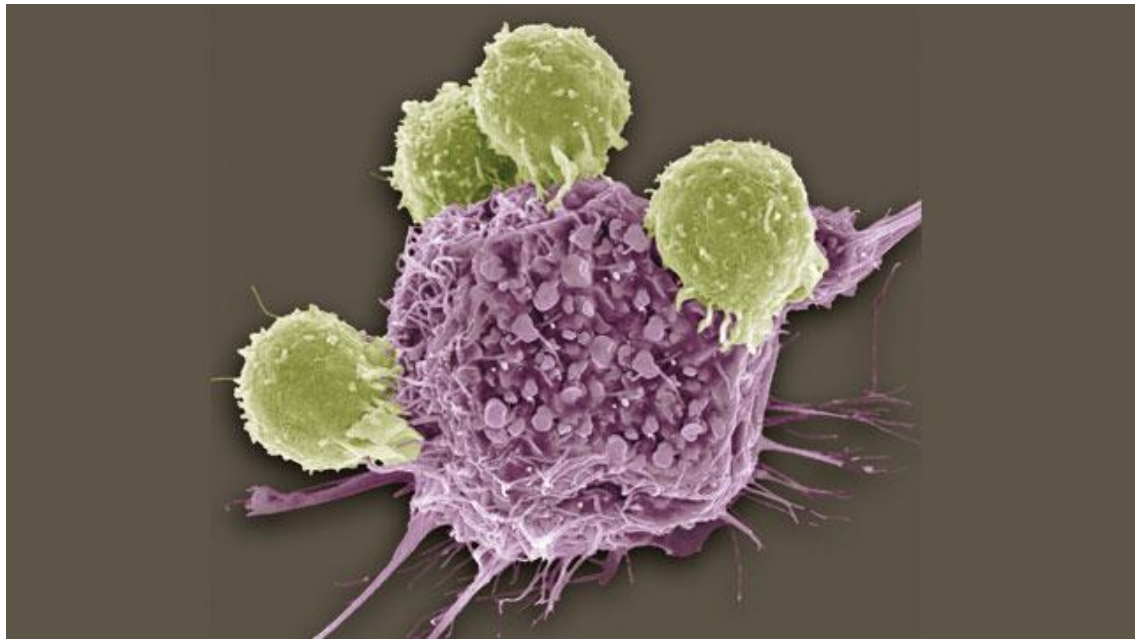
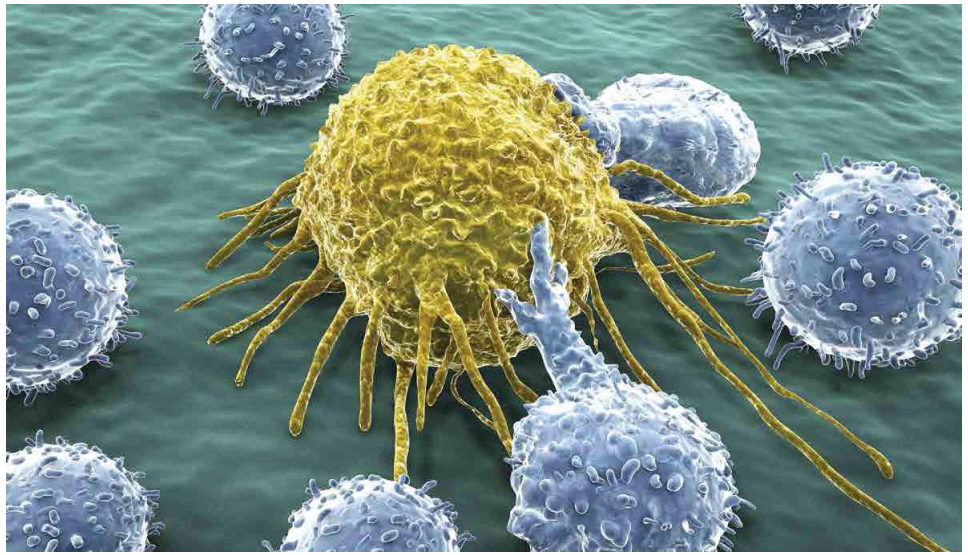
National Cancer Institute

Immune Cell Engineering for the Extramural Community: Recent Progress at the Frederick National Laboratory for Cancer Research

Rose Aurigemma, PhD
Deputy AD, Developmental Therapeutics Program
DCTD, NCI





July 13, 2020
FNLAC

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
National Institutes of Health

National Cancer Institute

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES

National Institutes of Health

Topics

- Brief Introduction to Cell Therapy
- Project updates
- Capability updates
- Facility upgrades
- Filling the queue

Biopharmaceutical Development Program (BDP)
at the Frederick National Laboratory for Cancer Research

- The BDP is contractor-operated cGMP manufacturing facility supporting phase I/II clinical trials
- Extramural/Intramural research discoveries translated to patient therapies
- Process development, manufacturing, fill/finish, QC, QA and regulatory affairs
- Biologic product types include: MAbs, recombinant proteins, oncolytic viruses, AAV, plasmids, vaccines
- NCI investment in BDP to establish cGMP capabilities to support cell therapy
- Extramural access to resource through NCI NExT program (next.cancer.gov)

National Cancer Institute

A look back at FNLAC October 2018

Anthony Welch: 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.

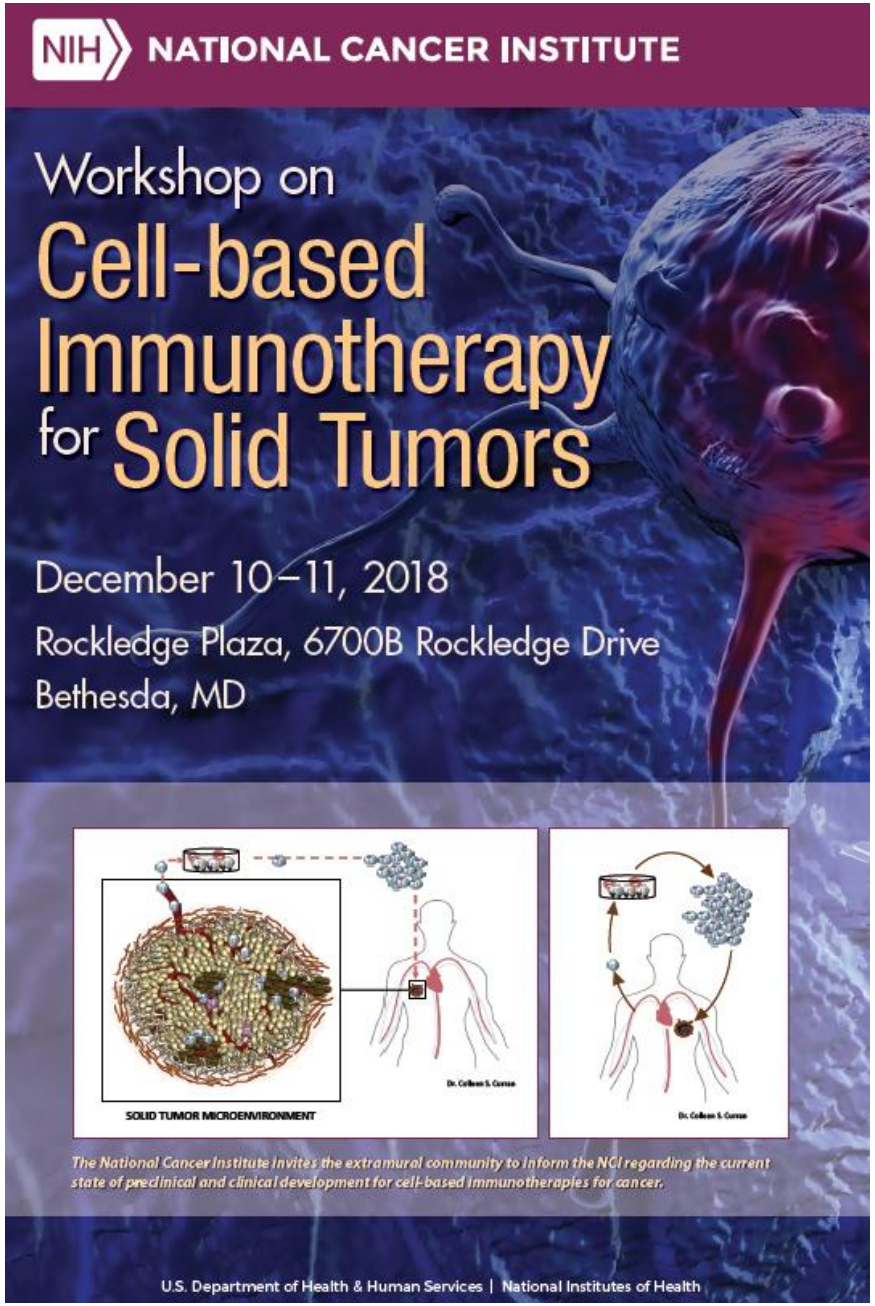
- Convert B2300/B2310 labs into cell therapy production suite to re-utilize VPF for virus production. Renovations estimated for completion in Sept 2019.

- 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.

National Cancer Institute

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
National Institutes of Health

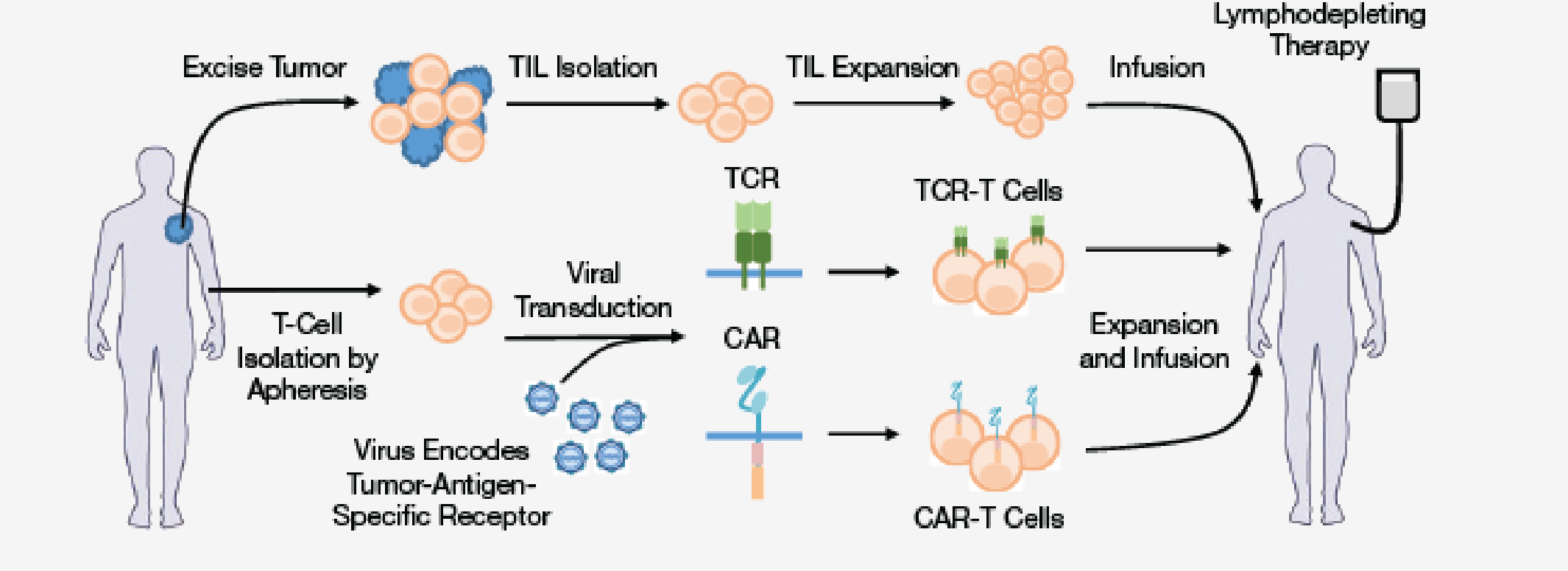


- Research needed:
  - Targeting, affinity, cross-reactivity
  - Improve trafficking and tumor penetration, non-invasive imaging
  - Overcome inhibitory TME
  - Explore autologous vs. allogeneic products; CAR-T vs. TIL vs. NK vs. DC vaccine, etc.
  - *In vivo* gene editing of immune cells
- Clinical development challenges
- Technology challenges:
  - gene transfer, cell production
- Regulatory challenges

2nd Workshop planned for December 2020

National Cancer Institute

Tumor Infiltrating Lymphocytes (TIL) vs T Cell Receptor-T cells (TCR-T) vs Chimeric Antigen Receptor-T cells (CAR-T)

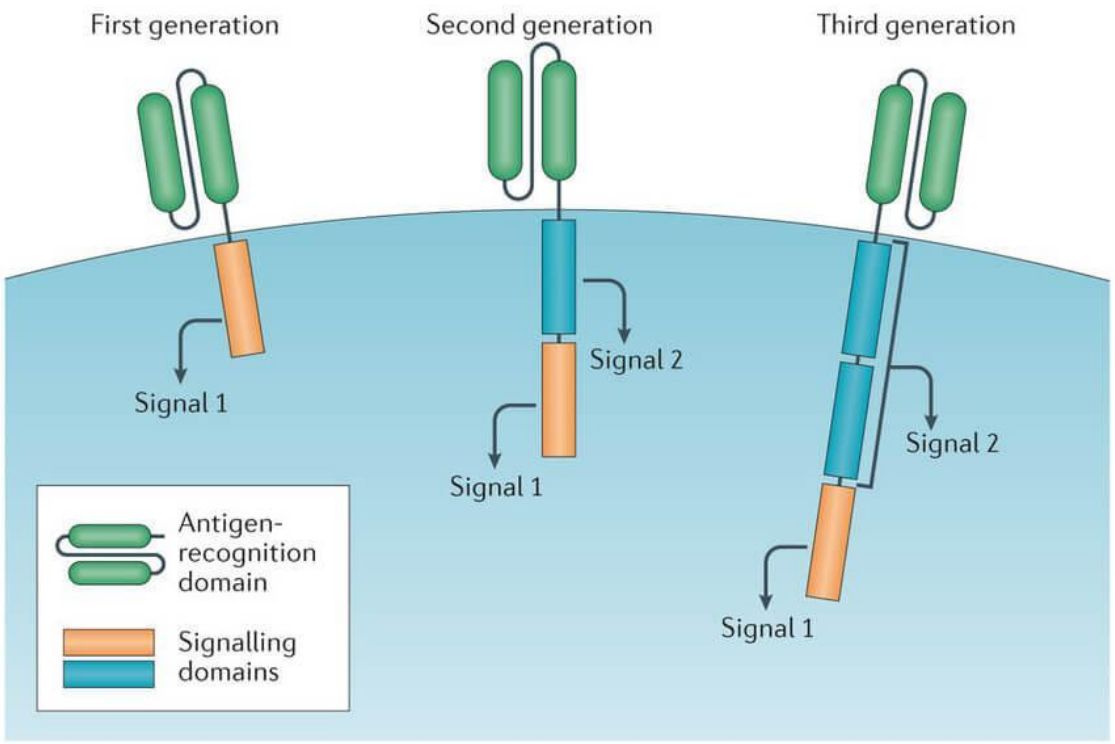


National Cancer Institute

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES

National Institutes of Health

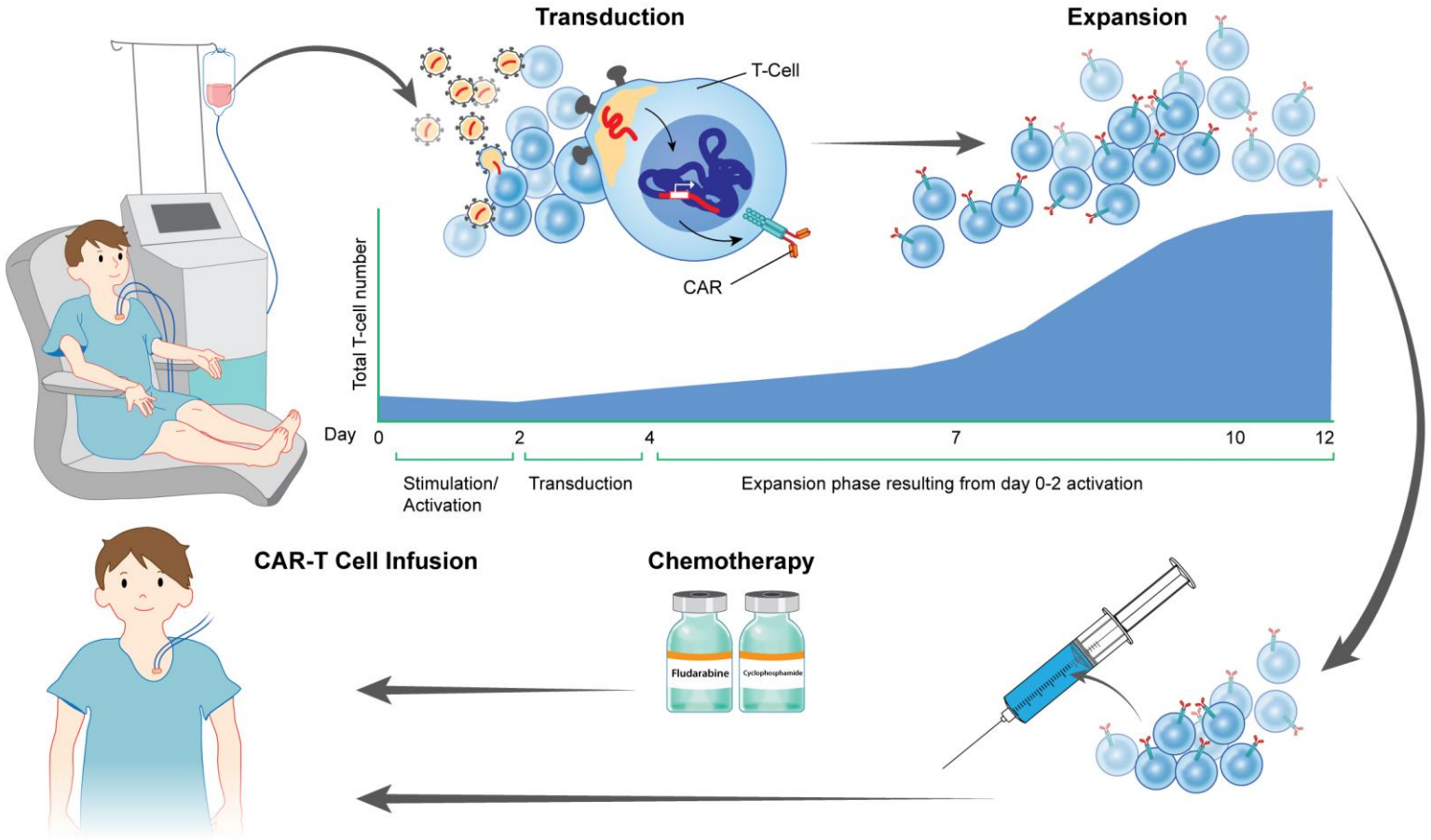
What is CAR-T Cell Therapy?



Nature Reviews | Clinical Oncology

- Chimeric Antigen Receptor
- Customized receptor:
  - Extracellular antigen-binding domain
  - Intracellular signaling domain of T cells
- Retains the functionality of a T-cell with the antigen recognition properties of antibody and is MHC-independent.

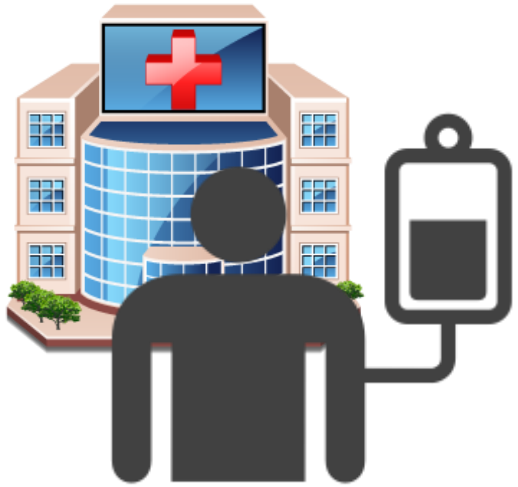
CAR-T Cell treatment is complex



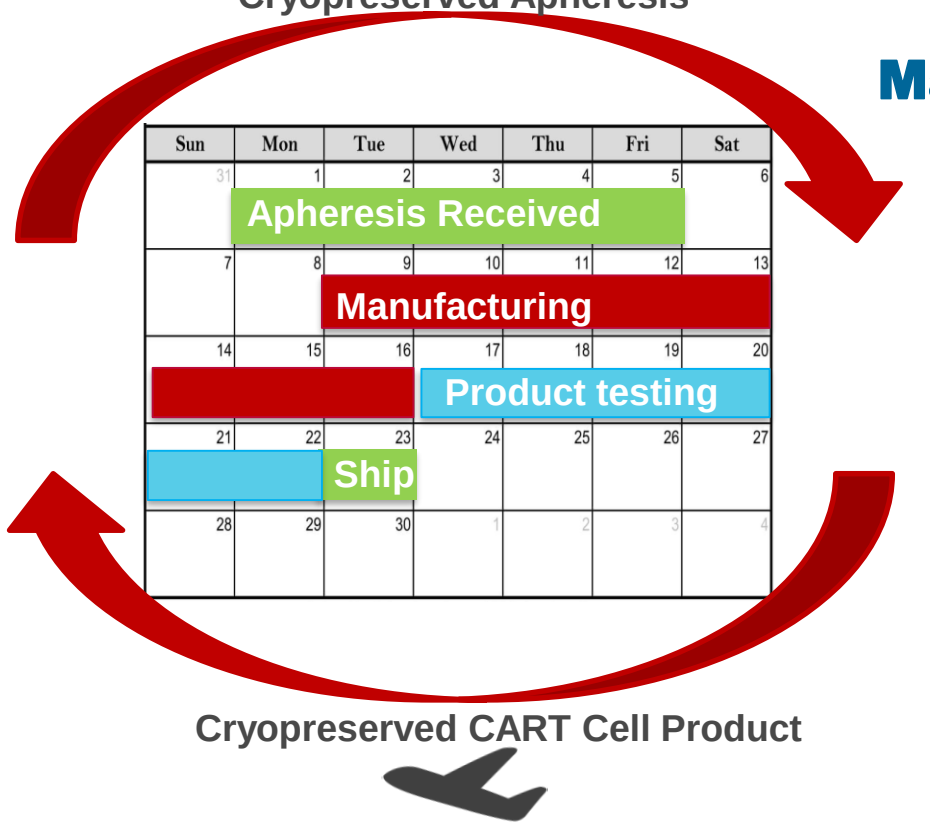
1) Apheresis, then select T-cells
2) Stimulation and Transduction
3) Expansion
4) QC and Safety Testing
5) Lymphodepletion
6) Infusion

Cyro-to-Cryo Manufacturing Logistics

Clinical Sites



Cryopreserved Apheresis



Cryopreserved CART Cell Product

Manufacturing Site: BDP

NCI Biopharmaceutical Development Program at the Frederick National Laboratory

Harnessing the BDP to support cell therapy for cancer

- cGMP manufacturing provides QA rigor and reproducibility for cell therapy products when "*the process = the product*".
- Experience with product chain logistics to support multi-center trials
- Provide cGMP-grade transduction vectors: lentivirus, gamma retrovirus, AAV
- Collaborate with FDA, NIST, other stakeholders to standardize product characterization assays and provide to the cell therapy community

CAR-T manufacturing in ATRF Virus Production Facility

National Cancer Institute

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
National Institutes of Health

Status of clinical trials supported by centralized manufacturing at NCI/FNLCR

Clinical Trial	Sponsor	Status
Phase 1/2 Study of Anti-CD33 Chimeric Antigen Receptor Expressing T-Cells (CD33CART) in Children and Young Adults with Relapsed/Refractory AML	Pediatric Blood & Marrow Transplant Consortium (PBMTC)	• IND: July 2019 • Site Activation: January 08, 2020 • First Subject Enrolled: February 12, 2020 • Two patients infused to date
GD2-CAR PERSIST: Production and Engineering of GD2-Targeted, Receptor Modified T Cells for Sarcoma and Neuroblastoma to Increase Systemic Tumor Exposure	NCI/CTEP Pediatric Cancer Immunotherapy Trials Network (PED-CITN)	• IND submission target August 2020

CD33CAR-T for pediatric acute myeloid leukemia (AML)

Clinical Trial sponsor:

NATIONAL MARROW DONOR PROGRAM® BE THE MATCH®

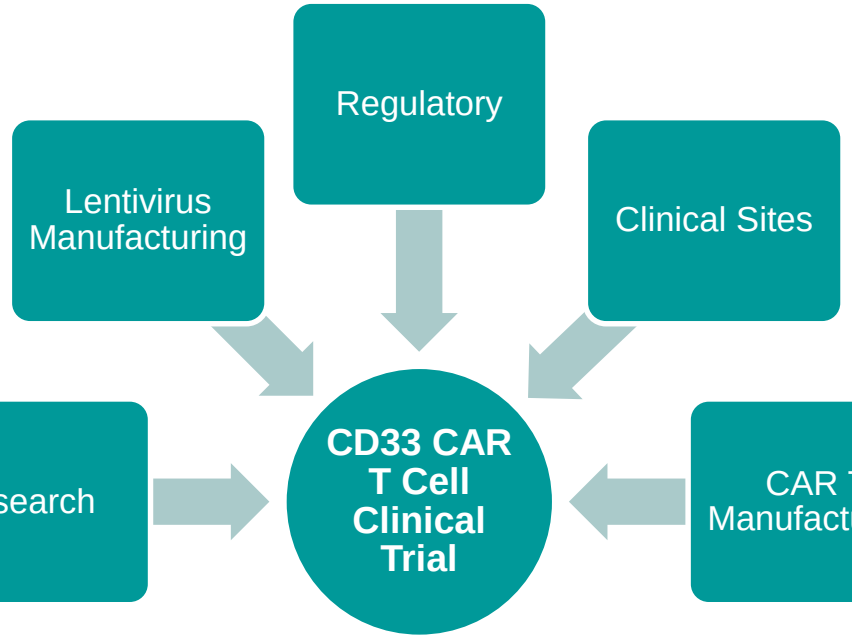
pediatric blood & marrow transplant consortium

Clinical Trial Co-PIs:

Nirali Shah (NCI, CCR)

Richard Aplenc (CHOP)

Children's Hospital of Philadelphia



Clinical Trial Sites:

NIH Clinical Center

Children's Hospital of Philadelphia

Children's Hospital Colorado

Seattle Children's Hospital

Children's Hospital Los Angeles

Boston Children's Hospital

CD33 CAR T Preclinical research:

Terry Fry (NCI/Colorado)

Sarah Tasian (CHOP)

NCI FNLCR:

Anthony Welch (NCI)

George Mitra (BDP)

NIH DTM/CCE:

Dave Stroncek

Steve Highfill

National Cancer Institute

CD33 CART is manufactured with 7-day process

Process flow and timeline are project-specific

Process Flow

Cryopreserved Apheresis product

Day 0
CliniMACS Prodigy:
Platelet Wash,
$CD4^+/CD8^+$ Enrichment,
Culture Initiation,
Activation

Day 1
Transduction with CD33CAR Lentivirus (MOI 20)

Day 3
Transduction Stop:
3 Culture Washes

Day 5
Feed Port
Final Process Volume 250 mL

Day 6
Media exchange (Vol ± 125 mL)

Day 7
Culture Harvest

Cryopreservation
CryoStor CS5

Samples for Testing

Day 0 - Remove Sample
Flow Cytometry ($CD3^+$, $CD4^+/CD8^+$, $CD33^+$), Count, Viability

Day 6 – Remove Sample
Flow (Transduction Efficiency – Protein L)
Total Cell Count

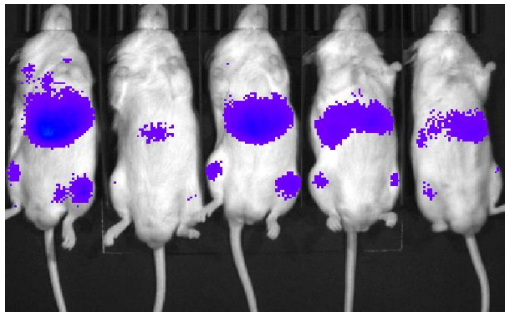
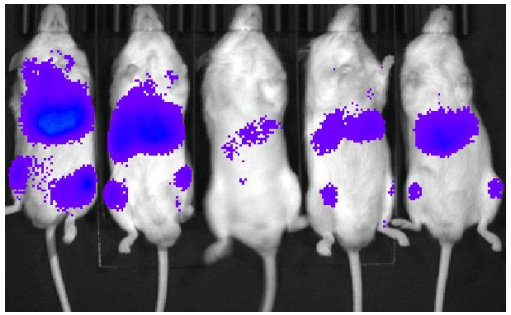
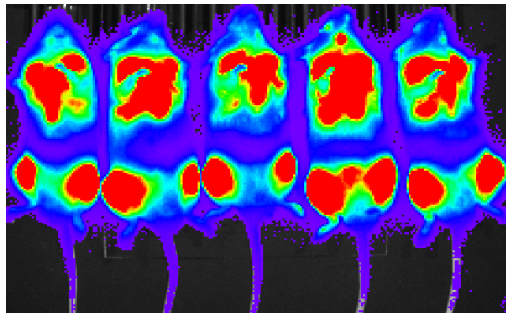
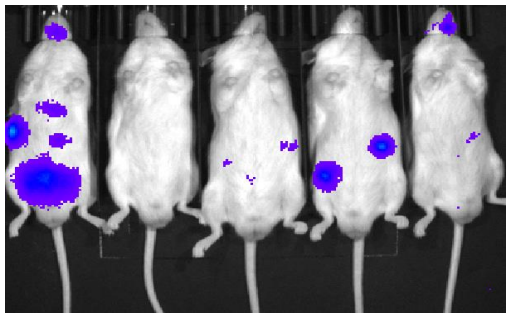
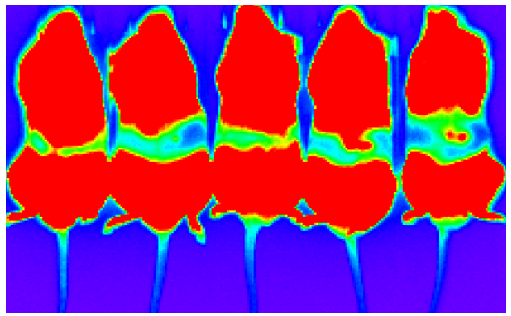
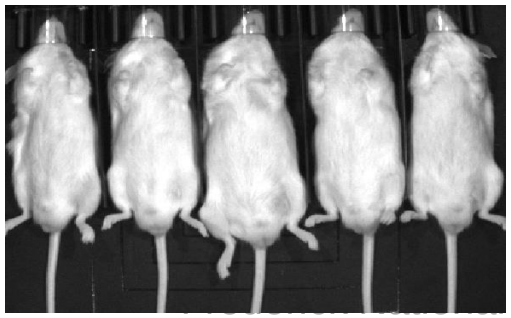
Day 7 – Remove Sample
Flow ($CD3^+$, $CD4^+/CD8^+$, $CD33^+$,
Transduction Efficiency – Protein L,
Identity – CD33Fc) Count, Viability

Gram Stain, Mycoplasma qPCR, VCN,
RCL-qPCR, Sterility, Endotoxin LAL

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
National Institutes of Health

Prodigy-manufactured CD33 CART retain potency

Day 0: IV 1E6 MOLM14-GL
Day 3: ADT 5.2E6 CAR^{+} T cell

	G1: Mock T	G2: CD33 CART
ADT Day 3		
Day 10		
Day 17		

Study Performed by
Haiying Qin, NIH/CCR

National Cancer Institute

CD33 CART production using Prodigy demonstrates:

Robust selection for $CD3^+$ population using AML patient apheresis samples with low or high CD33+ blast cells present

	Process Parameters	Healthy volunteer run1	Healthy volunteer run2	AML Patient with low CD33+ blasts	AML Patient with high CD33+ blasts
Day 0	$CD3^+$	59.3%	56.4%	78.8%	1.4%
	$CD33^+$	<1.38%	<1.38%	1.41%	30.08%
Post CD4/CD8 selection	$CD3^+$	92.08%	94.92%	78.6%	69.8%
	$CD4^+/CD8^+$ Ratio	1.8	1.8	0.49	2.89
	Seeding density	1e8	1e8	1e8	1e8
	Lentivirus MOI	20	20	20	20
Day 7 Harvest	TE (% protein L)	42.2%	41.8%	34.7%	23.7%
	TNC	20.7e8	15.0e8	12.4e8	10.5e8
	$CD3^+$(% total cells)	99.5%	99.5%	99.4%	97.6%
	Fold expansion	20.6	14.9	12.3	10.2
	$CD4^+/CD8^+$ Ratio	2.1	1.7	2.0	9.2
	Viability	91.4%	88.6%	92.2%	93.2%

GD2-CAR-T for sarcoma and neuroblastoma

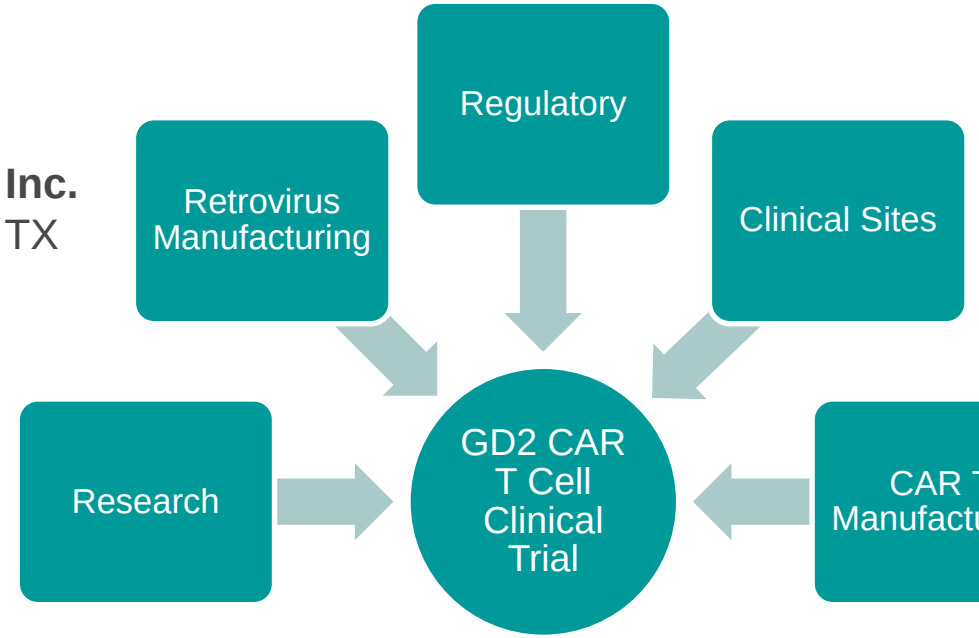
*Gamma-retrovirus vector transduction

Clinical Trial sponsor:

CTEP/Peds-CITN

Clinical Trial Co-PIs:
Crystal Mackall (Stanford)
Rosie Kaplan (NCI, CCR)

Bellicum, Inc.
Houston, TX



Clinical Trial Sites:
NIH Clinical Center
Stanford
Peds-CITN network

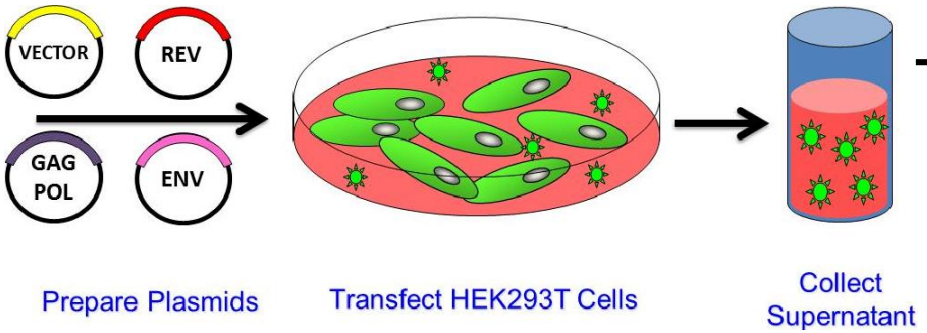
GD2 CAR T Preclinical research:
Crystal Mackall Lab
Stanford

NCI FNLCR:
Anthony Welch (NCI)
George Mitra (BDP)
Tech transfer from Stanford
Steve Feldman, Shabnum Patel

National Cancer Institute

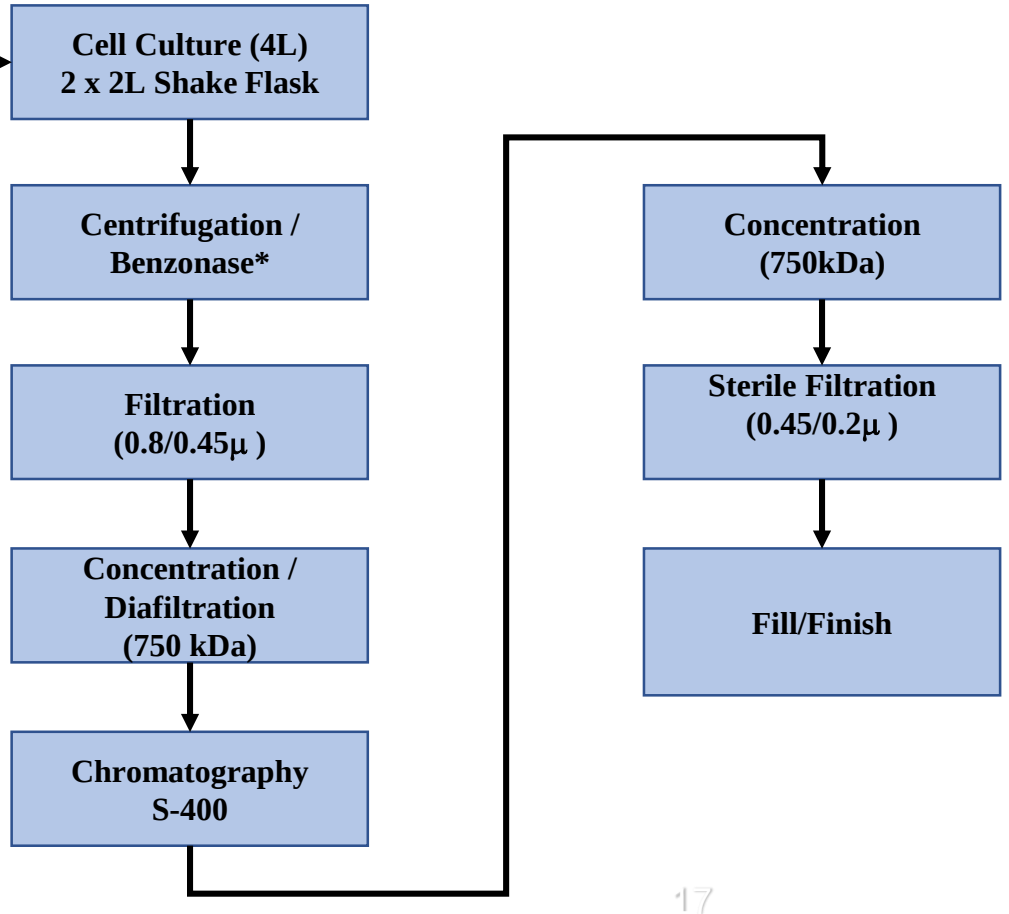
Current NCI/FNLCR lentivirus production platform

A scalable 4-plasmid lentivirus production platform has been developed



Pilot project: *IL12 lentivirus genetically engineered myeloid cells (GEMys) to limit metastatic progression of solid tumors*

Rosandra Kaplan, MD
Pediatric Oncology
Branch, CCR, NCI, NIH



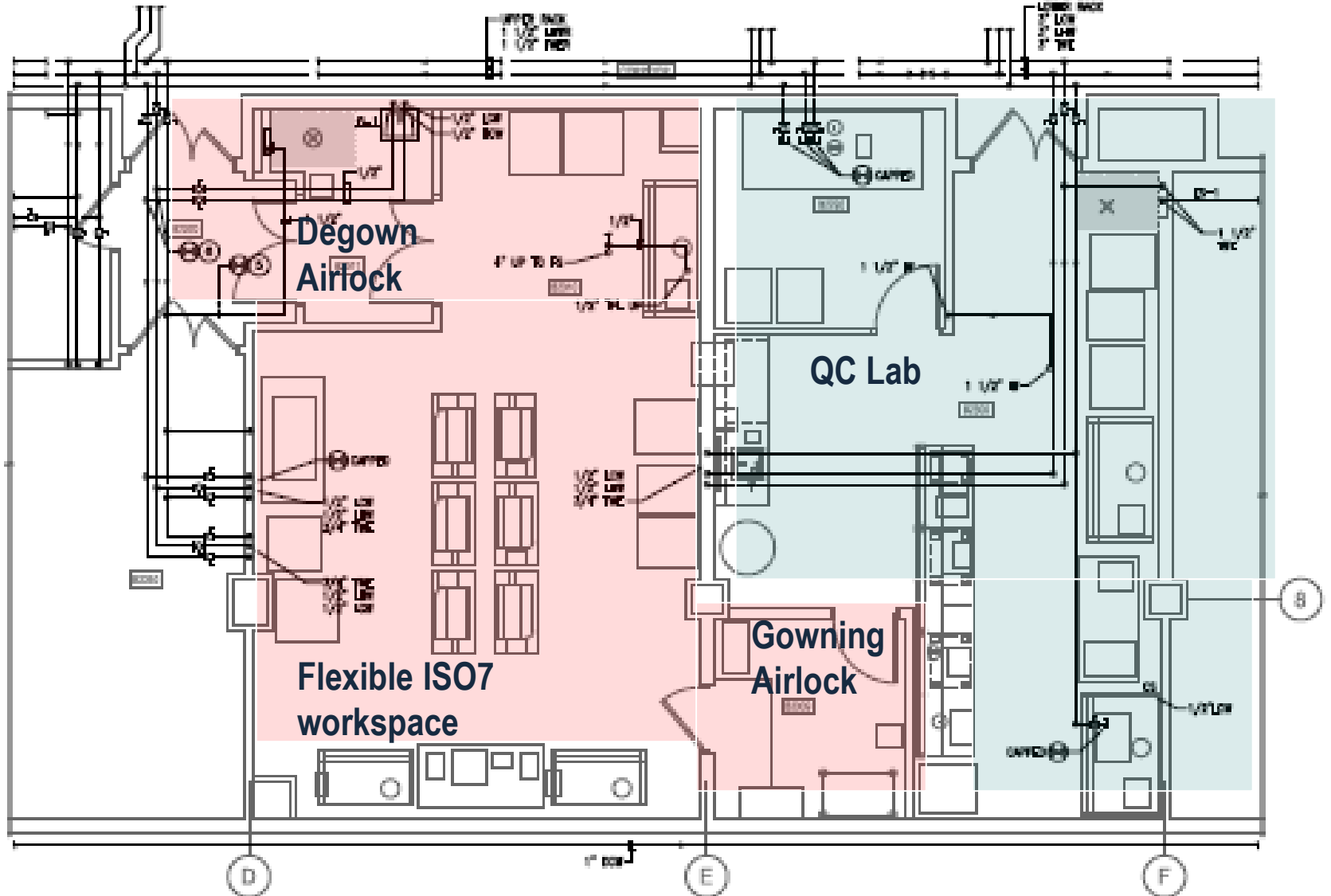
National Cancer Institute

FNLCR Cell Therapy & Virus Manufacturing Capacity

Product Type	Suite	Status/Capacity
Autologous cell therapy	***B2310 Cell Therapy Suite***	• Prodigy-based only • 2 Prodigy units produce 4/month
Lentivirus vectors	***Virus Production Facility (VPF)***	• 1st cGMP production in Q1 2021 • 4 campaigns per year (lenti or retro)
Gamma retrovirus vectors	***Virus Production Facility (VPF)***	• Process development ongoing • cGMP capability by Q2 2021 • 4 campaigns per year (lenti or retro)
Cell therapy or virus vectors	***New construction 3rd floor ATRF***	• 3 new suites expected on-line 2022 • 12 virus campaigns/year • Capacity for cell therapy production is product-type dependent (i.e. autologous or allogeneic, manufacturing platform)

National Cancer Institute

Renovations completed for ATRF B2300/B2310 ISO7 production suite, flexible workspace and adjacent QC testing capabilities



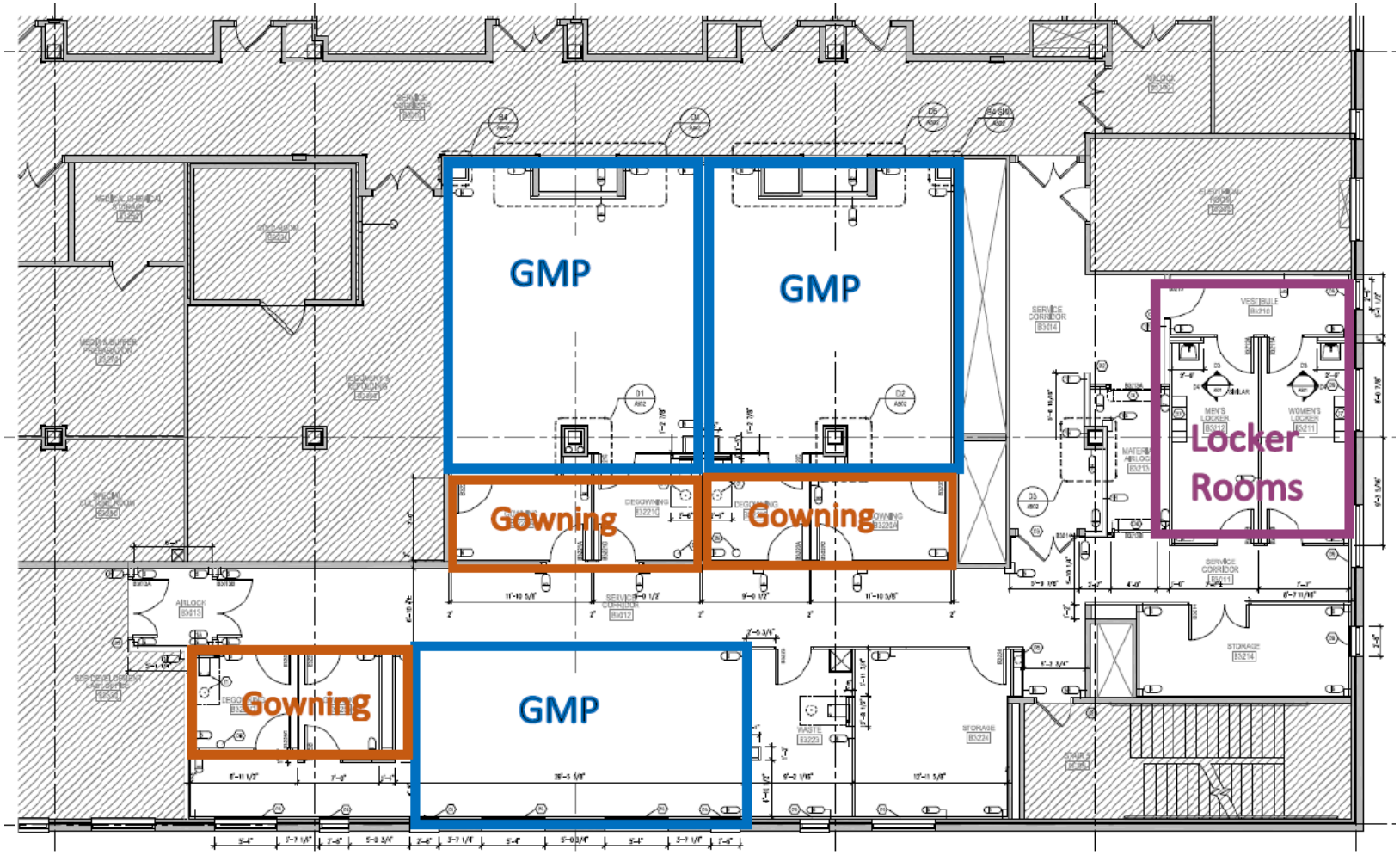
ATRF B2300/B2310 renovation completed

- Validation ongoing but delayed due to COVID shut-down
- Will be ready for cGMP manufacturing in fall 2020

National Cancer Institute

Beginning Fall 2020: Renovations to ATRF 3rd floor B wing

- Expand flexible cell therapy manufacturing space with 3 cGMP rooms
- Provide cGMP production for transduction vectors: lentivirus, gamma retrovirus, AAV



Timeline for cell therapy and vector production

CELL THERAPY DEVELOPMENT AND IND FILING

2 months	Project initiation & MTA
3 months	Tech transfer and assay development
2-3 months	Pilot production, efficacy confirmation
2 months	IND filing
3-6 months	Site activation & patient accrual
~12-15 months	**TOTAL**

VECTOR DEVELOPMENT AND MANUFACTURING

2 months	Project initiation & MTA
2-4 months	Identify and procure packaging plasmids
2-3 months	Feasibility (when GOI plasmid, testing protocols provided)
1 month	GMP production run
2-3 months	Testing and release (QC/QA).
9-13 months	**TOTAL**

National Cancer Institute

Future of Cell Therapy and Vector Production

- New autologous CAR-T projects take 10-12 months to reach IND.
  - Production platforms other than Prodigy will take additional time to establish
  - Cell therapies other than CAR T will take additional time to establish
- Site-activation and intitial accrual may take an additional 3-6 months.
- cGMP vector production projects (lenti, retrovirus, AAV) take about 9-12 months to complete development, cGMP manufacturing and product release.
- Gene-editing (e.g. CRISPR/Cas9) technology development for cell-based immunotherapy is being launched in September 2020

A queue must be established to generate cost-effective manufacturing capabilities.

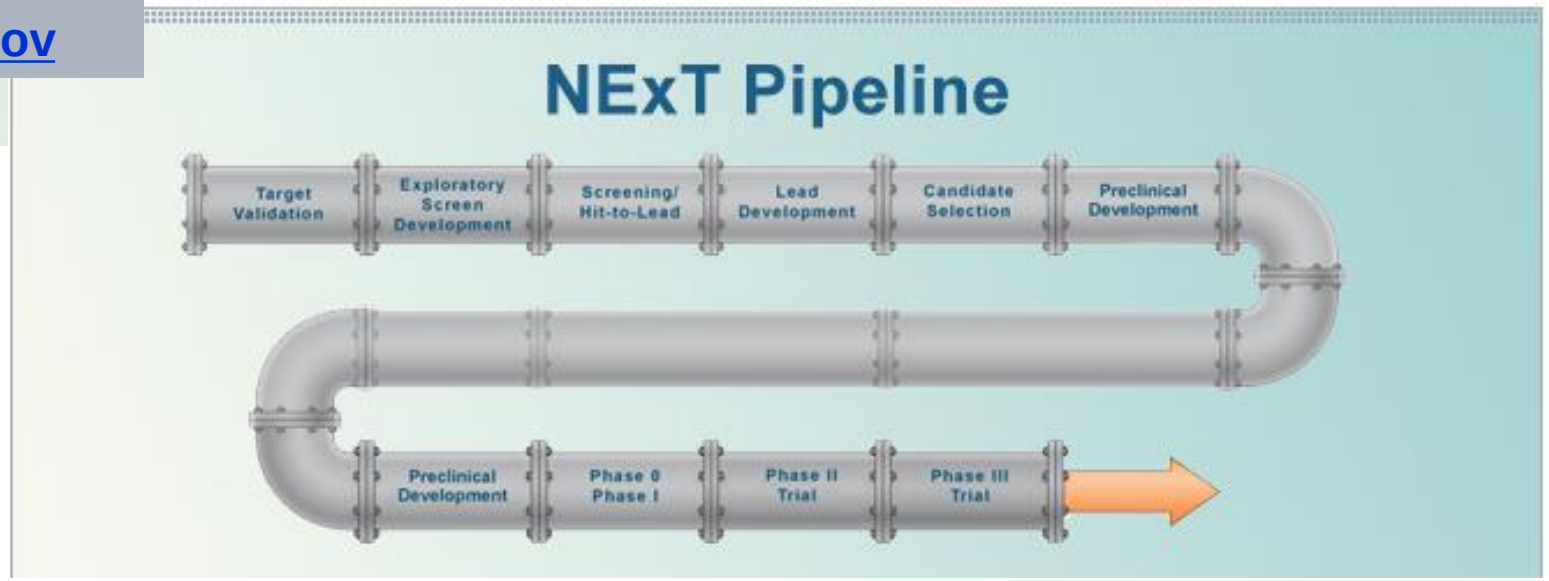
National Cancer Institute

Extramural innovators can access cell therapy manufacturing (including viral vectors) through the NCI NExT program

About NExT

The mission of the NExT Program is to advance clinical practice and bring improved therapies to patients with cancer by supporting the most promising new drug discovery and development projects.

https://next.cancer.gov



Services for developing small molecules, biologics and cell therapies

Acknowledgements

BDP:
George Mitra
Doug Gaum
Sheryl Ruppel
John Roach
Trevor Broadt
Julie Blake
Senthil Sivagurunathan
Marie Elena Fraley
Ramarao Vepachedu
Naga Selvaraj
Brain Bowser
Kevin Urak
Vinay Vyas
Xiaoyi Yang
Greg Feaga
Alokesh Ghosal
Hui Dong
Cynthia Ng

NCI/NIH:
Jim Doroshow
Anthony Welch
Jason Yovandich
Rachelle Salomon
Ray Harris
Carrie Laurencot (now at FDA)
Nirali Shah
Dave Stroncek
Steve Highfill
Haiying Qin
Sandhya Panch

CCR Clinical Trial Co-PIs:
Nirali Shah (NCI)
Rosie Kaplan