“And Now We Know!”

Lessons from establishing North Dakota’s first Cellular Therapy Laboratory

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Sanford Health Fargo

SANFORD HEALTH

Objectives

1. Define key terms relevant to cellular therapy and bone marrow transplantation.

2. List laboratory equipment commonly used by a hospital cellular therapy lab in support of bone marrow transplant.

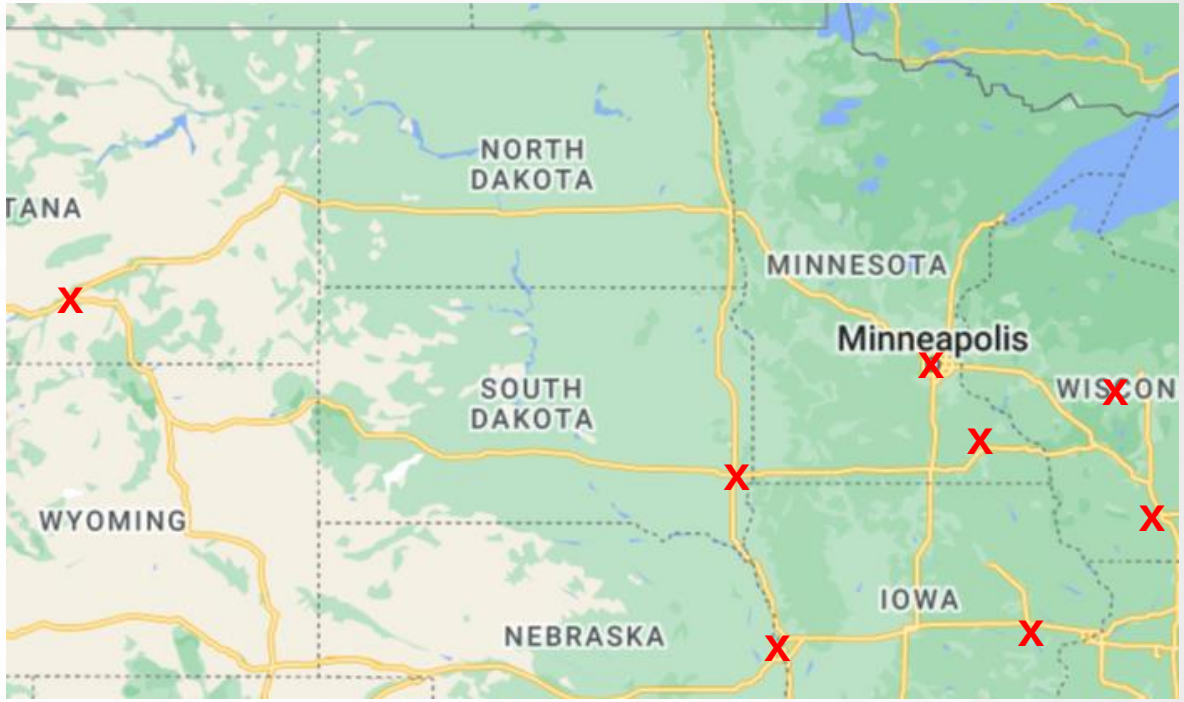
3. Name the accrediting bodies with standards pertinent to cellular therapy labs.

4. List key people and services inside and outside of the laboratory required for a successful transplant program.

Hematopoietic Stem Cell Transplant: A Local Need

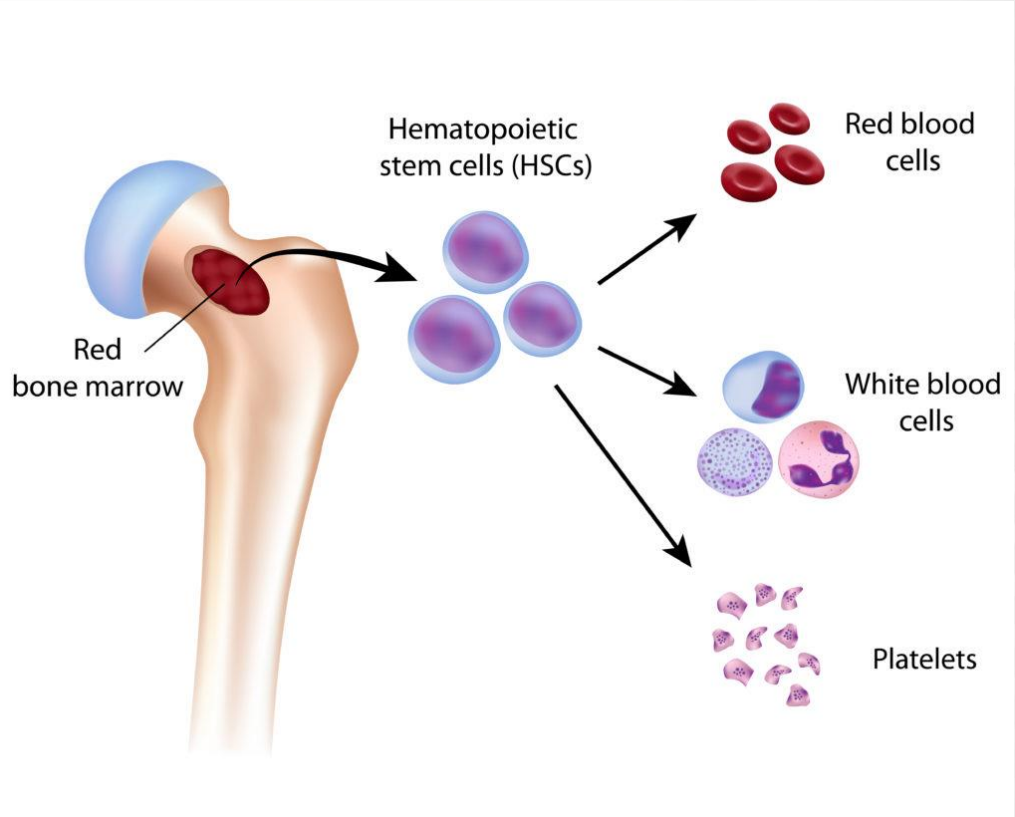
- Observed incidence, per 100,000 persons (US, 2018)[1]:
  - Multiple myeloma – 6.4
  - Non-Hodgkin's lymphoma – 18.5
  - Leukemia – 12.7

- North Dakota population (est. July 2021)[2]:
  - 774,948



Background: HSCT

- Synonyms:
  - Hematopoietic Stem Cell Transplant (HSCT)
  - Stem Cell Transplant
  - Bone Marrow Transplant (BMT)
- HSCT consists of conditioning chemotherapy, followed by infusion of liquid cell product
- **Engraftment:** sustained, measurable evidence of hematopoiesis



Background: HSCT Donors & Sources

- Two main categories of HSCT based on cell donor:
  - Autologous (donate for self)
  - Allogeneic (donate for other)

- Sources of HSCs:
  - Bone marrow
  - Umbilical cord blood
  - Peripheral blood (apheresis)

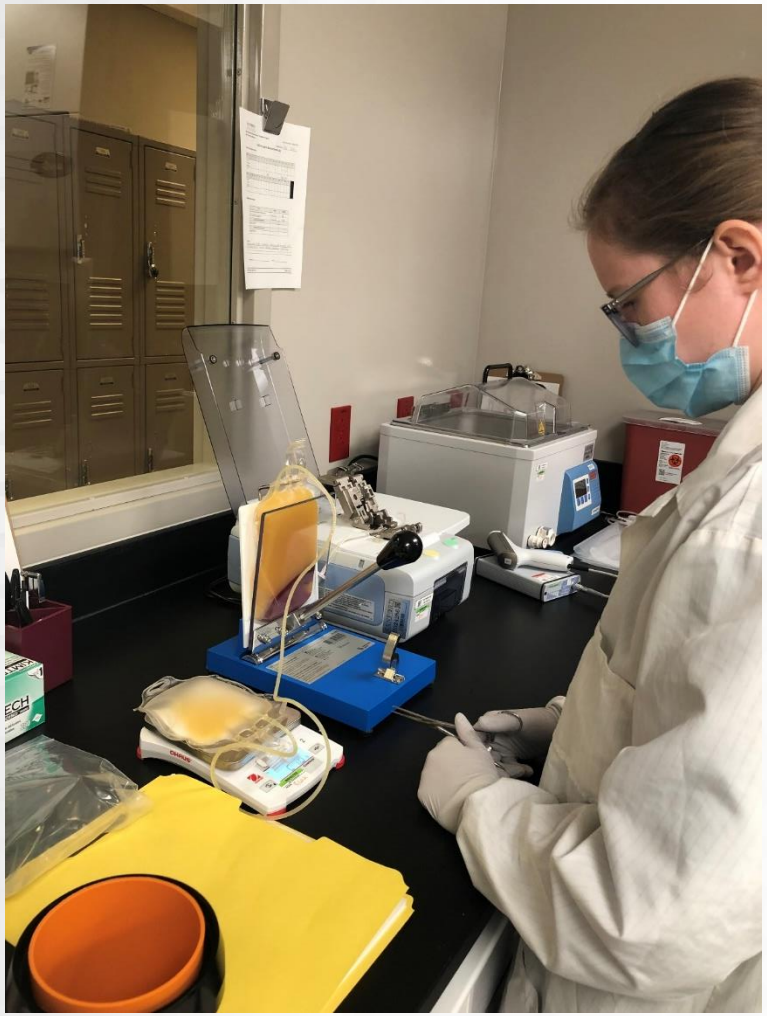
Background: What is Cellular Therapy?

- **Cellular therapy:** the use of human cells, tissues, or cell & tissue-based products (HCT/Ps) to treat, or support treatment, of disease
- HCT/Ps are regulated as biologics by the US Food & Drug Administration (FDA)
- Growing field that includes:
  - Cell products for HSC/BMT
  - Immune effector cells (e.g. CAR-T cells)
  - Orthobiologics (mesenchymal stem cells, MSCs)
- Does not include blood, blood components, and whole organs

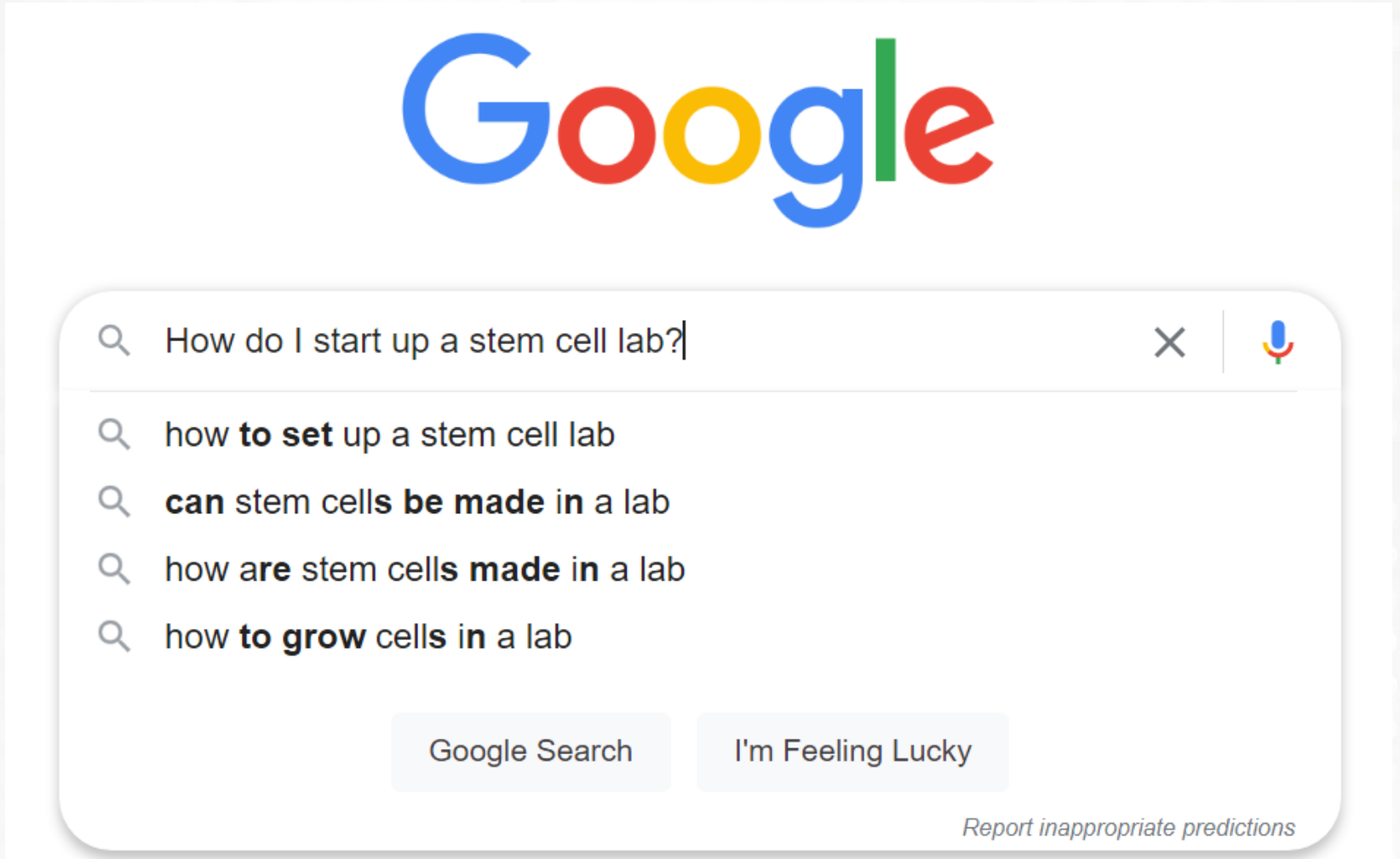
Background: What does a Cellular Therapy Lab (CTL) do?

- Manufactures HCT/Ps as needed for customers:
  - Cell recovery (collection/isolation)
  - Product processing/modification, cell expansion
  - Labeling
  - Storage
  - Distribution for use
  - Shipping/receiving
- Compliance with Good Tissue Practice (GTP; 21 CFR 1271) and Good Manufacturing Practice (GMP; 21 CFR 600), as applicable
- Accrediting bodies: Foundation for Accreditation of Cellular Therapy (FACT), AABB, CAP

Not all CTLs are alike!



https://www.labmanager.com/product-news/ge-healthcare-launches-multifunctional-cell-processing-system-4292



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Step 1: Setting Goals

- Sanford Health Fargo wants to establish **FACT-accredited**:
  1) Autologous HSCT program
  2) Allogeneic HSCT program
  3) CAR-T program
  4) Apheresis collection facility
  5) Bone marrow collection facility

Are these goals attainable, and when?

- 2019 – Expert Consultant Review Findings:
  - Adequate patient volume in region
  - Provided equipment list, cost estimates
  - Starting point for timelines, budgeting, to-do lists

- Focused goals:
  - Perform first autologous HSCT at Sanford Fargo in July, 2021
  - Obtain FACT accreditation for auto HSCT, allo HSCT, and CAR-T all at once (estimated 2024)

Step 2: Assemble a Team

- FACT-required positions:
  - BMT Clinical Program Medical Director
  - Apheresis & Processing Facility Medical Directors
  - BMT Program Quality Manager
  - Adequate staff trained for inpatient care, apheresis collection, and laboratory manufacturing
- Need a “quarterback” – keep whole team working toward goal
- Need “coaches” – knowledgeable in HSCT and FACT standards

Sanford's Starting Lineup…

- "Quarterback" – Director of Advanced Therapeutics & Research (RN)
- "Coach" – expert consultant with FACT inspector experience (RN)
- Directors:
  - Interim Program Director – Hematology-Oncology physician
  - Apheresis & CTL Director – BB/TM trained physician
- CTL Techs: two chosen with ~3 years blood bank experience
- Lab Quality Specialist assigned to CTL

Step 3: Create a Road Map

- Envision what the process might look like given existing processes, available tools and resources
- Representatives from all relevant groups (lab, oncology, facilities, IT, etc.) helps identify problems & solutions
- Key to do before writing policies & procedures

Auto-HSCT Road Map

Patient evaluation

- Referral process
- BMT visits (multiple)
- Labs & diagnostics
- Evaluation by other specialists as needed
- Multidisciplinary candidacy meeting

- Questions: acceptance criteria? Which insurers will work with us?

Cell Collection

- Need catheter placement
- Borrow apheresis machines from dialysis

- Questions: Where to perform?
- Whose staff performs?
- Staff training?
- Order sets and documentation flowsheets?
- Patient safety parameters?

Cell Processing

- Build lab at Broadway Medical Campus
- Utilize existing labs to perform necessary product testing

- Questions: Equipment and supplies to purchase?
- How to train staff?
- LIS vs. paper documentation?
- Materials for validation?

Transplant

- Occur on inpatient oncology floor
- Patient hospitalized from conditioning chemo through neutrophil engraftment (2-3 weeks)

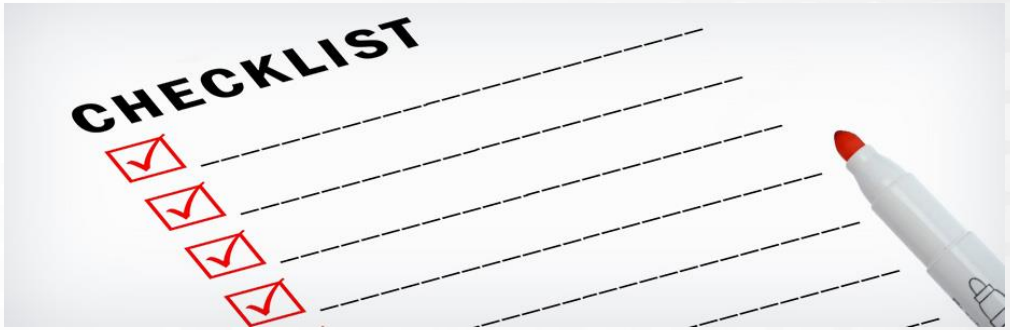
- Questions:
- Are current rooms sufficient?
- Pharmacy experienced with required chemo regimens?
- How to document in the EMR?
- Staff training?

What “extras” will an auto-HSCT patient need?

- Specialists to evaluate & manage other medical comorbidities
- Facilities: positive pressure inpatient rooms, filtered water for bathing
- Long-term lodging (~2 weeks) for close post-transplant follow up
- Allied health professionals: dietician, psychologist, financial advocate, social worker, patient educators, environmental services
- Laboratory: contracted services (equipment service, backup processing, donor infectious disease testing)

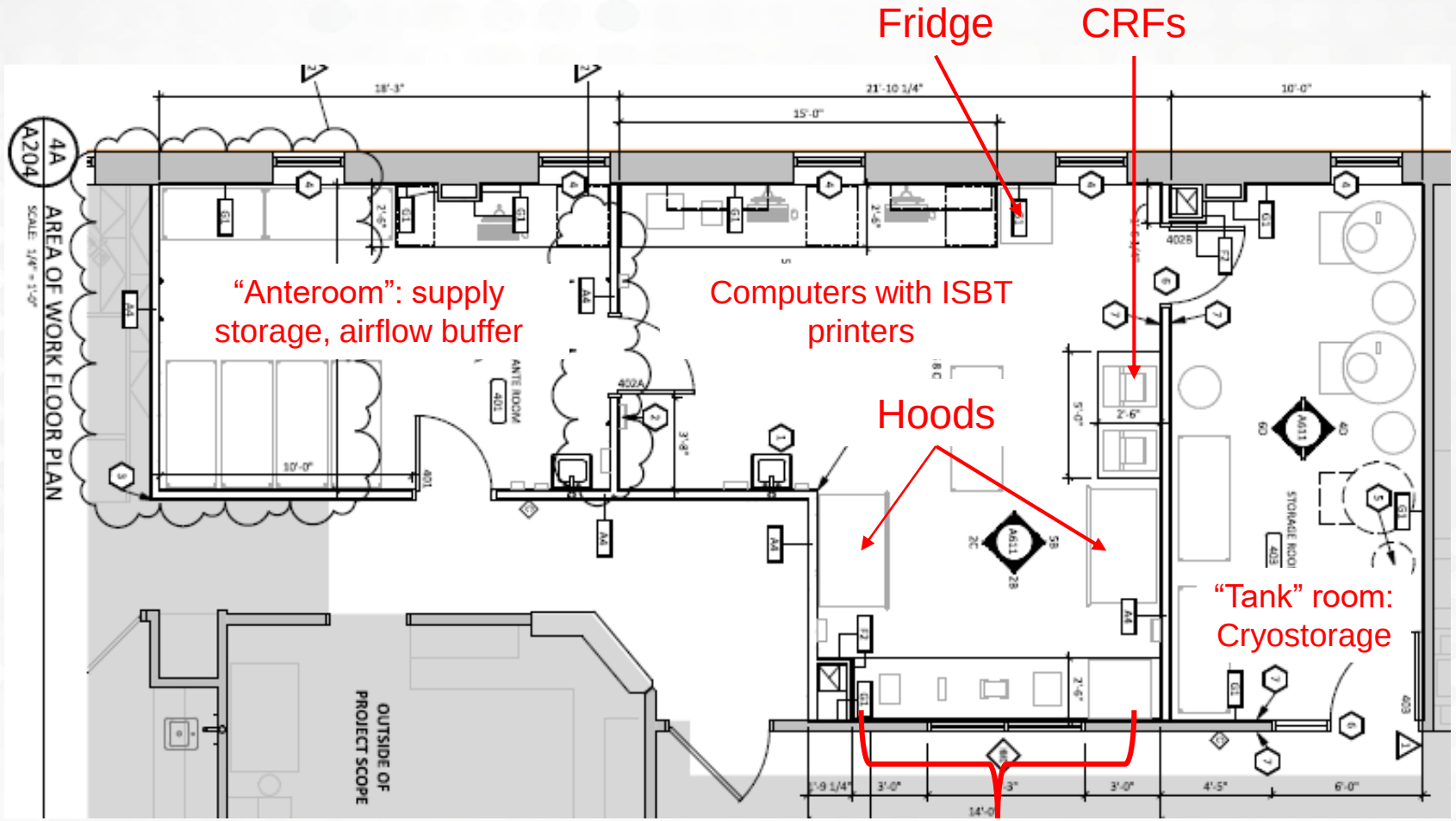
Step 4: Construction

- CTL Planning Projects:
  - Design & construction of CTL
  - SOP writing
  - Equipment purchasing & install
  - Supply purchasing & organization
  - Staff education & training
  - Process validation plans



Designing the CTL

- **Primary Function:** preparing peripheral blood HSC for cryopreservation and infusion, long-term cryostorage
- **Key Regulations & Standards:** space must be adequate to prevent product mix ups and contamination; environmental monitoring required: temperature, humidity, microbial cultures
- **Other Features:** positive-pressure to keep contaminants out; seamless surfaces to allow for decontamination, if needed
- **Long-term concerns:** cryostorage space; manufacturing equipment


Fridge
CRFs
“Anteroom”: supply storage, airflow buffer
Computers with ISBT printers
Hoods
“Tank” room: Cryostorage
Bench with sterile dock, centrifuge
4A
A204
AREA OF WORK FLOOR PLAN
OUTSIDE OF PROJECT SCOPE

Tank Room

Processing Lab

Anteroom

Writing CTL SOPs & Forms

- **Primary Function:** clear instruction for staff, comprehensive documentation, and ultimately positive patient outcomes
- **Key Regulations & Standards:** document-controlled SOPs and forms for all manufacturing activities; documentation allows tracking of cells from donor to recipient
- **Other Features:** using online document control system (Policy Tech), all paper processing records
- **Long-term concerns:** adaptable for future products; record storage & access; cell therapy LIS

No Comprehensive Resources

- HSC products are not FDA licensed; labs must validate their own processes
- Published Resources:
  - Leemhuis T, et al. Essential requirements for setting up a stem cell processing laboratory. *Bone Marrow Transplantation*. 2014;49:1098-1105.
  - Areman E and Loper K, editors. *Cellular Therapy: Principles, Methods, and Regulations*, 2nd Ed. Bethesda, MD: AABB, 2016.
  - Creer MH, Lemas MV, Mathew AJ. *Practical Handbook of Cellular Therapy Cryopreservation*. Bethesda, MD: AABB, 2015.
  - FACT Accreditation Manual; AABB Standards for Cellular Therapy Services
- A little help from your friends:
  - Policies shared by other institutions
  - Cell therapy lab Google group

BJC HEALTH CARE

Cryopreservation Lab
(314) 454-7673
One Barnes-Jewish Plaza
St. Louis, MO 63110

Autologous
PBSC
Storage

ATTACH TAG HERE

Name:
SSN:
DOB:
Protocol#:
Physician:
Coord.:
Diagnosis:

Patient ABO/Rh: BBTR or SLCH BB
Act wt:

STORAGE DATA	#1	#2	#3	#4	#5	CUM.
Date:						
Product #:						
Vol. Processed (Ml):						0
Volume (Ml):						
Cells/uL:						
Platelet/uL:						
TNC:	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	
TNC/Bag:	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
TNC/Kg:	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
TNC/Kg/Bag:	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
# of Bags:						0
CD34+ /ul.:						
CD34 Absolute:	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	
CD34+/Kg:	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
CD34+/Kg/Bag:	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
Viability:						
MNC/kg:	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
Product Neutrophil %:						
Product Lymphocyte %:						
Product Monocyte %:						
Product ABO/rH:						
Bag Location:						
Vials:						
Liters to be processed						
Peripheral CD34/ul:						
Prediction CD34/Kg:	#DIV/0!	#DIV/0!	#VALUE!	#DIV/0!	#VALUE!	
Product Yield:	#DIV/0!	#DIV/0!	#VALUE!	#DIV/0!	#VALUE!	
CD34+ (Yield) Check:	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
LOT#/ EXP. Bag Lot #:						
Bag Exp:						
Plasmalyte-A Lot #:						
Plasmalyte-A Exp:						
DMSO Lot #:						
DMSO Exp:						
Heparin Lot #:						
Heparin Exp:						
60 ml Syringe Lot #:						
14 G Needle Lot #:						
3 ml Syringe Lot #:						
300 ml bag Lot #:						
600 ml bag Lot #:						
Sample Port Lot #:						
TECH						

Policies & forms from other centers serve as a starting point or provide ideas.

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Auto PBSC Processing
Worksheet B - Calculator
Form #CTL-801-2-C Issued: 9/21
Revised: N/A Version #: 1

Validation
Date:
Due:

Cell Therapies Lab (701) 234-2234
737 Broadway N, Rte 1102, Fargo, ND 58122

DAY:

Patient Information

Name: ABO/Rh:
DOB: Weight: kg
MRN: CD34 dose goal: x 10^6/kg
(Write or Place Patient Sticker Here) *Weight and dose from Processing Order*

Product Information

Product DIN (write or place sticker)
Collection Date / Time:
Expiration Date / Time:
(combined) Product Volume (mL):
Blood volume processed (mL):

Product Testing Results

Deliver hematology specimen first. Add copies of result reports from Beaker to patient paper file as available.

ABO/Rh: ☐ ABO/Rh matches patient history.
☐ ABO/Rh does not match; complete Deviation Report Form.

Hematocrit: % **Differential - PMNs:** %
TNC/µL: cells/µL *(aka WBC)* **Differential - MNCs:** %
CD34/µL: cells/µL
Viability: % ☐ Viability <80%; contact CTL Medical Director & complete Deviation Report Form.

Date: Time: Tech:

Calculations

0	=		x	0	/	100
Product RBC Volume (mL)		Combined Product Vol (mL)		Hct (%)		

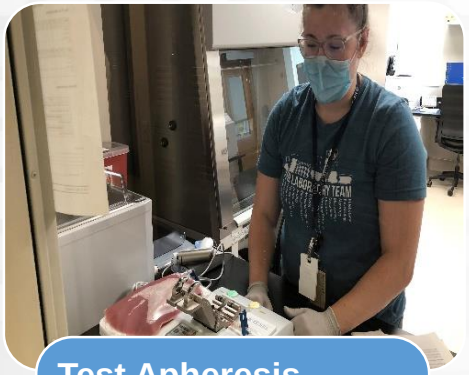
☐ RBC Vol. ≥25 mL in apheresis product; complete Process Deviation Report.

0.00E+00	=	0	x	0	x	1,000
Absolute Product TNC (cells)		Comb. Product Vol (mL)		TNC/µL		
#DIV/0!	=	0.00E+00	/	0	/	10^8
TNC Dose (cells x 10^8/kg)		Absolute Product TNC		Weight (kg)		
0.00E+00	=	0	x	0	x	1,000
Absolute CD34 (cells)		Comb. Product Vol (mL)		CD34/µL		
#DIV/0!	=	0.00E+00	/	0	/	10^6
CD34 Dose (cells x 10^6/kg)		Absolute Product CD34		Weight (kg)		

Calculations performed by: Date: Time: Tech:
Calculations verified by: Date: Time: Tech:

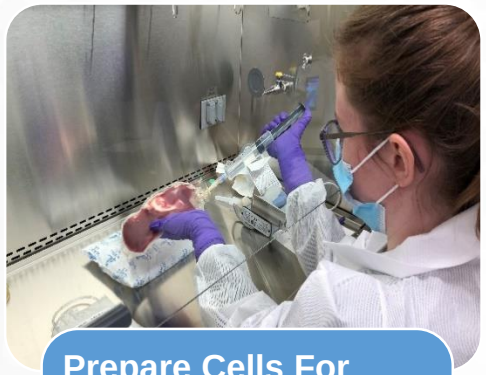
Calculations may be performed using validated spreadsheet. Results must match before reporting or initialing form.

Auto-HSCT Processing In a Nutshell…



Test Apheresis Product

- How many cells are present?
- Does ABORh match donor?
- Is there baseline microbial contamination?



Prepare Cells For Freezing

- Remove excess plasma
- Add cryoprotectant solution
- Divide into aliquots and QC vials
- Repeat culture
- ISBT 128 labeling

Freeze & Store

- Mechanical freezer or controlled rate freezer
- Liquid/vapor nitrogen storage

Thaw & Infuse

- Product release procedure
- Transport to floor in frozen state
- Thaw immediately before infusion in body temperature water bath

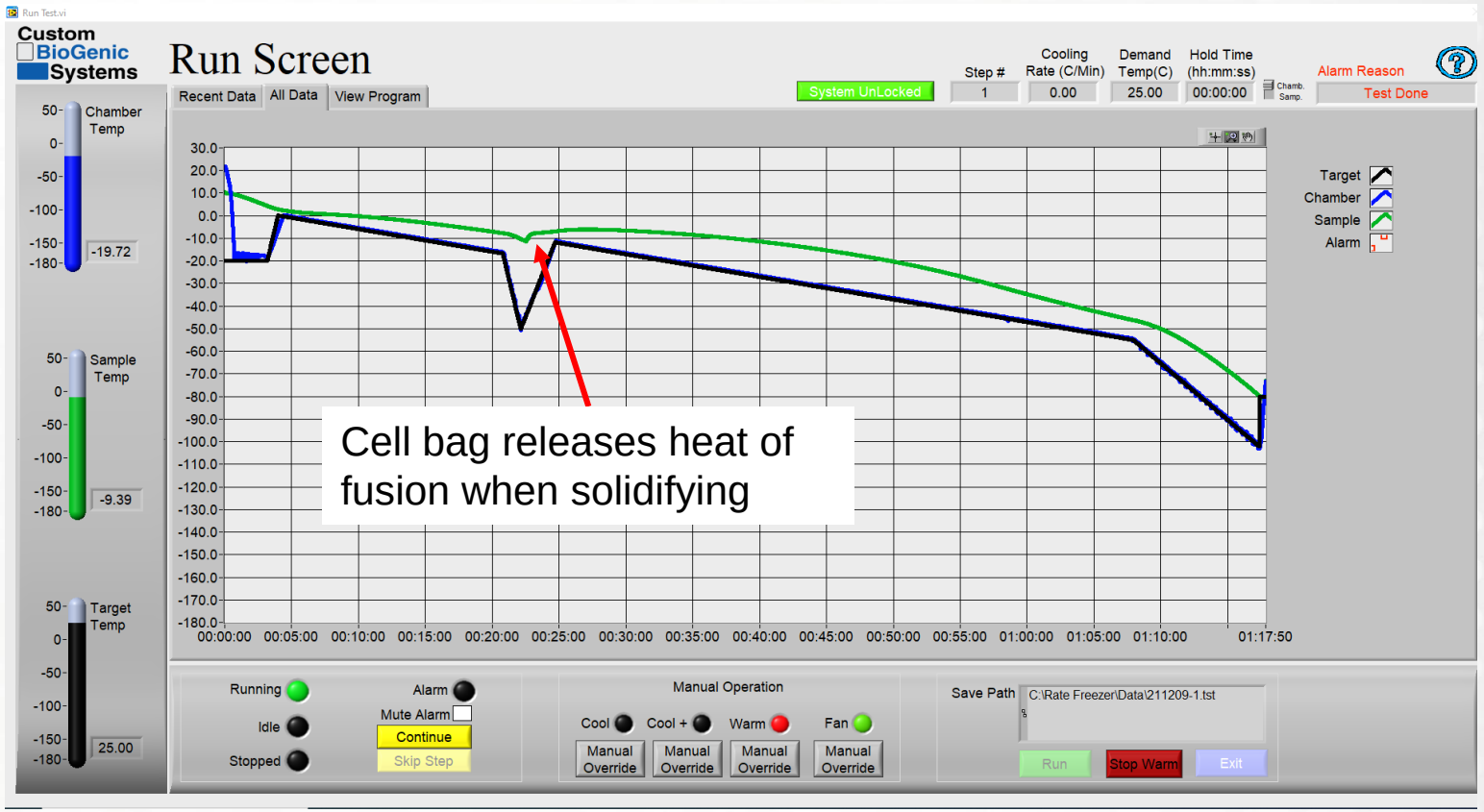
CTL Equipment

- **Primary Functions:** aseptic processing of peripheral blood HSCs, cryopreservation, short & long-term storage
- **Key Regulations & Standards:** must be adequate to perform the required manufacturing steps
- **Other Concerns:** redundancy in case of equipment failure
- **Key equipment:** class I biosafety cabinet, sterile connection device, RF tubing sealer, refrigerated centrifuge, controlled rate freezer, blood bank refrigerator, vapor nitrogen storage tank

Cryopreservation

- Freezing kills cells through mechanical damage and toxic solute concentrations
- HSCs survive freezing best when cooled 1 to 2 ^{o}C per minute in the presence of cryoprotectant solution
- Mechanical freezers may cool cells too rapidly early on, and not rapidly enough as the cells approach the freezer’s set temperature
- Controlled rate freezers use temperature probes and a computer program to inject liquid nitrogen into a cooling chamber

CRF and Example Cooling Curve



HSCs may be stored indefinitely in liquid/vapor nitrogen (-196ºC) freezer

Supply Chain, Anyone?

- Lab construction is mostly complete, equipment being moved into the space for install
- **Where are our freezers???**
- Stainless steel shortage → no freezer racks
- Wait it out? Buy from a different company? **Delay program start?**
- Received shipping notification just in time…

CTL Supplies

- **Primary Functions:** must be sterile and high quality to prevent patient harm
- **Key Regulations & Standards:** all critical supplies must meet acceptance criteria and lot numbers recorded in processing records
- **Other Concerns:** cryopreservation media may require lot-to-lot verification; assessing adequacy of supply vendors
- **Key supplies:** blood transfer packs, cryopreservation bags, cryovials, cryopreservation media, needle-free bag & vial spikes

Trying Something New

- Majority of labs make own cryopreservation cocktail
  - Dimethyl sulfoxide (DMSO) +/- hydroxyethyl starch (HES)
- Requires lot verification, opportunity for contamination
- Cheap, trusted → INERTIA

- USP-grade product available
- Cost $$$
- No lot verification, can transfer with sterile connection device
- Great fit for Sanford

Staff Education & Training

- **Primary Functions:** produce competent staff and quality patient care
- **Key Regulations & Standards:** staff are adequately trained to perform assigned tasks; receive annual HSCT-related education
- **Other Concerns:** CTL processing not included in standard technologist training programs; finding a host lab may be difficult
- **Training Activities:** virtual lab visit, in-person lab training, AABB Certificate in Cellular Therapies course, medical director lectures, participation in program planning and validations

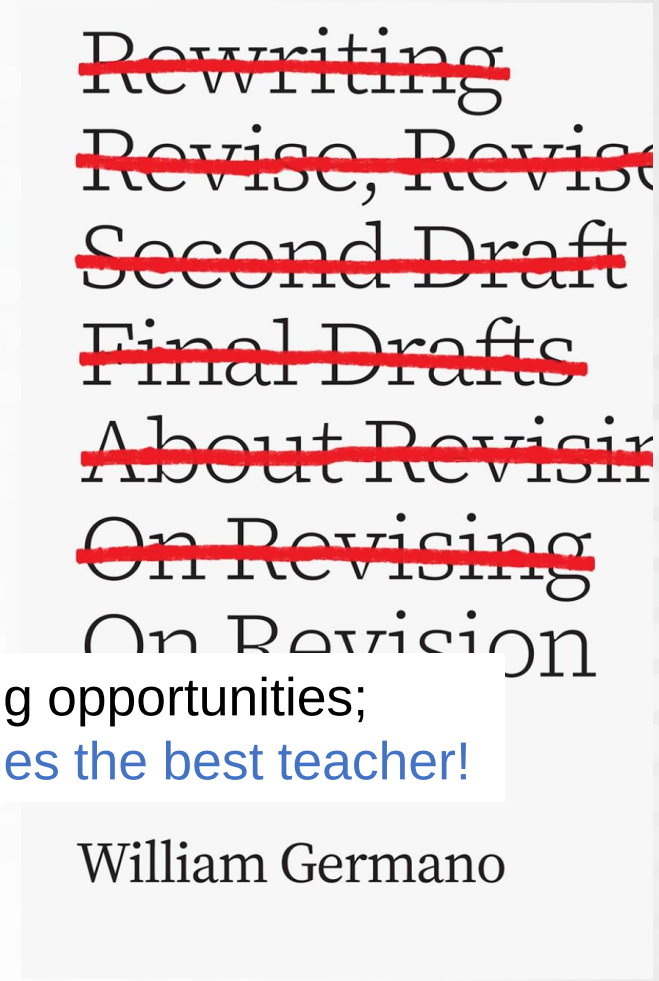
Validation Plans

- **Primary Function:** demonstrate process/equipment performs as expected and meets facility needs
- **Key Regulations & Standards:** validations of equipment, processes, and tests are required
- **Other Concerns:** must design own protocol and acceptance criteria; obtaining materials
- **Major Validations:** Cryopreservation & cryostorage, aseptic processing, apheresis collection, sterility culture, CD34 enumeration by flow cytometry, labeling (quality & stock)

Step 5: Practice and Revise

- Lab construction complete
- Installed & qualified equipment
- Began lab process validations
- Participated in joint mock runs

These are learning opportunities;
failure is sometimes the best teacher!

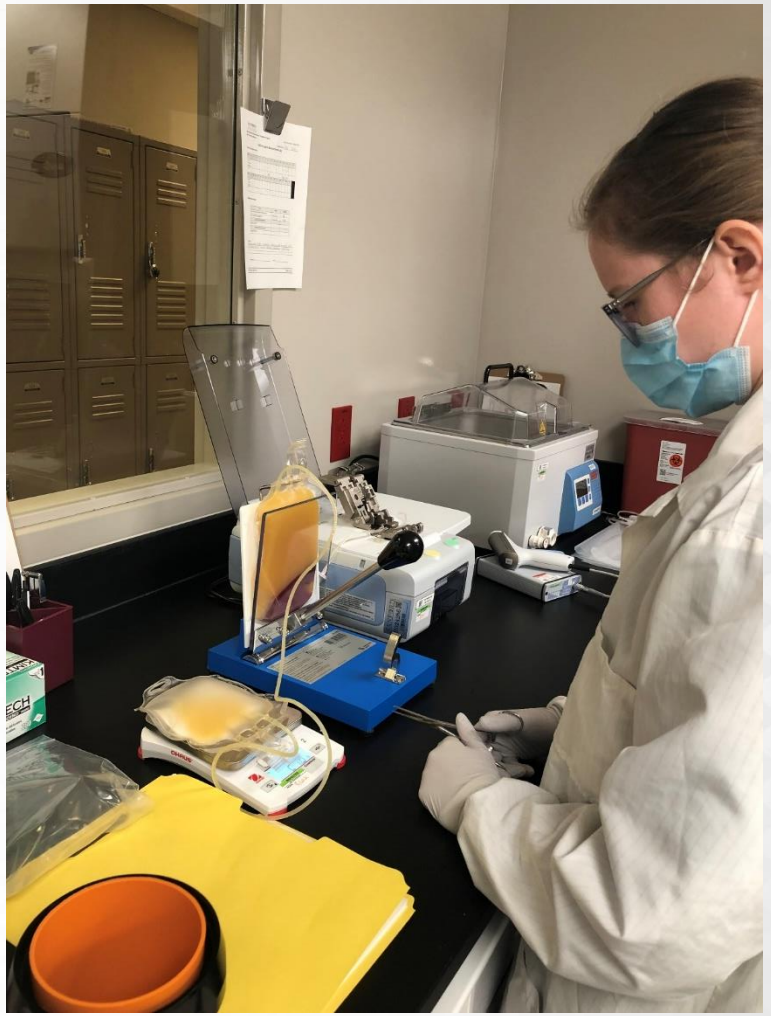


A short list of lessons learned during mock & validation runs…

- **Verify product volumes by weighing them.**
- Don’t expect slaughterhouse bovine blood to be sterile…
- Don’t use rigid centrifuge balance weights.
- We should have bought bigger hoods…
- Freezing bags alone in the cassette yields better freezing curves
- Incidentally, we now have half the freezer storage space…
- Writing validation acceptance criteria is hard.
- There are different types of liquid nitrogen cylinders, and yes, it matters!

Trust, But Verify

- Apheresis MNC product collected by blood center
- 400 mL was written on bag label
- When volume reducing ahead of cryopreservation, was about 100 mL “short”
- Blood center staff had added plasma volume twice—real volume was only 300 mL
- **Adjusted our SOP and processing records to include weight checks to confirm volume**

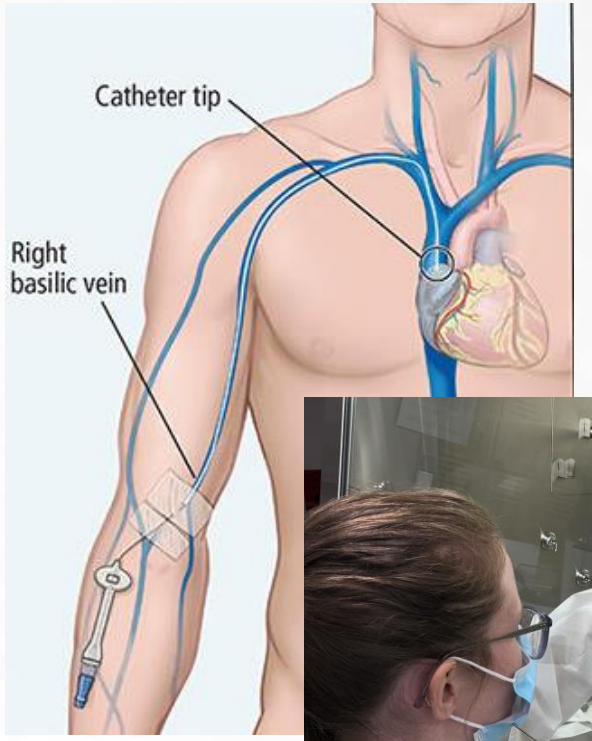


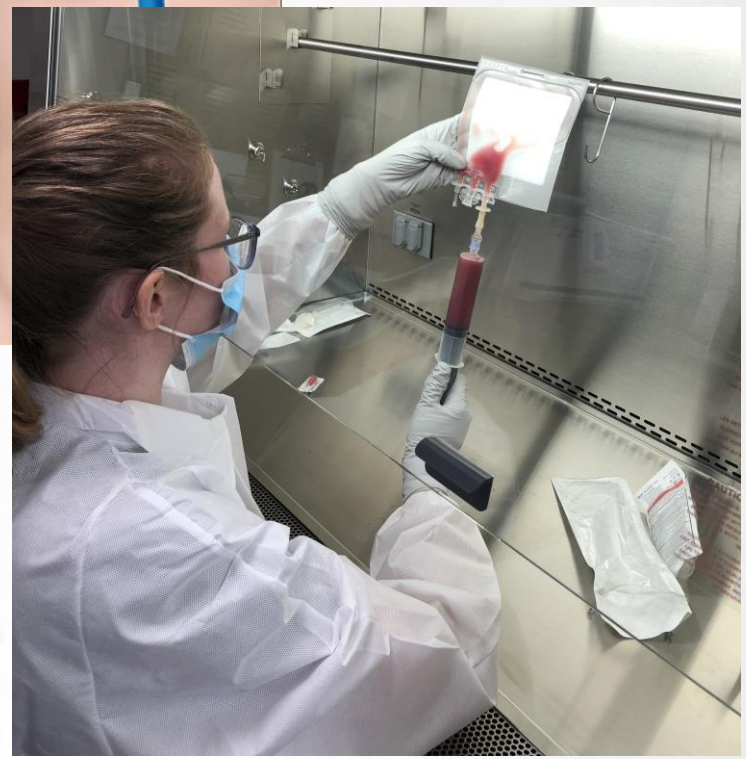
Step 6: The First Transplant

- 55 y.o. man from NW Minnesota with multiple myeloma
- Underwent 2 days of apheresis collection
- Received high-dose melphalan on 10/20/21
- HSC infusion scheduled on 10/21/21

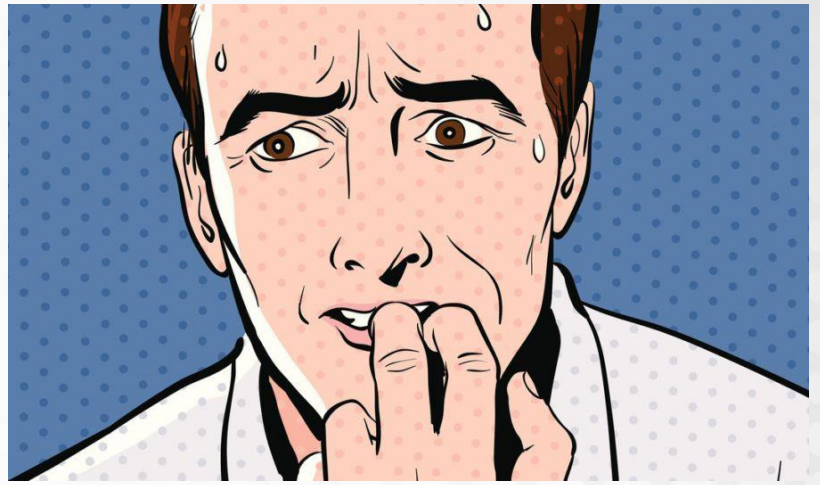
The Cells Are Stuck…

- The first bag is thawed, spiked and hung for infusion by gravity
- PICC line was kinked intravascularly—cells would not flow!
- Took remaining frozen bags back to lab for thaw & transfer to syringes under hood
- Cells pushed in via infusion pump





The Long Wait…



- 10 days after transplant:
  - WBC 0.1 x K/uL (Ref. 4.0 – 11.0 K/uL)
  - Hgb 10.4 g/dL (Ref. 13.5 – 17.5 g/dL)
  - Plt 11 K/uL (Ref. 140 – 400 K/uL)
- New onset fever (100.6F)
- C-reactive protein spiking to 20.8 mg/L (Ref. <5 mg/L)

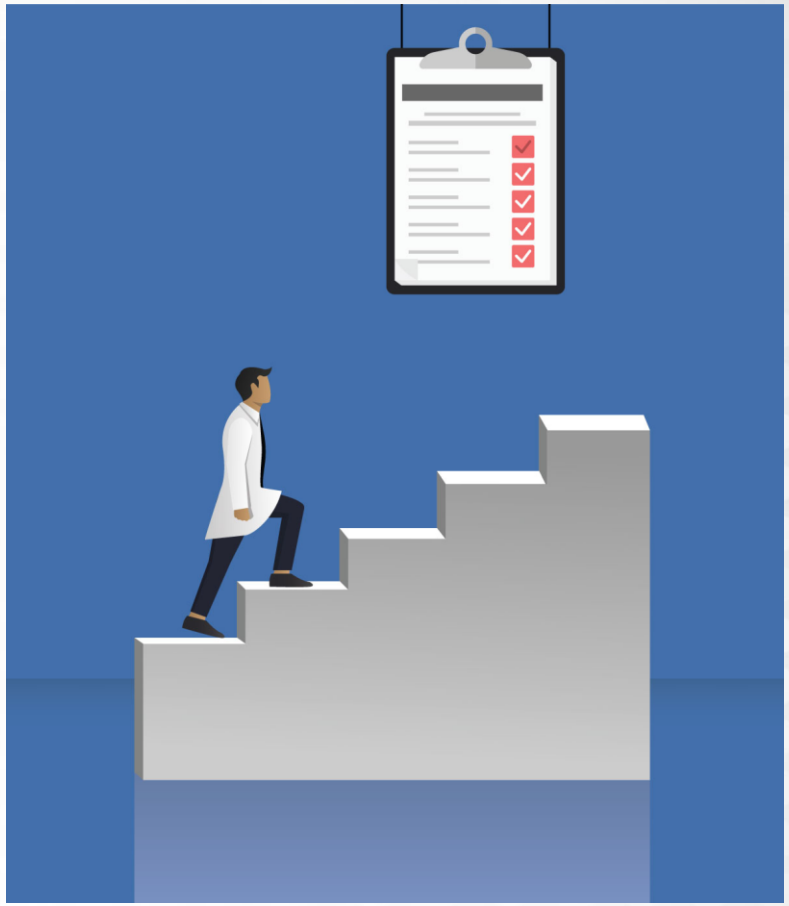
Houston, we have lift off!

- Engraftment of neutrophils and platelets on day +12
- Discharged on 11/4/21
- 8 successful auto-HSC transplants, and counting!

	10/31/2021 0507	11/1/2021 0617	11/2/2021 0445	11/3/2021 0620	11/4/2021 0600	11/5/2021 0804
CBC						
WBC	0.1 *	0.5 *	1.5 *	3.3 *	3.2 *	3.5 *
RBC	3.59	3.39 *	3.25 *	3.40 *	3.36 *	3.60 *
Hemoglobin	10.4	10.0 *	9.6 *	9.8 *	9.8 *	10.1 *
Hematocrit	30.3	28.6 *	27.3 *	28.8 *	28.5 *	30.2 *
MCV	84.4	84.4 *	84.0 *	84.7 *	84.8 *	83.9 *
MCH	29.0	29.5 *	29.5 *	28.8 *	29.2 *	28.1 *
MCHC	34.3	35.0 *	35.2 *	34.0 *	34.4 *	33.4 *
RDW-CV	14.5	14.7 *	14.7 *	15.1 *	15.3 *	15.8 *
RDW-SD	45.3	45.4 *	45.4 *	46.5 *	47.2 *	48.1 *
Platelet Count	11	18 *	32 *	56 *	87 *	149 *
MPV	9.7	10.1 *	10.6 *	10.8 *	10.6 *	11.0 *

Next Steps…

- Building our quality system
- Refining SOPs and forms
- Training additional CTL techs
- Preparing for allogeneic transplants (July 2022)
- Preparing for FACT accreditation (2023-24)



Words of Wisdom – Lab Style

- Be flexible, things will change from the initial plan. And that's OK.
- Roll with the Punches! Things outside of your control will go wrong from time to time.
- Get the bigger hood. ☺
- Become friends with the IT department! ☺
- Utilize your resources. You don't always have to re-invent the wheel.
- Look ahead to what we want to become, but don’t over/under prepare for it; find a happy medium.

Heartfelt thanks to:

Kayleigh Buescher, MLS

and

Stephanie Hidalgo, MLS

First Cellular Therapy Technologists in North Dakota!

References

- National Cancer Institute Surveillance, Epidemiology, and End Results Program (SEER). Cancer Stat Facts. https://seer.cancer.gov/statfacts/. Accessed 3/17/2022.
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Questions?

- Email: nicholas.gau@sanfordhealth.org