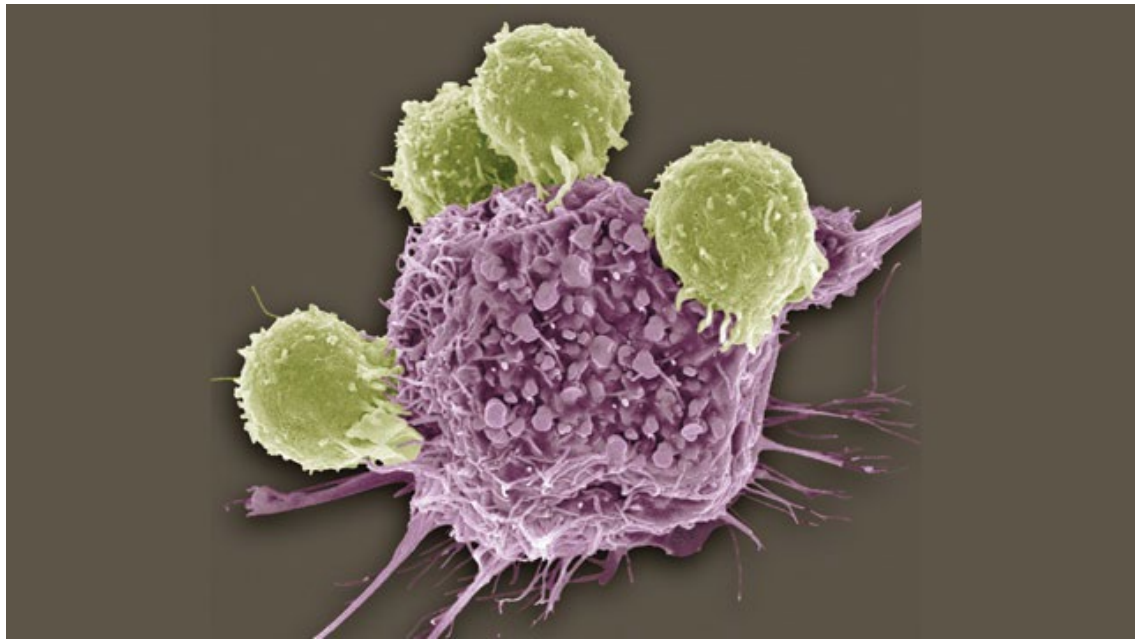
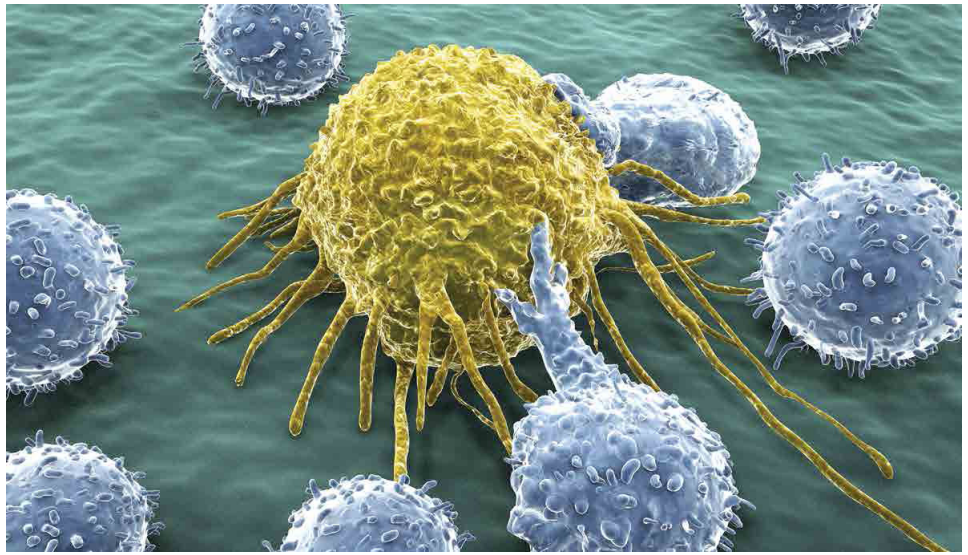
National Cancer Institute

Cell Therapy: CMC considerations to prepare a bridge from preclinical to clinical

Anthony R. Welch, PhD
Biologics Resources Branch
DTP/DCTD/NCI





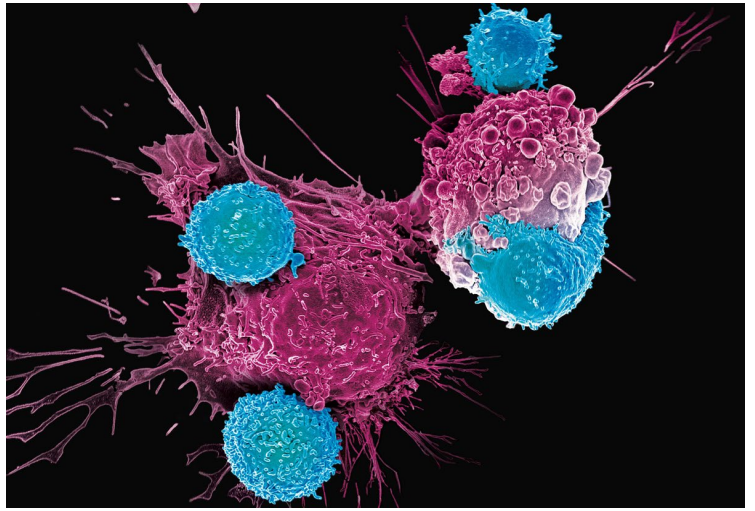
September 10, 2021
DTP Drug Development Workshop

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

National Cancer Institute

Cell and gene therapy for cancer is regulated by FDA

- The FDA Office of Tissues and Advanced Therapies (OTAT), Center for Biologics Evaluation and Research (CBER) regulates cell therapy.
- See current FDA guidances at: https://www.fda.gov/vaccines-blood-biologics/biologics-guidances/cellular-gene-therapy-guidances
- Early interaction with FDA is valuable to clarify CMC expectations:
  - INTERACT meetings
  - Pre-IND meetings



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

Cell therapy manufacturing is complex and thus, CMC issues will be product specific

Autologous single product lot

Large scale allogeneic cell bank-based product

Common concerns

Mechanism of action, material qualification, challenges establishing specifications, manufacturing facility, product shipping/handling, major manufacturing changes

Specific concerns

- Product tracking and segregation
- High product variability
- Limited material or time for testing
- Short shelf life
- Manufacturing logistics
- Scale-out

- Donor eligibility
- Qualification of cell banks
- Reproducibility of replacement bank
- Stability of cell banks and intermediates
- Scale up

Position your preclinical research for translation:

- *Interact with both the clinical and GMP manufacture teams ASAP*
- *This is a critical point since it is not easy to "GMP-ify" your cell therapy product like a Hulk-ification*

Position your preclinical research for translation:

- Identify important critical quality attributes of your product.
- Identify identity, purity, potency, safety assays to release product.
- Decide on cryo/fresh logistics early in process so you can use to support preclinical data
- Determine how you will ‘count cells’ for your dose, and whether you will flat dose or dose/kg.
- Where will you get your virus vector? Use a similar quality for your preclinical studies. Who will do the stability testing on the vector?

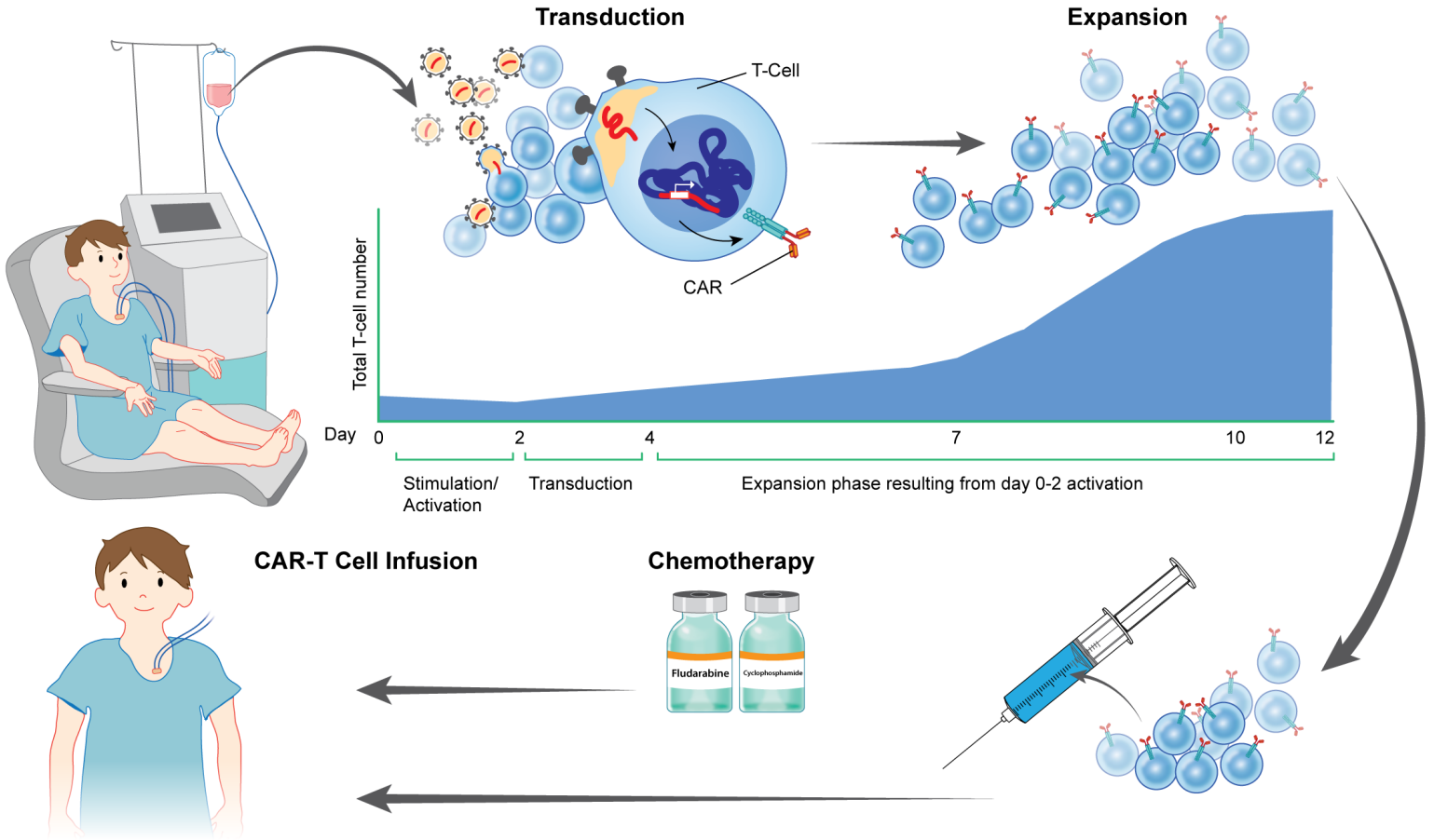
National Cancer Institute

An example that highlights CMC Challenges: CD33CART for pediatric acute myeloid leukemia (AML)

- **Process Qualification:** Demonstrate cells manufactured with GMP process work in preclinical model. Evaluate production reproducibility and robustness. Evaluate stability pre-cryopreservation and post-cryopreservation.
- **Aseptic process validations:** Split process into unit operations for validation.
- **Rapid product release:** QC/QA systems to review and ship cryopreserved product 6 days post-harvest (minimum 3-day sterility data with all other safety tests). Justify to FDA and include detailed Action Plan in case of post-infusion positive result.
- **Shipping stability study:** Demonstrate product is stable after shipping.

National Cancer Institute

CAR-T Cell treatment is complex



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

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

Why target CD33?

- CD33 is expressed on >85% of childhood AML
- Bright CD33 surface expression is enriched in high risk AML subtypes and associated with inferior outcomes
- Addition of gemtuzumab ozogamicin (anti-CD33 MAb) to chemotherapy improved EFS in many patient subsets on COG AAML0531 and other trials

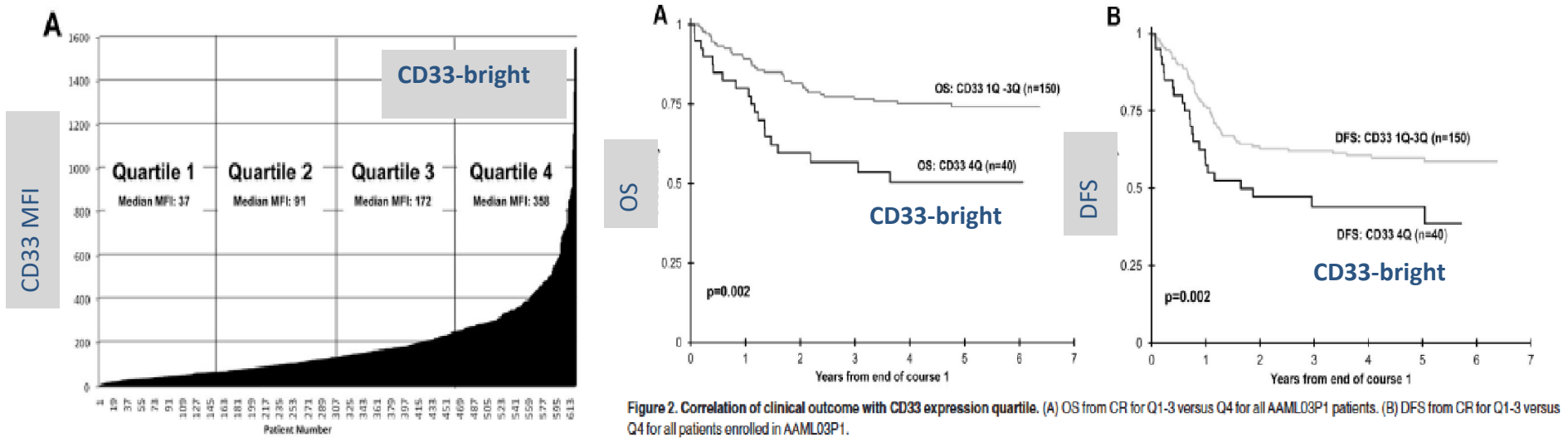


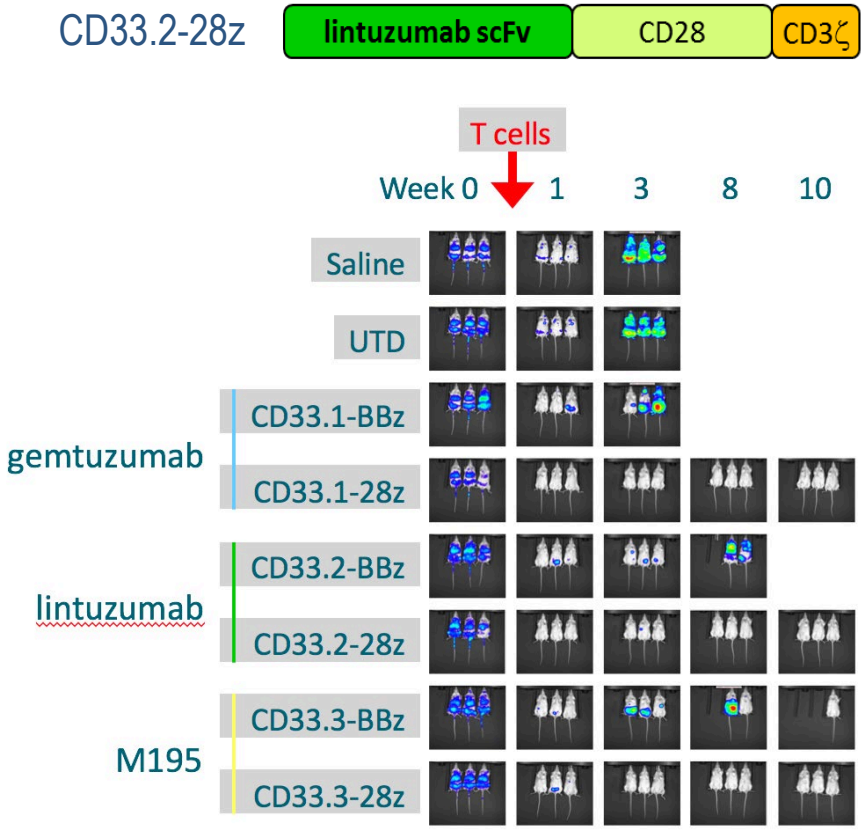
Figure 2. Correlation of clinical outcome with CD33 expression quartile. (A) OS from CR for Q1-3 versus Q4 for all AAML03P1 patients. (B) DFS from CR for Q1-3 versus Q4 for all patients enrolled in AAML03P1.

Gamis *et al* JCO 2014, Pollard *et al* Blood 2012 and JCO 2016

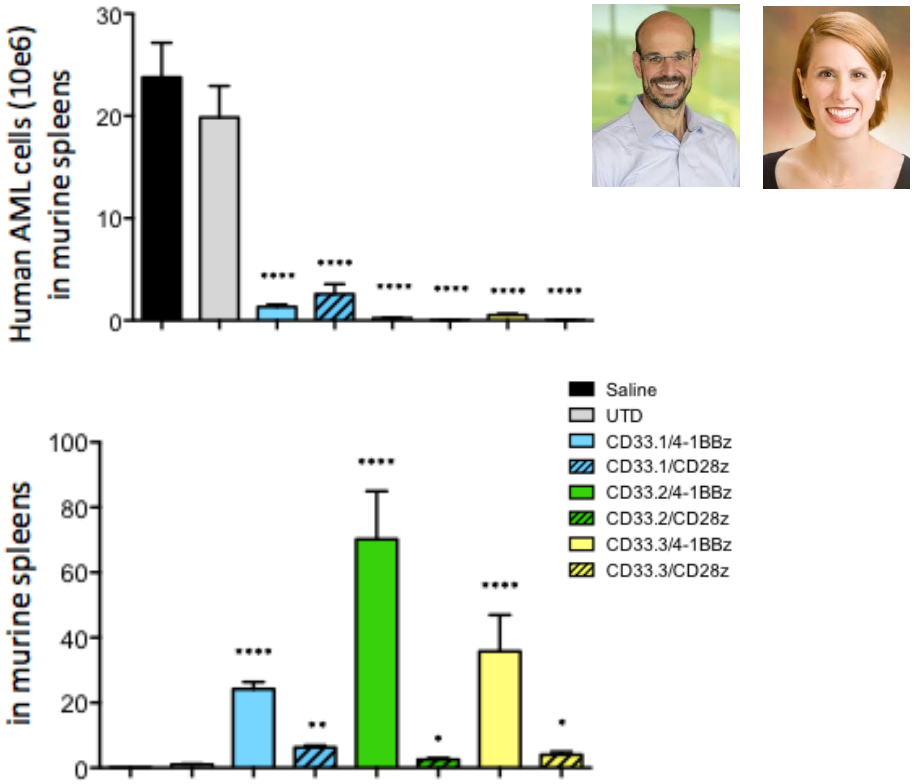
National Cancer Institute

Preclinical evaluation of CD33CARTs:

CD33.2-28z construct was chosen due to efficacy and shorter persistence



AML PDX model (JMML117) at
3 weeks post CART treatment



Tasian, et al. 2021 Journal for ImmunoTherapy of Cancer, accepted for publication

How do we manufacture the CAR T cells?

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

MACS Miltenyi Biotec

- **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**

National Cancer Institute

Example CART project:
CD33 CART 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

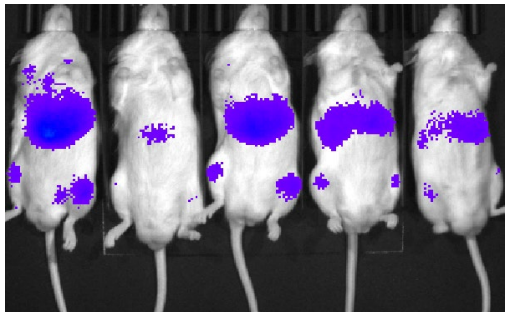
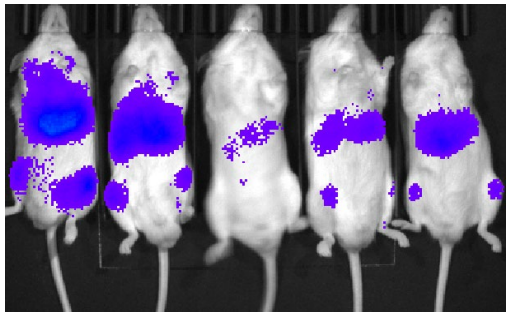
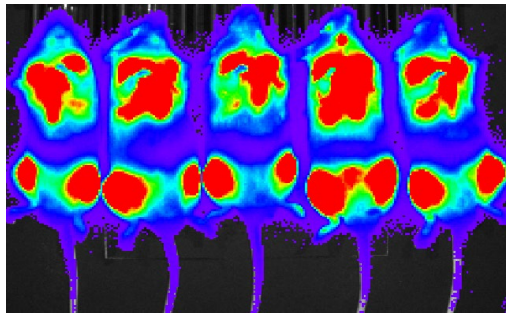
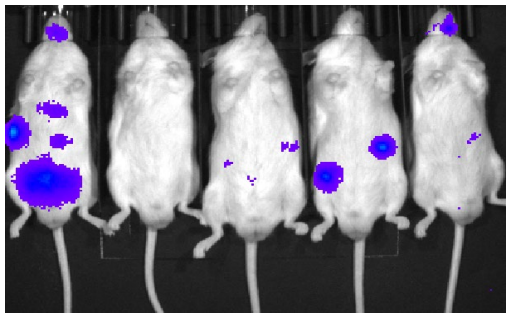
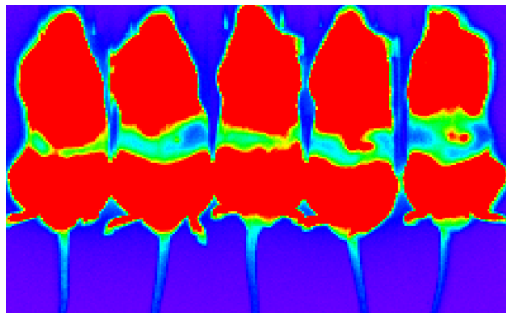
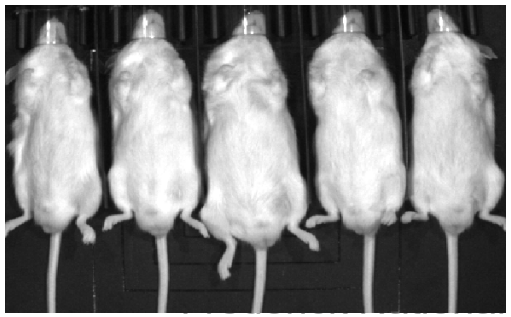
Day 6 – Remove Sample
Flow (Transduction Efficiency – Protein L)
Total Cell Count

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

Does your full-scale manufactured product retain activity?

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

	Mock T	CD33 CAR (Prodigy)
ADT Day 3		
Day 10		
Day 17		

National Cancer Institute

Is your production reproducible and robust?

	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%

Is your final product stable before and after thaw?

Dose formulated in CryoStor CS5 is stable for up to 4 hours at room temperature

(pre-cryopreservation in-process hold time)

Lot	% Viability			CAR expression (% Protein L)		
	0 Hr	2 Hr	4 Hr	0 Hr	2 Hr	4 Hr
L1901001RD	91.2	89.6	90.1	42.2	42.9	42.4
L1901006RD	88.8	86.9	86.7	41.8	41.7	41.8

Final product is stable up to 6 hours post-thaw at room temperature

(post-thaw stability at clinical site)

Lot	% Viability (post-thaw)			
	0 Hr	2 Hr	4 Hr	6 Hr
L1901001RD	84.4	87.2	86.2	84.7
L1901006RD	84.0	81.9	82.3	80.9

Aseptic process validation: Unit operations validated separately

Unit operation 1
Day 1: Apheresis washing
Open process done in BSC

Unit operation 2
Day 1-7: Prodigy manufacturing
Closed process

Unit operation 3
Day 7: Dose formulation in CryoStor CS5
Open process done in BSC

Example COA shipped with cryopreserved product

Note: Final COA is published following 14-day sterility test completion

Interim
Certificate of Analysis
Genetically Modified Autologous T-cells from Patient Apheresis

DIN:	W092116060161	Protocol/IND#:	TBD when IND approved
Recipient ID:	CD33-12345-6789	Principal Investigator:	Dr. Nirali Shah
Dose/Kg:	3×10^5	Recipient Weight:	95 kg
Harvest Date:	April 2, 2019	Release Date:	April 5, 2019
BDP Lot #	L1903007RD		

Product Content, Appearance, and Label Verification

Test	Method / SOP # / QCTR #	Result	Units / (P/F)
Appearance	Visual Inspection / SOP 22925 / QC-060953	Milky white fluid w/no visible aggregates	Pass
Total Viable Nucleated Cells (TNC)	Trypan Blue / SOP 12227 / BPR	81.8×10^6	Cells
Total Viable anti-CD33 CAR Transduced T-cells	Flow Cytometry / SOP 22206 / QC-060946	28.5×10^6	Cells
Bag Volume (mL)	N/A	25	mL
Verification of Bag Label ID and Content	Final Product Label Inspection / SOP 21913	Conforms to Specification	Pass

Product Characterization Results

Test	Method / SOP #	QCTR #	Specification	Result	Pass/Fail
Post-thaw Viability	Trypan Blue / SOP 12227	060954	≥ 70%	86.2%	Pass
Pre-freeze Viability	Trypan Blue / SOP 12227	060954	≥ 70%	94.8%	Pass
% viable CD3*	Flow Cytometry / SOP 22206	060956	Report %	99.7%	N/A
% viable transduced CD3*	Protein L Flow / SOP 22207	060946	≥ 15%	34.7%	Pass
Identity	CD33Fc Flow / SOP 22211	060980	Positive (%)	33.8%	N/A
% viable CD33*	Flow Cytometry / SOP 22213	060955	≤ 5%	0.4 %	Pass

Product Safety Test Results

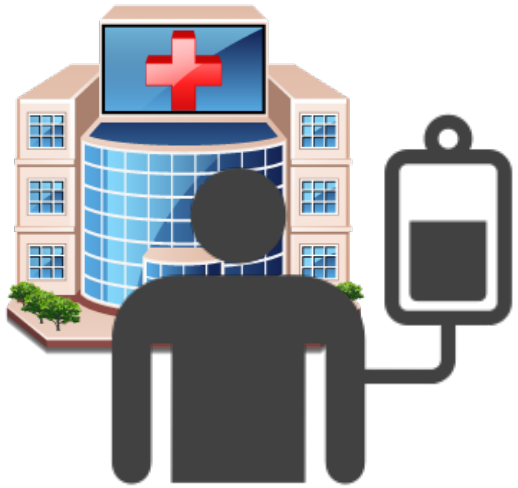
Test	Method / SOP #	QCTR #	Specification	Result	Pass/Fail
Sterility (Preliminary, Day+3)	21 CFR 610.12 / DLM-STS	060958	No growth*	No growth	Pass
Sterility (Final, Day+14)	21 CFR 610.12 / DLM-STS	060958	No growth*	*Pending*	Pending
Mycoplasma	qPCR / SOP 22208	060961	Negative*	Negative	Pass
Gram Staining	Gram Stain / SOP 22137	060962	Negative*	Negative	Pass
Endotoxin	KC LAL / SOP 22135	060963	≤ 5 EU/mL	<0.5 EU/mL	Pass
Replication Comp. Lenti.	VSV-G qPCR / SOP 22209	060960	Negative	Negative	Pass
Vector Copy Number/Cell	ddPCR / SOP 22970	060959	≤ 5	0.47 Copies/cell	Pass

*Positive results will be speciated.

All Release Criteria are:	MET	QC Reviewed By/Date:	"Mock Signature" QC Director
All Release Criteria are:	MET	QA Reviewed By/Date:	"Mock Signature" QA Manager
All Release Criteria are:	MET	BRB Reviewed By/Date:	"Mock Signature" BRB

Cyro-to-Cryo Manufacturing Logistics

Clinical Sites



Cryopreserved Apheresis

Sun	Mon	Tue	Wed	Thu	Fri	Sat
31	1	2	3	4	5	6
	Apheresis Received					
7	8	9	10	11	12	13
	Manufacturing					
14	15	16	17	18	19	20
			Product testing			
21	22	23	24	25	26	27
		Ship				
28	29	30	1	2	3	4

Cryopreserved CART Cell Product

Manufacturing Site: BDP

NCI Biopharmaceutical Development Program at the Frederick National Laboratory

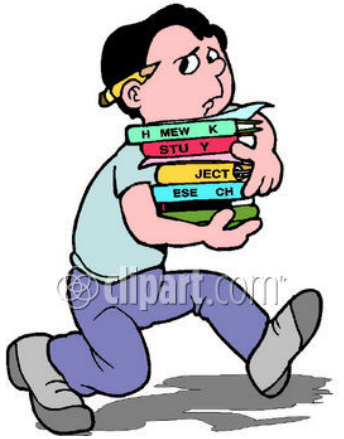
Is your product stable during shipping?

2 identical doses were generated from single Prodigy run. 1 product shipped to clinical site and shipped back to manufacturer. Both products thawed and tested together. Cryoport was the shipper.

Assay	Cryopreserved and Shipped	Cryopreserved, not shipped
TNC/dose (FACS)	3.43e7	3.83e7
Viability (7-AAD, FACS)	91.4%	94.4%
Viability (trypan blue)	84%	82%
Viable $CD3^+$	99%	99%
$CD4^+/CD8^+$ ratio	1.5	1.5
Percent CAR T (protein L)	34.6%	34%
Bioburden	No Growth	No Growth
Gram stain	Negative	Negative

National Cancer Institute

Some take home points



- Interact with both the clinical and GMP manufacture teams ASAP.
- Engage the FDA early in the process (INTERACT and/or pre-IND)
- Identify critical quality attributes and monitor them through scale-up.
- Reproduce preclinical efficacy studies with cells made using GMP process.

Questions? **Contact me anytime:**

Anthony Welch, PhD

welcha@nih.gov

301-846-5691