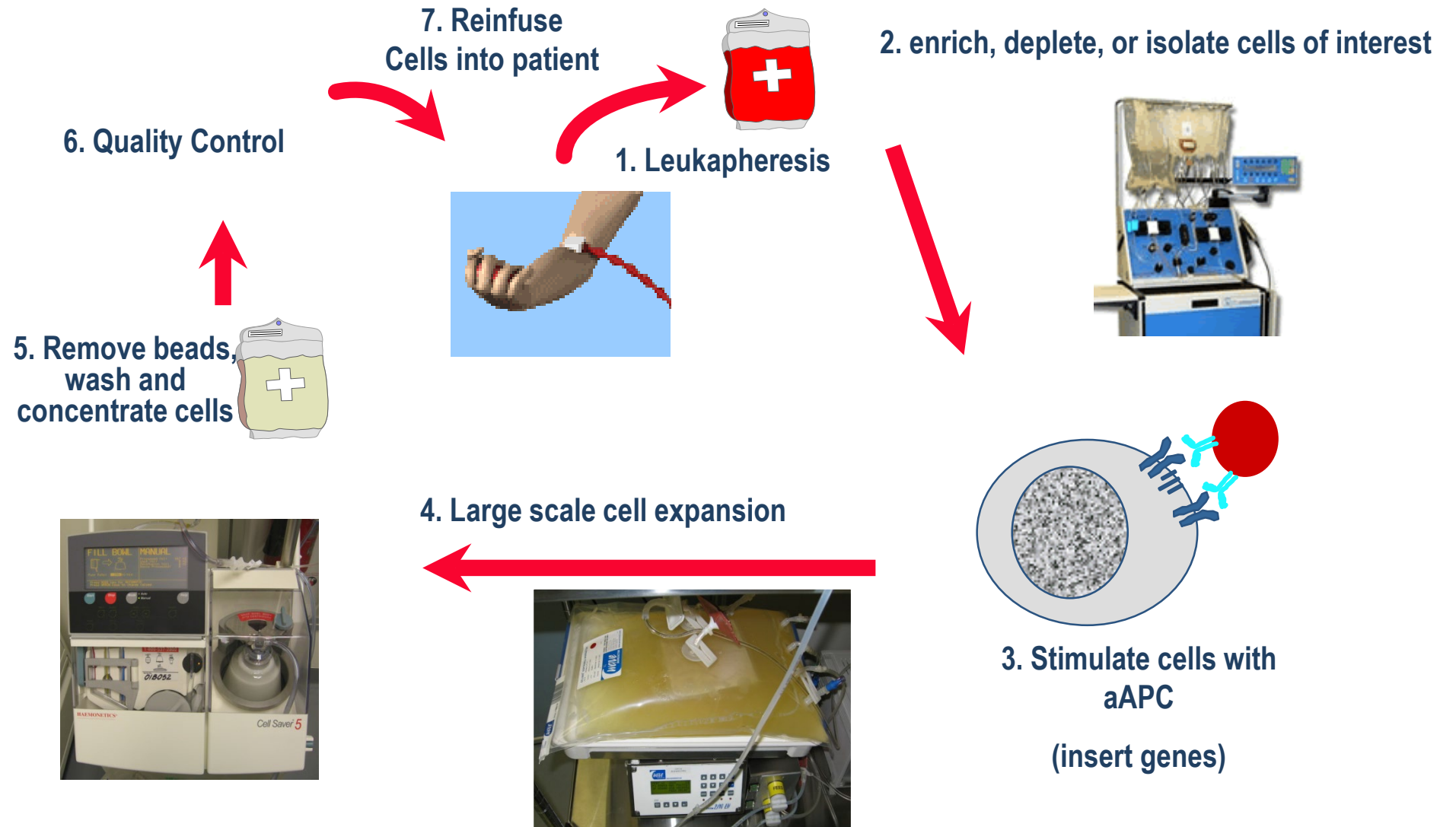
# Making a CAR-T Cell (autologous transplant)



1. Apheresis, then select T-cells
2. Stimulation and Transduction
3. Expansion
4. Lymphodepletion
5. Infusion



# Traditional 'open' manufacturing production process



# ***DCTD/NCI Cell Therapy for Cancer at FNLCR***

- Current open production platform has capacity and reproducibility challenges
- Reproducible manufacturing and testing results in reproducible science
- Use of closed system at FNLCR can support multi-center trials and/or tech transfer for point-of-care
- cGMP production capability for virus, plasmid, and cell products
- Collaborate with FDA, NIST, other stakeholders to standardize product testing



## CliniMACS® Prodigy – Automated Cell Processing System for GMP Cell Manufacturing

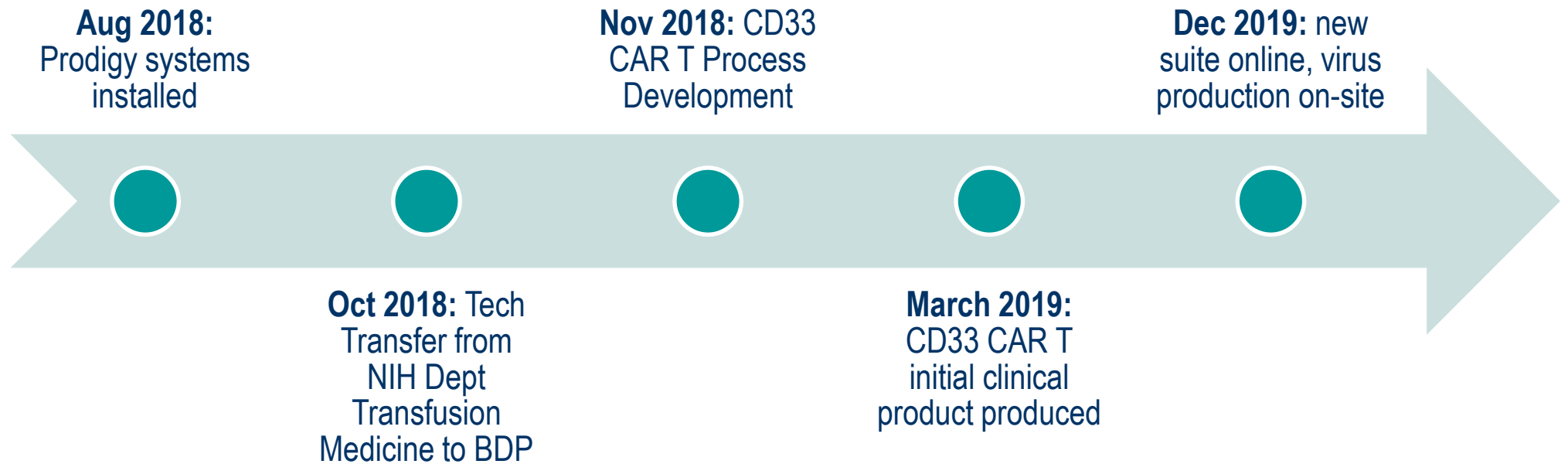


- Integrated cell processing from starting material to final cellular product:
  - Sample preparation
  - Cell washing & density gradient separation
  - MACS cell separation
  - Cell activation
  - Genetic modification
  - Cell culture
  - Final product formulation
- Enabling complex processes
  - Automated & controlled system
  - **Closed single-use tubing set**





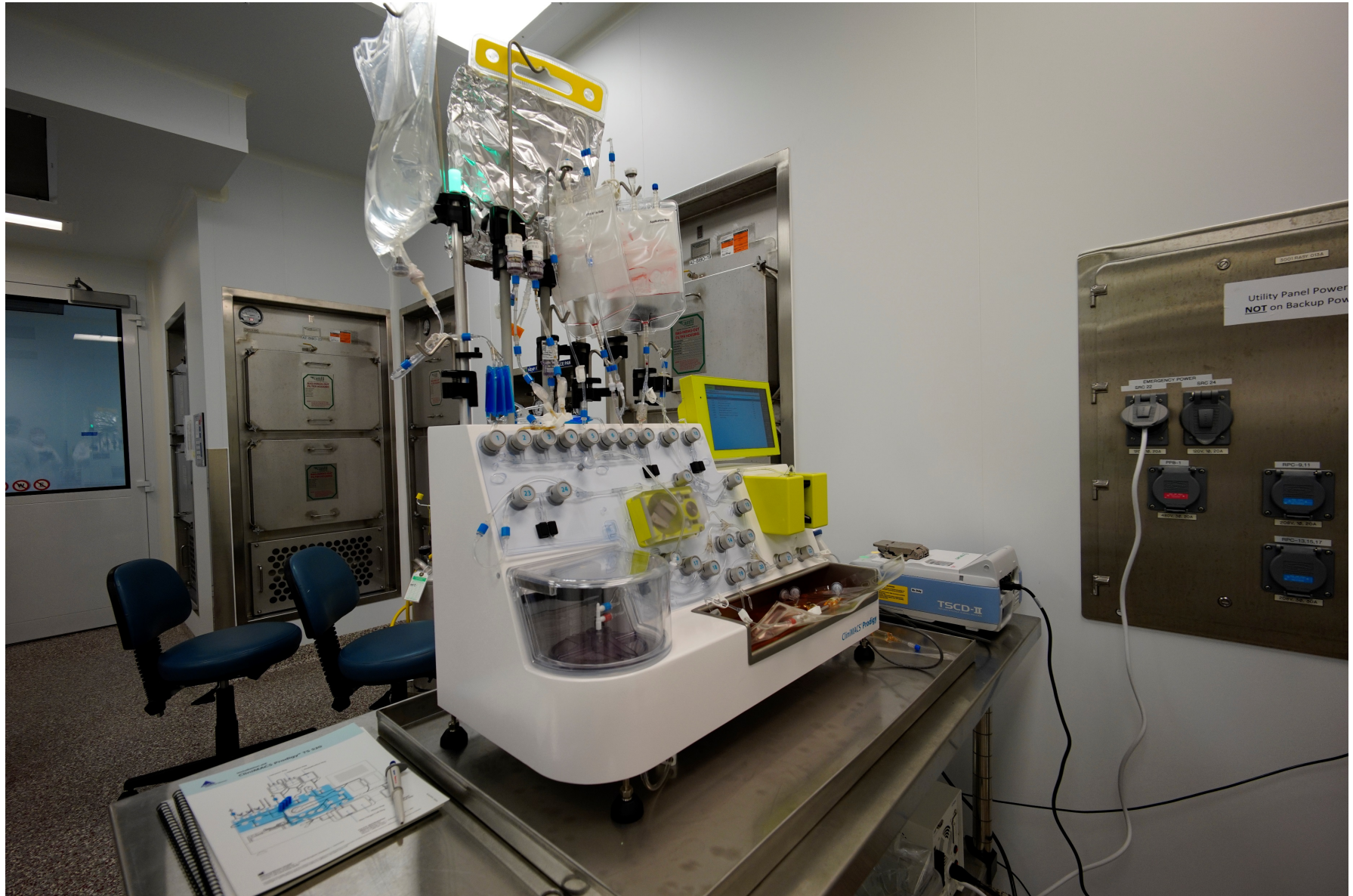
# Timeline for cell therapy resources at DCTD/BDP



# Decision Points and Progress to Date

Decision	Progress
Use closed manufacturing system	<ul style="list-style-type: none"><li>• Miltenyi Prodigies acquired</li><li>• BDP staff trained</li></ul>
Initiate process development in ISO7 cGMP space	<ul style="list-style-type: none"><li>• Prodigies installed in the BDP Virus Production Facility (VPF)</li></ul>
Tech transfer existing CAR T manufacturing and testing	<ul style="list-style-type: none"><li>• Tech transfer of CD19/CD22 CAR T-cells from Dept Transfusion Medicine NIH Clinical Center to BDP ongoing</li><li>• Assay development for product release ongoing</li></ul>
Manufacture CAR T for clinical trial	<ul style="list-style-type: none"><li>• Process development for CD33 CAR T-cell production initiated</li><li>• BDP is manufacturing site for CD33 CAR T-cell for pediatric AML trial sponsored by National Marrow Donor Program</li><li>• NCI PI is Dr. Narali Shah</li><li>• Multi-site trial will result in establishing centralized manufacturing logistics</li></ul>
Increase cell/virus production capacity	<ul style="list-style-type: none"><li>• Renovations to B2300/B2310 initiated</li></ul>

## Prodigy system has a small footprint in VPF





BDP current capacity: 2 Prodigy units in BDP = 4 patients/month





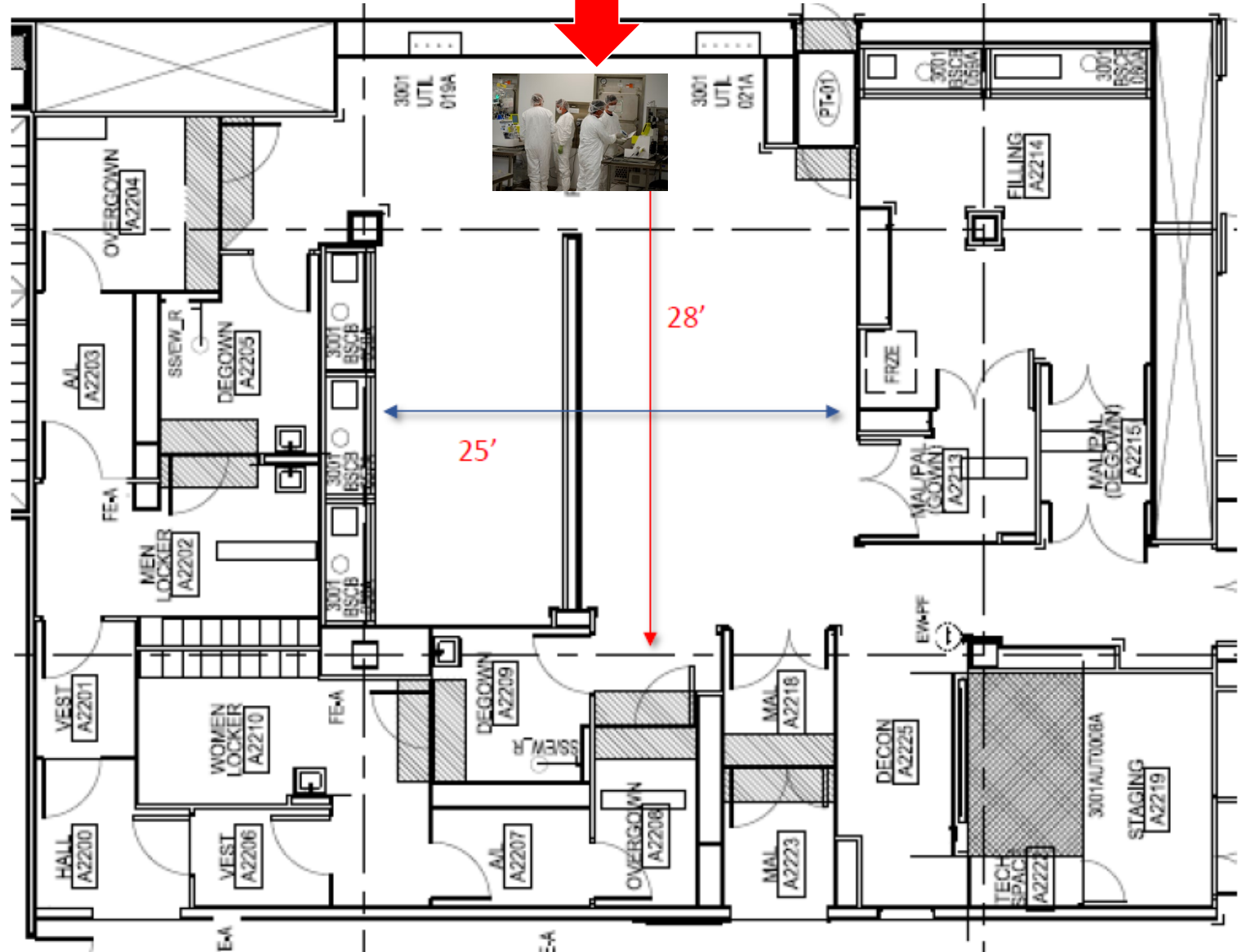
## Plenty of space in VPF to add additional Prodigy units



## Scale of current workspace demonstrates space available to increase capacity

### BDP Virus Production Facility (VPF)

- ISO7 manufacturing resource
- Independent HVAC system
- Locker rooms with unidirectional flow
- Fill/finish area

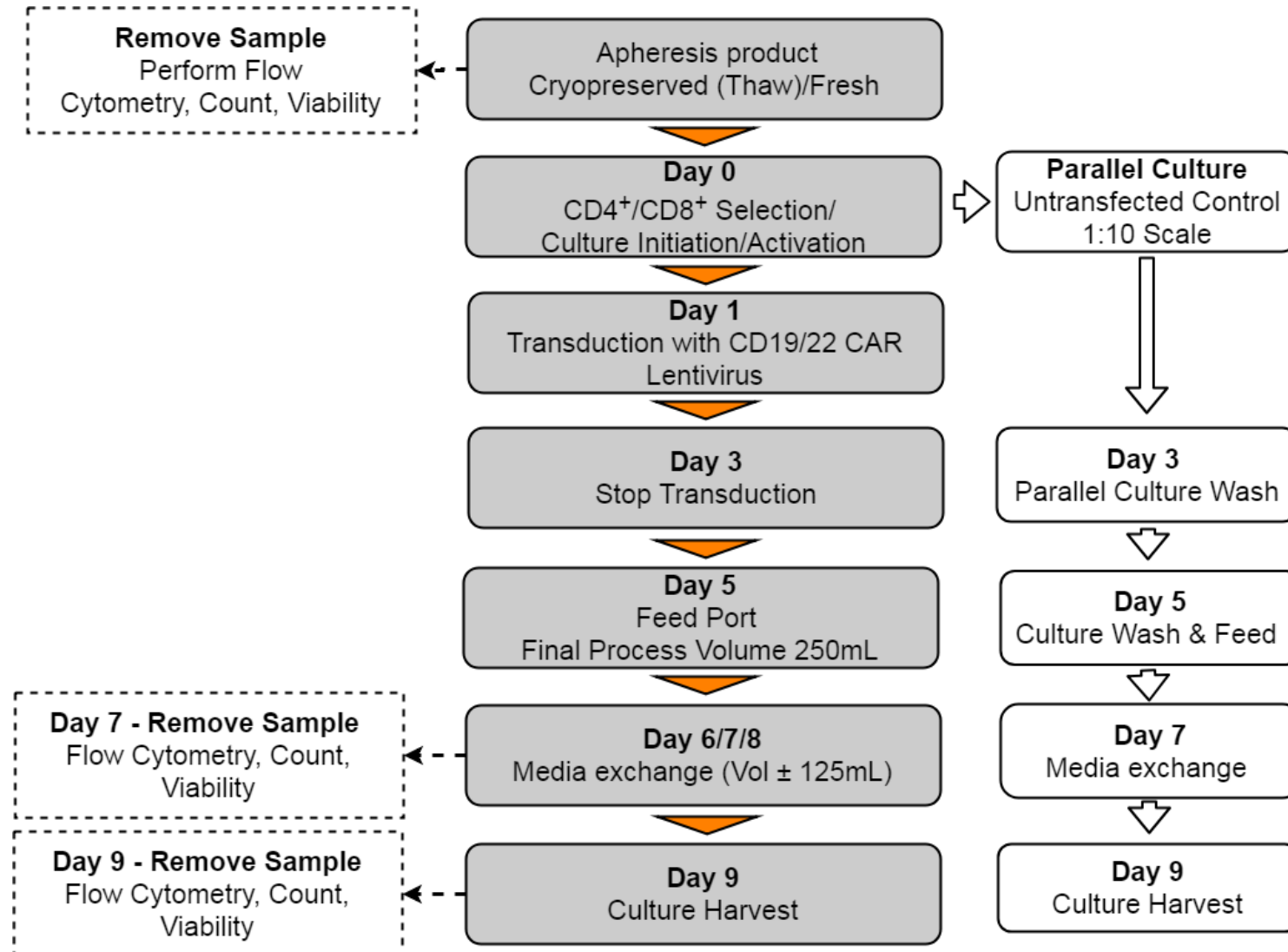




## Tech transfer: CD19/CD22 CAR T-cell production at BDP



# Anti-CD19/22 CAR T-Cell Manufacturing Tech Transfer Process Flow (9-day process)



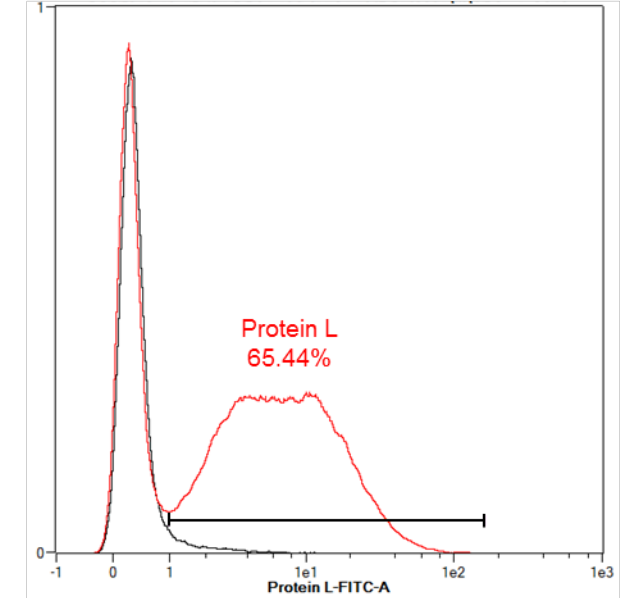
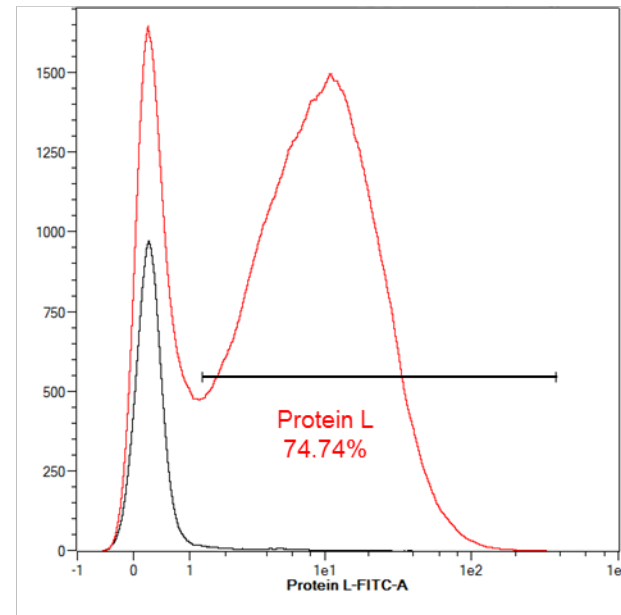
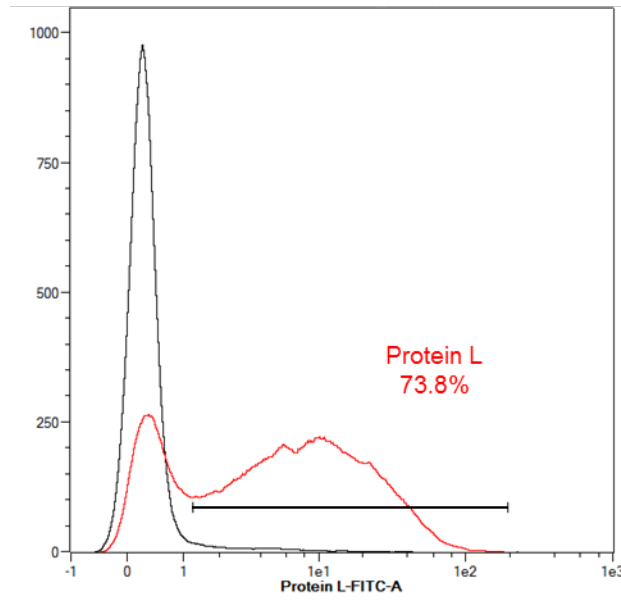
## CD19/22 CAR T-cell Tech Transfer

Process Parameters	BDP Run 1 (Cryopreserved apheresis - HemaCare)	BDP Run 2 (Fresh apheresis - NIH CC)	NIH Clinical Center (n=4)
Total Nucleated Cells Pre-selection ( $\times 10^9$ )	2.08	1.74	0.95 - 6.55
Total Nucleated Cells Post-selection ( $\times 10^8$ )	6.36	5.31	2.8 - 26.0
T-cell Recovery Post-selection (%)	51	60	66 - 84
CD4 <sup>+</sup> /CD8 <sup>+</sup> Purity Post-selection (%)	94.6	101.4	>95
B-cell Density Post-selection (%)	1.2	0.5	0.2 - 0.3
Fold Expansion	23.2	29.9	8.8 - 16.9
Total Nucleated Cells ( $\times 10^9$ )	2.77	3.29	1.01 - 1.88
Viable CD3 <sup>+</sup> (%)	99.6	99.7	>99
CD4 <sup>+</sup> /CD8 <sup>+</sup> ratio	0.74	1.64	1.5 - 2.5
B-cell Density Post-expansion (%)	0.3	0.0	0.0 - 0.1
Transduction Efficiency - Protein L (%)	74.7	65.4	54.6 - 76.5
<b>Transduced viable CD3<sup>+</sup> (CAR<sup>+</sup>) cells (<math>\times 10^9</math>)</b>	<b>2.06</b>	<b>2.15</b>	<b>0.5 - 1.4</b>



## CD19/CD22 CAR T-cell Transduction Efficiency similar to NIH DTM

— NIH DTM CD19/22 CAR T-cells    — BDP Prodigy Run 1 CAR T-cells    — BDP Prodigy Run 2 CAR T-cells  
— BDP Negative Control    — BDP Control Run 1    — BDP Control Run 2

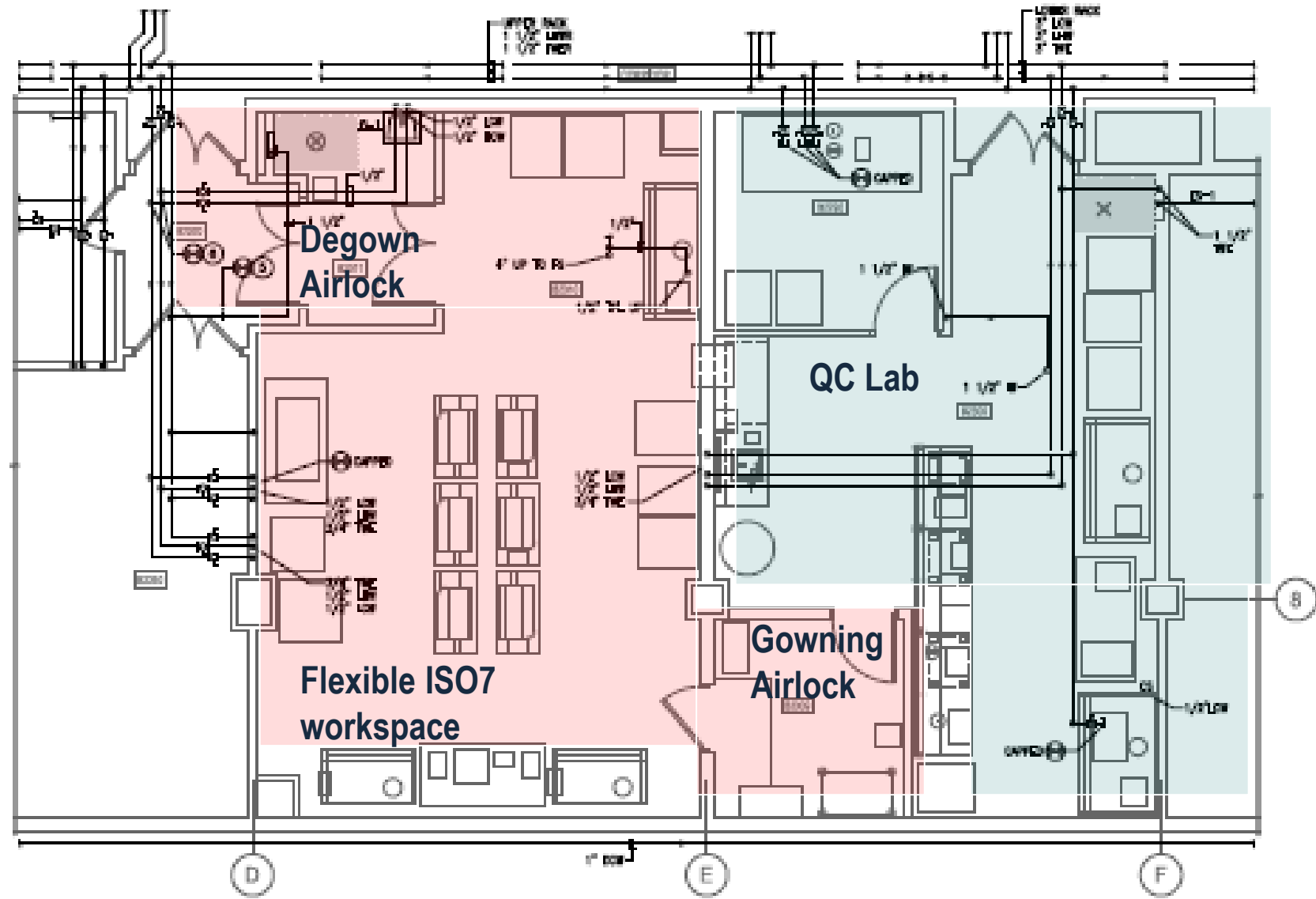


- The DTM Protein L staining and flow cytometry protocol was followed.
- Flow cytometry was performed using a Miltenyi Biotec MACSQuant Analyzer 10

## Future planning

- DCTD/NCI Workshop on Cell Therapy for Solid Tumors, Dec 10-11, 2018. Guidance from cell immunotherapy community, FDA and other stakeholders for continued NCI investment prioritization.
- Convert B2300/B2310 labs into cell therapy production suite to re-utilize VPF for virus production. Renovations estimated for completion in Sept 2019.
- Gain experience in centralized manufacturing logistics for supporting multi-center clinical trials from the DCTD/BDP.
- BDP development of in-process and release testing SOPs for improved reproducibility and faster tech transfer to point-of-care options.

Renovations to ATRF B2300/B2310 will result in ISO7 production suite, flexible workspace and adjacent QC testing capabilities





Logistics of centralized cell therapy manufacturing will be developed in support of initial CD33 CAR T-cell trial for AML  
(Sponsor is NMDP, sites: NIH CC, CHOP, Colorado, UCLA)

## COMPLETE LOGISTICS SUPPORT FOR REGENERATIVE MEDICINE PROGRAMS



## Summary

- Miltenyi Prodigy systems installed in BDP VPF, staff trained, units are under operation in ISO7 cGMP environment
- Tech transfer of CD19/CD22 CAR T-cells from DTM/NIH CC to BDP ongoing
- Assay development is underway at the BDP for in-process and release assays supporting CAR T-cell production
- Renovation design for new cGMP suite completed, construction initiation expected Nov 2018
- Upcoming DCTD/NCI workshop Dec 10-11, 2018



# Questions?

Contact me anytime:

Anthony Welch

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DTP/DCTD/NCI

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