NOVEL TECHNOLOGIES FOR THE PRODUCTION OF BIOPHARMACEUTICALS

Antonio Moreira

UMBC

March 20, 2019

1st International Symposium on Biopharmaceuticals

São Paulo, Brazil

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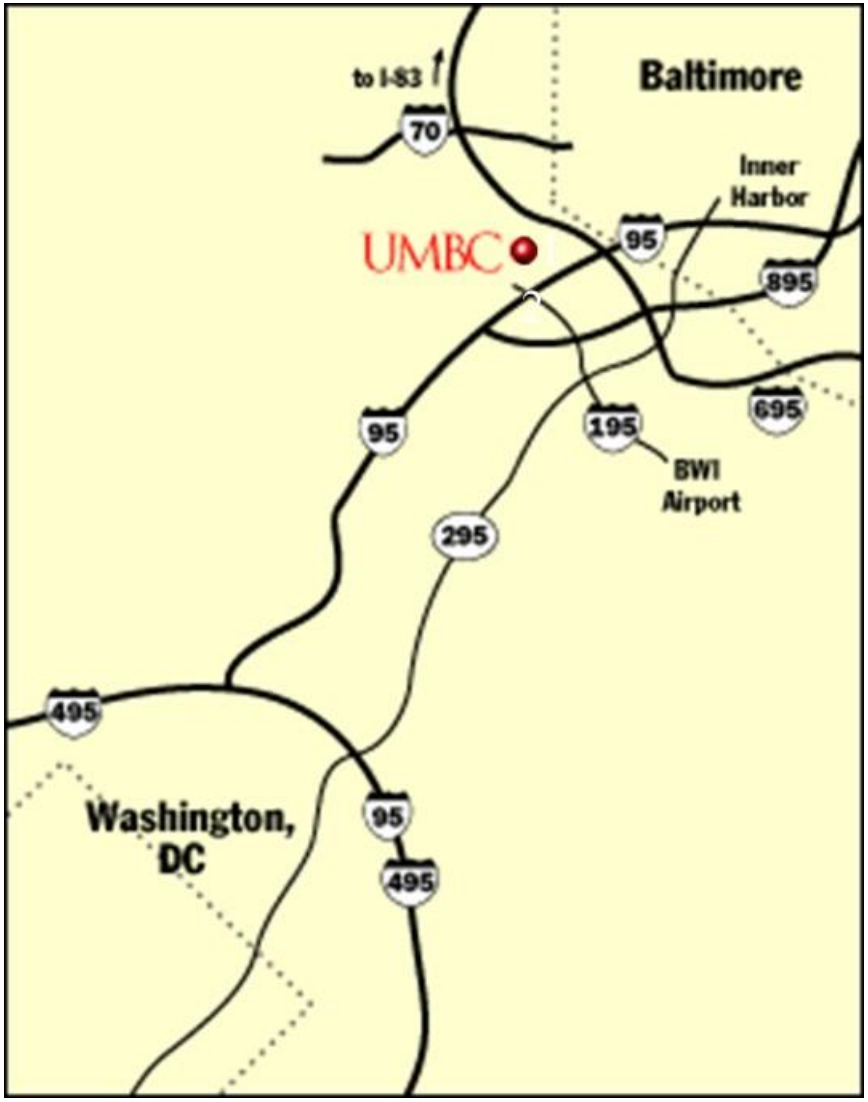
WHERE IS UMBC?



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Innovation that Matters

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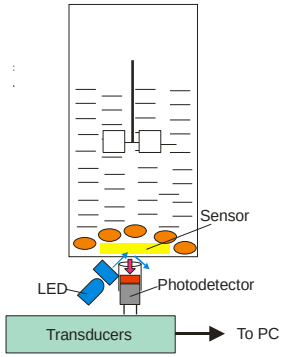
OUTLINE

- Production of biologics in cell-free systems
- Cell-based and gene therapies
- CAR-T Cell manufacturing
- Antibody-Drug Conjugates
- Combination Products
- 3D Printing

CENTER FOR ADVANCED SENSOR TECHNOLOGY

- Disruptive innovation leading to paradigm shifting practices
  - Low-cost integrated opto-electro-bio-mechanical device conceptualization and rapid prototyping
- Experience in bioprocessing, biomedical and environmental applications
- Many licensees for pO_2, pH, pCO_2, glucose sensors
  - Becton Dickinson, Scientific Industries, Inc., Sartorius-Stedim (GEHC sub)
- Rich list of corporate partners
  - DuPont, Genentech, Grace, Merck, Pfizer

Core non-invasive sensor technology led to miniaturization and cost reduction of bioreactors & enabled single-use bioreactors. CAST demonstrated similar performance with less cost, size, and energy used



Commercialized bioprocess products

CAST
Center for Advanced Sensor Technology

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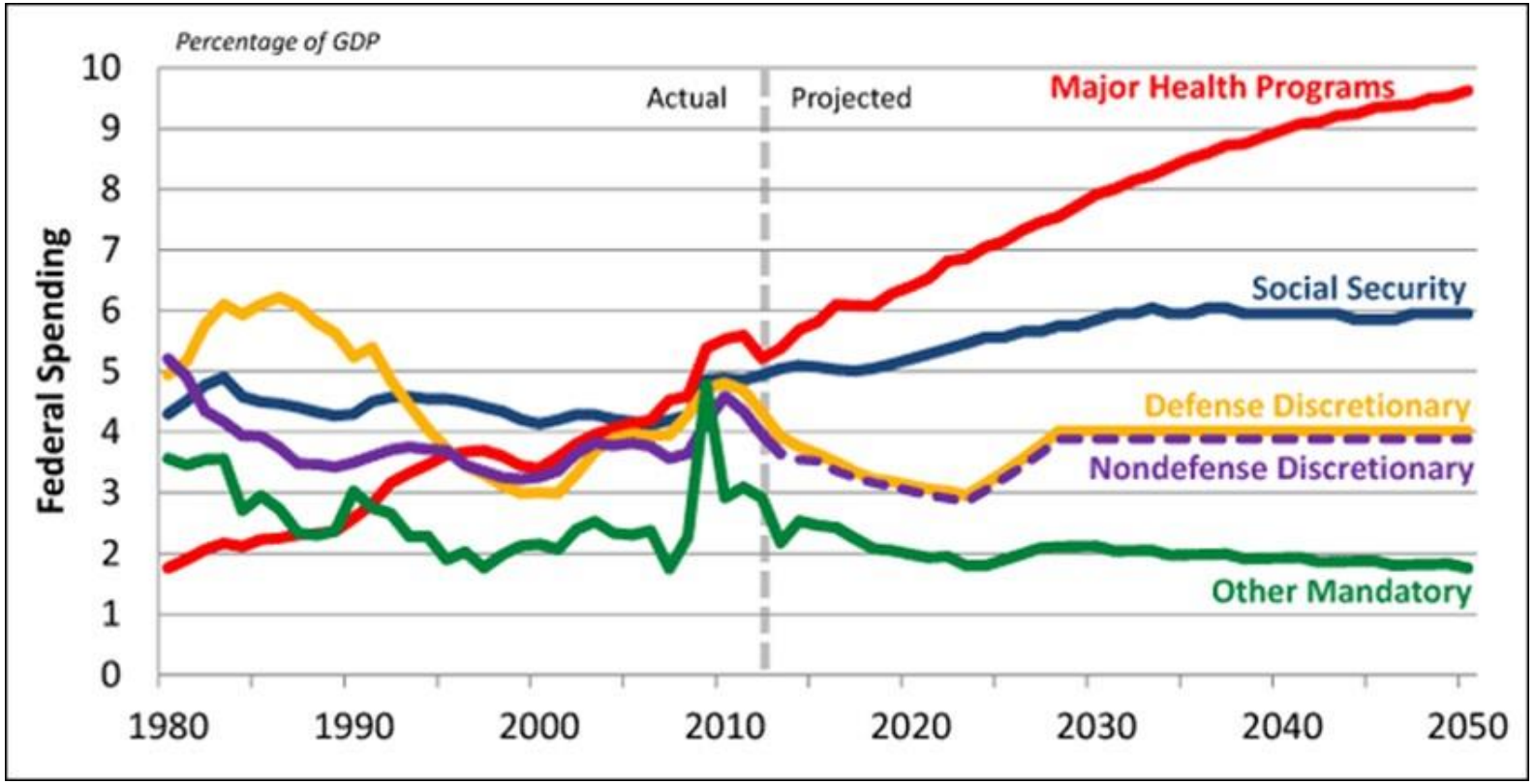
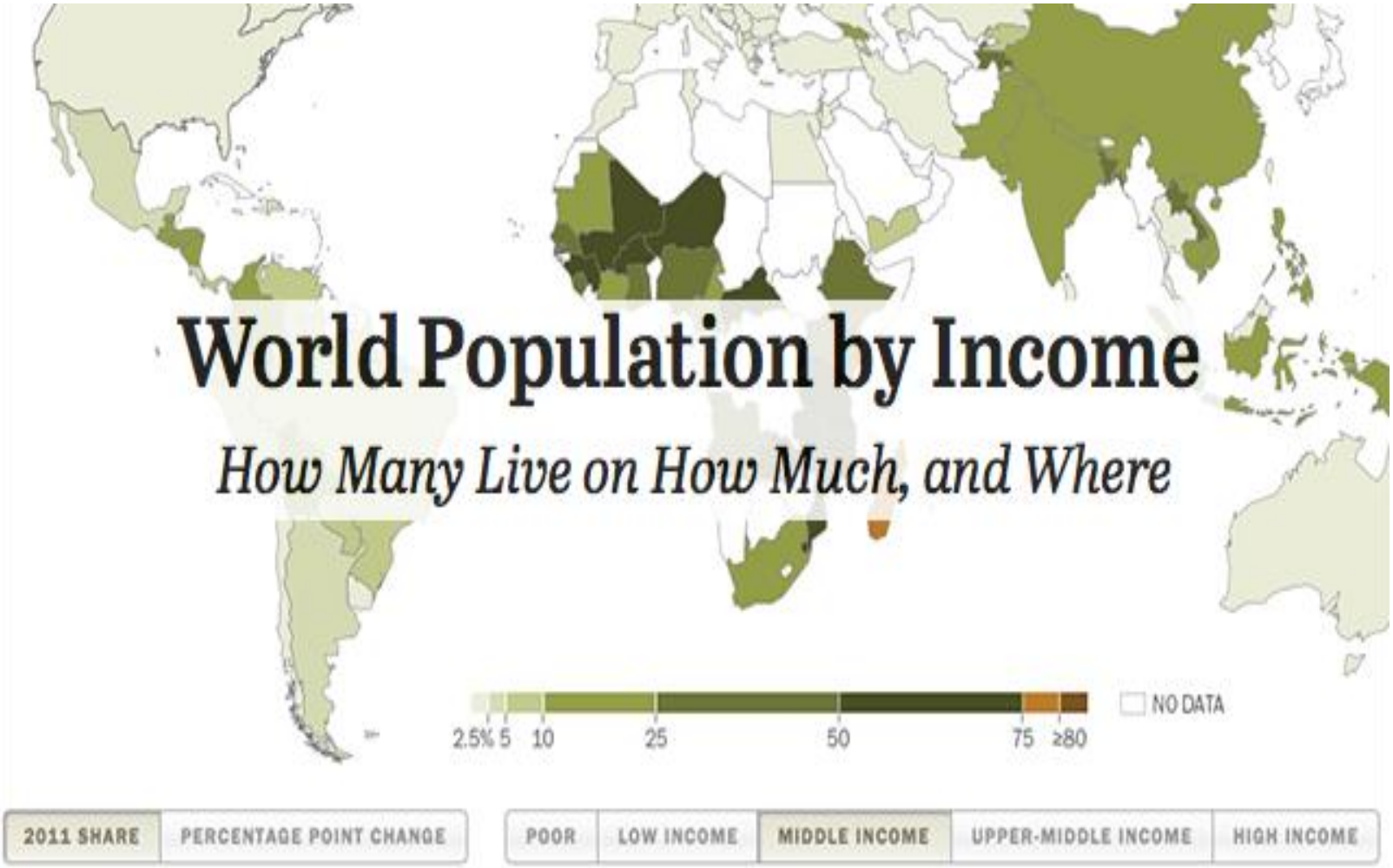
Per capita health spending is now >$10,000, or $3.07 trillion!



Population living on <$10/day or 71% of the World Population (5 Billion)
55.7% in US are High Income, defined as >$50/day (NOT a typo, Pew Research!)
CURRENT PRICING FOR HEALTHCARE IS UNSUSTAINABLE AND IRRATIONAL



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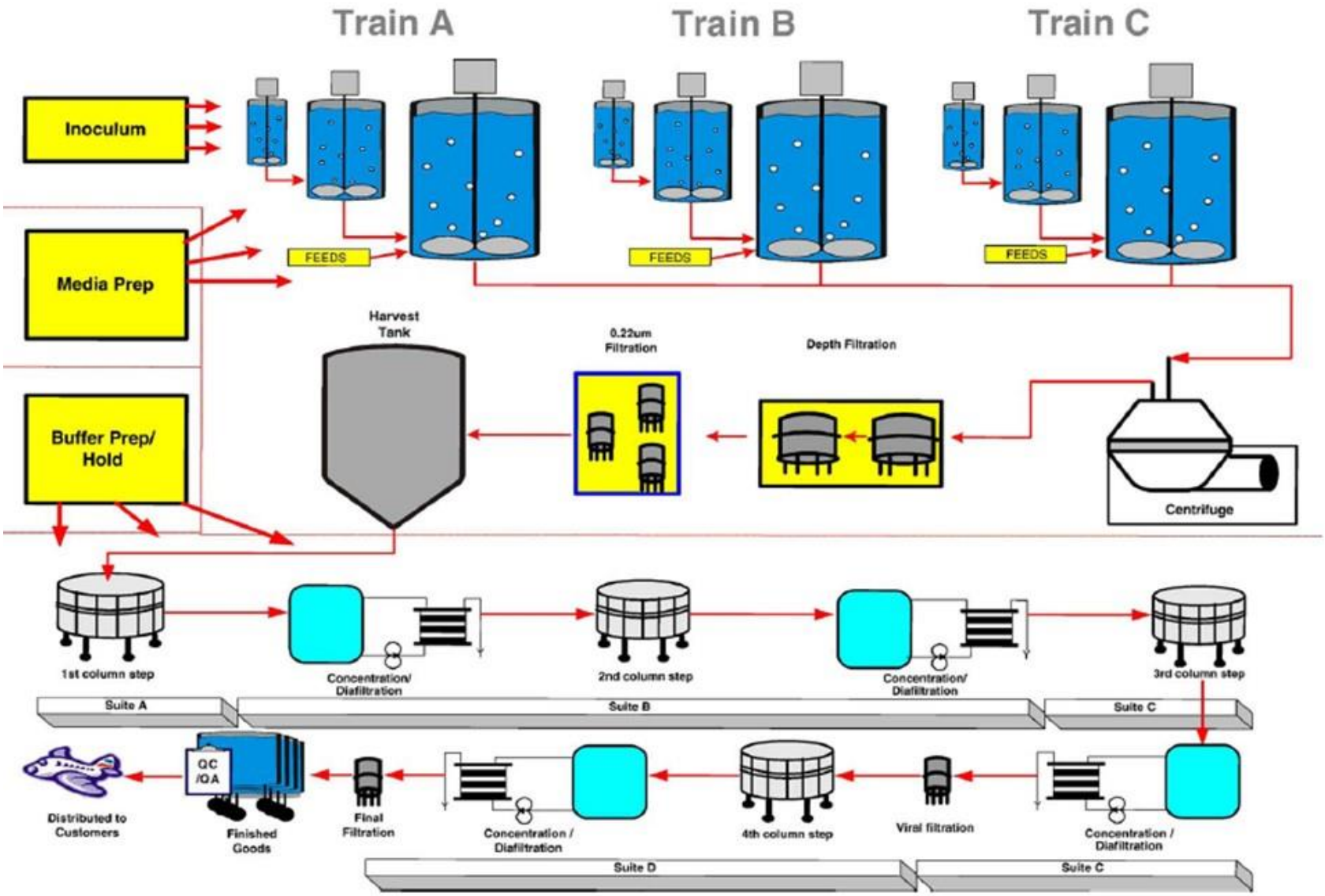
WHERE WE HAVE BEEN…



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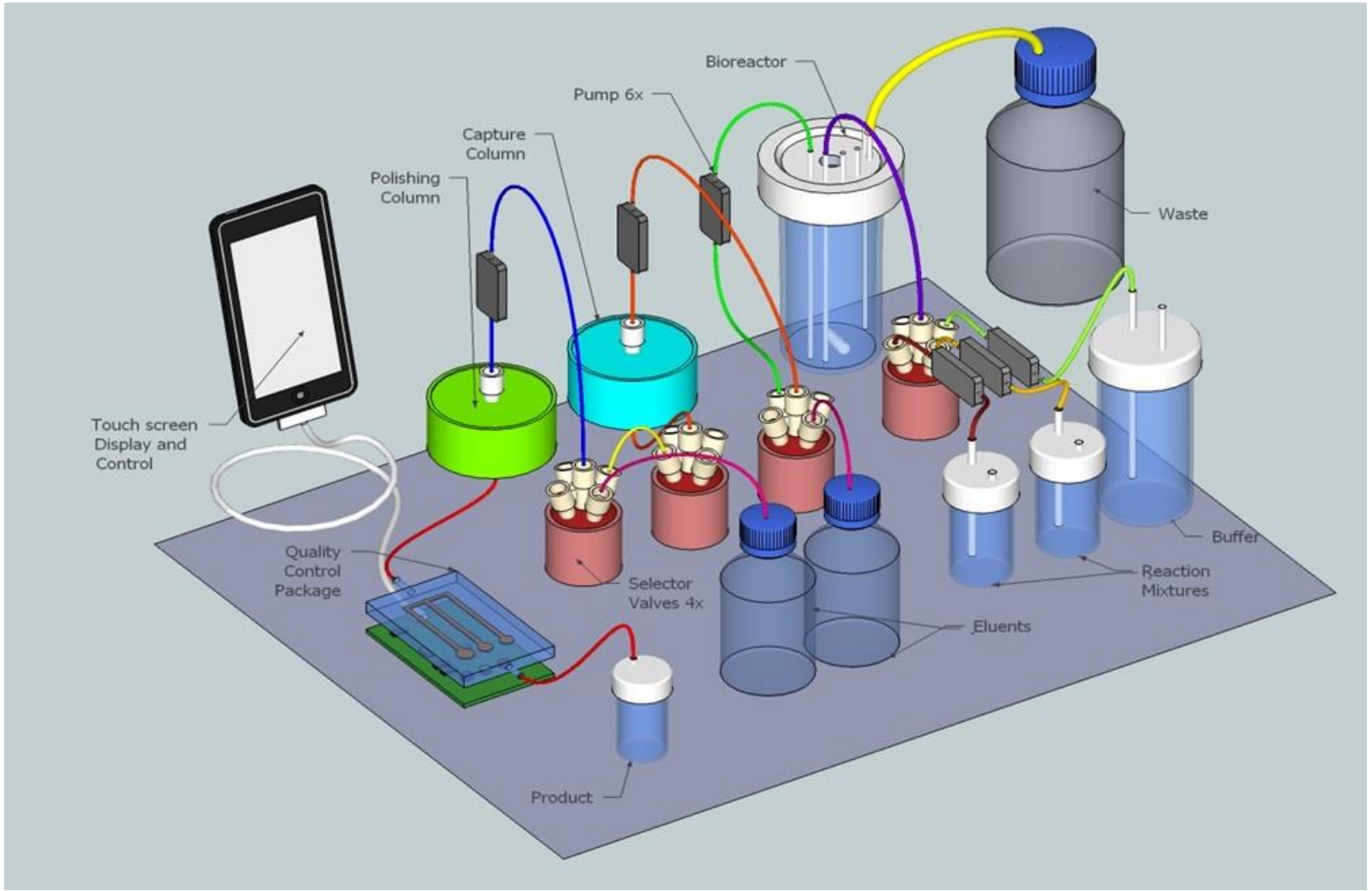
WHERE ARE WE GOING TO?

MODULAR, DISPOSABLE, GMP CAPABLE BIOLOGICS ON DEMAND



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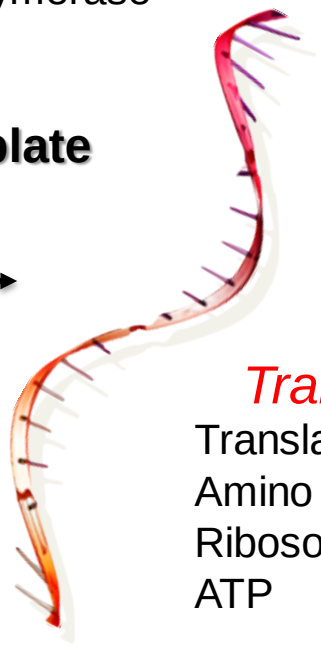
RAPID EXPRESSION VIA *IN VITRO* TRANSLATION (IVT)

CHO Cells

- 15.0L+ Bioreactor Batch Culture
- Lysis (Cavitation)
- Differential centrifugation to isolate critical organelles
- Lyophilized

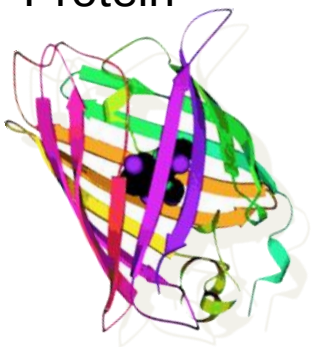
T7 RNA Polymerase
Nucleotides
ATP
DNA Template

RNA



Translation
Translation Factors
Amino Acids
Ribosomes
ATP

Protein



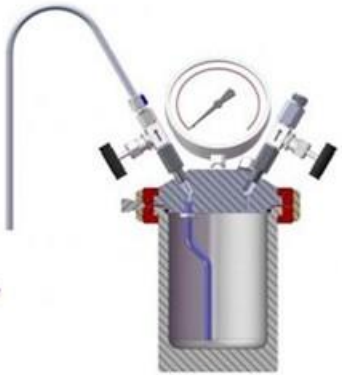
- Advantages of *in vitro* translation (IVT):
  - No cell line development
  - No seed train, passaging, or transfection
  - All live culture scale-up done off line in **generic** single batches

CHO CELL-FREE LYSATE MANUFACTURING PROCESS

Shaker flasks

15 L bioreactor

Harvest –
spin down cells and wash



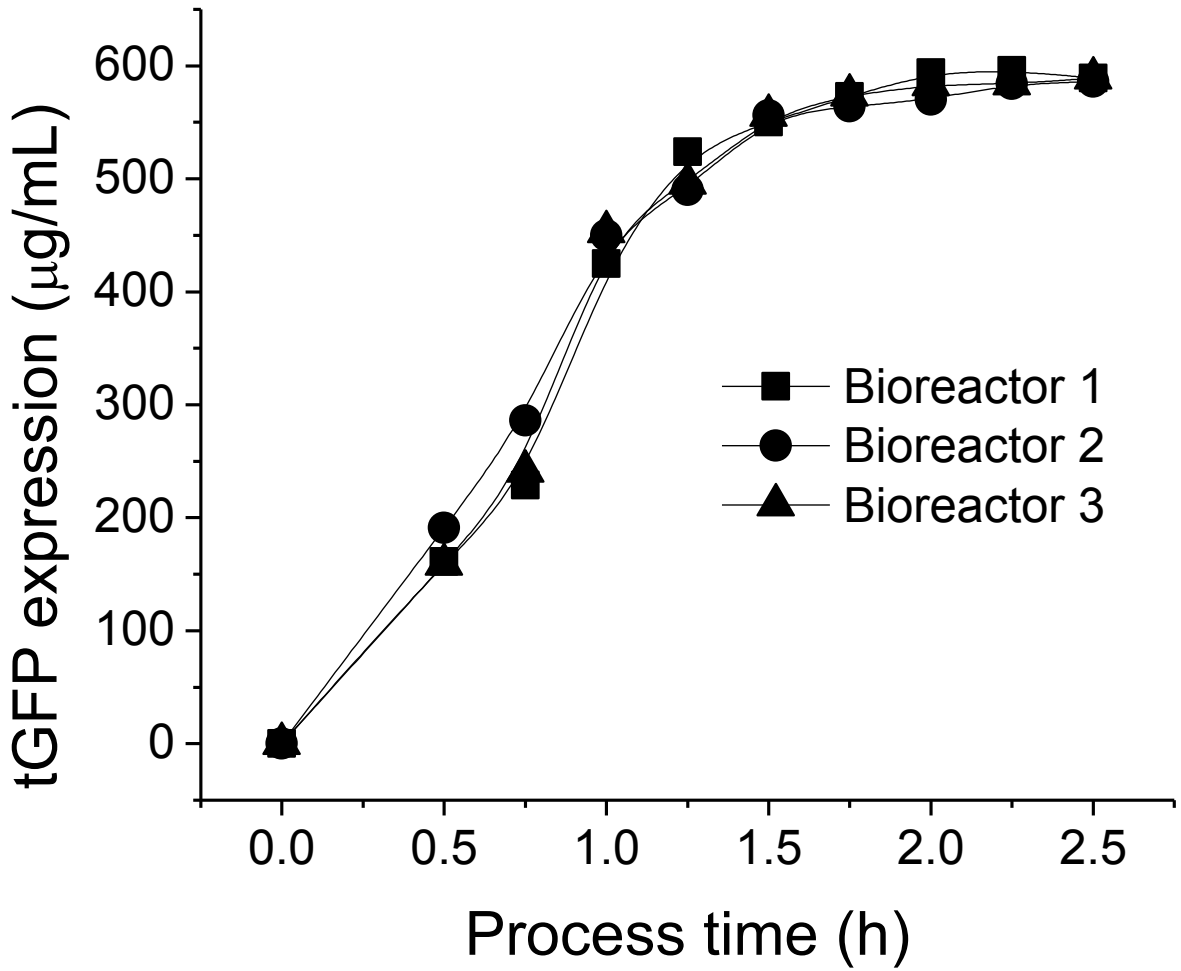
Lyse cells using
nitrogen cavitation

Spin to remove
cellular debris

lysate

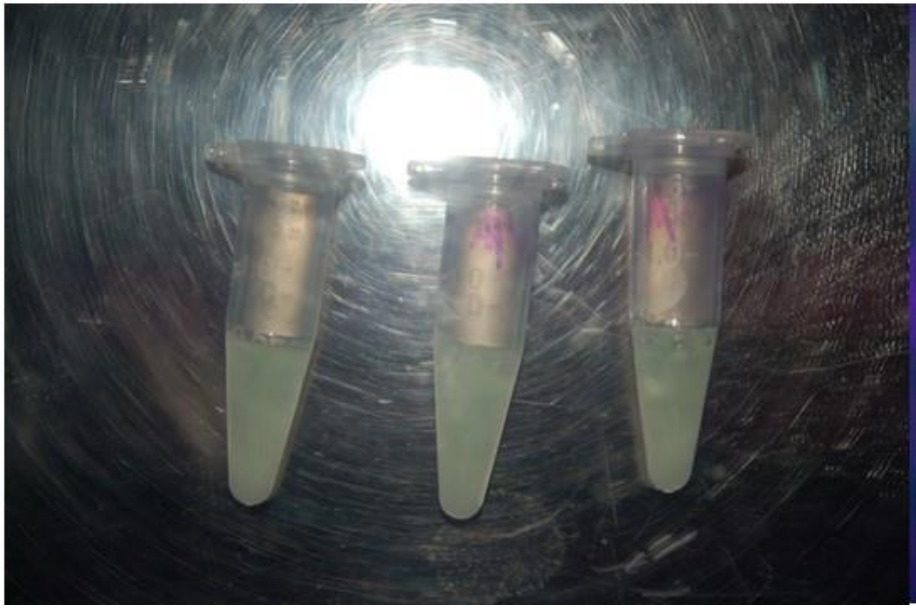
Lyophilized
product

EXPERIMENT 192K14

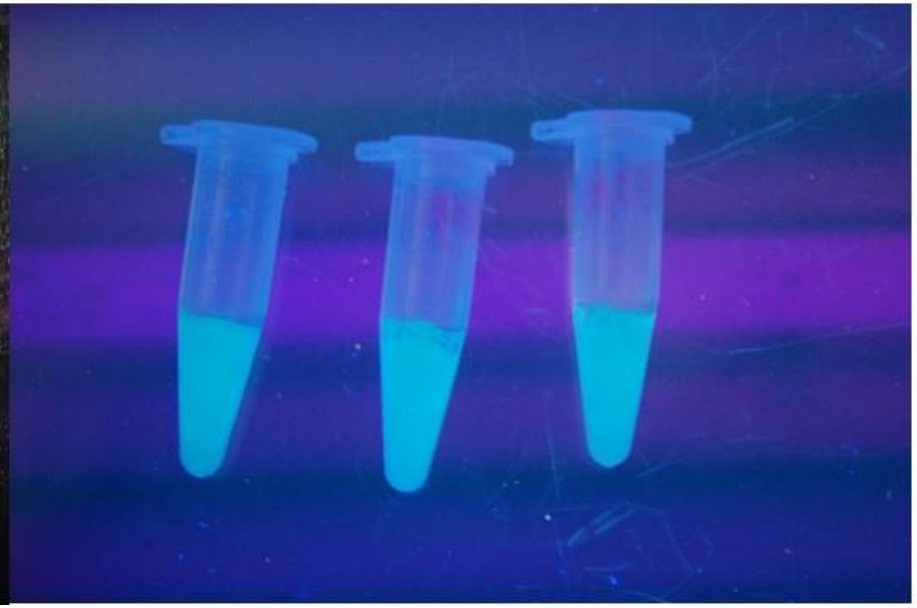


POST-RUN HARVEST VISUALIZATION (192K14)

Under normal light



Under UV light



8/25/15, DARLing 1.0!

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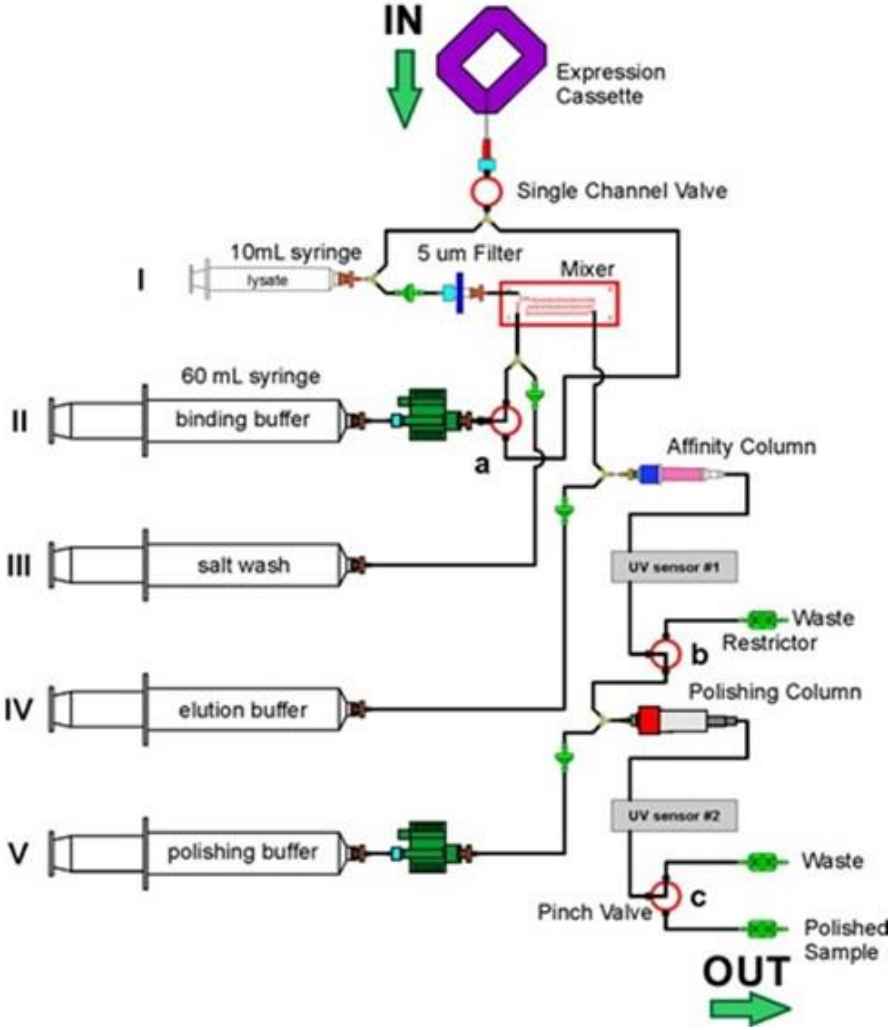

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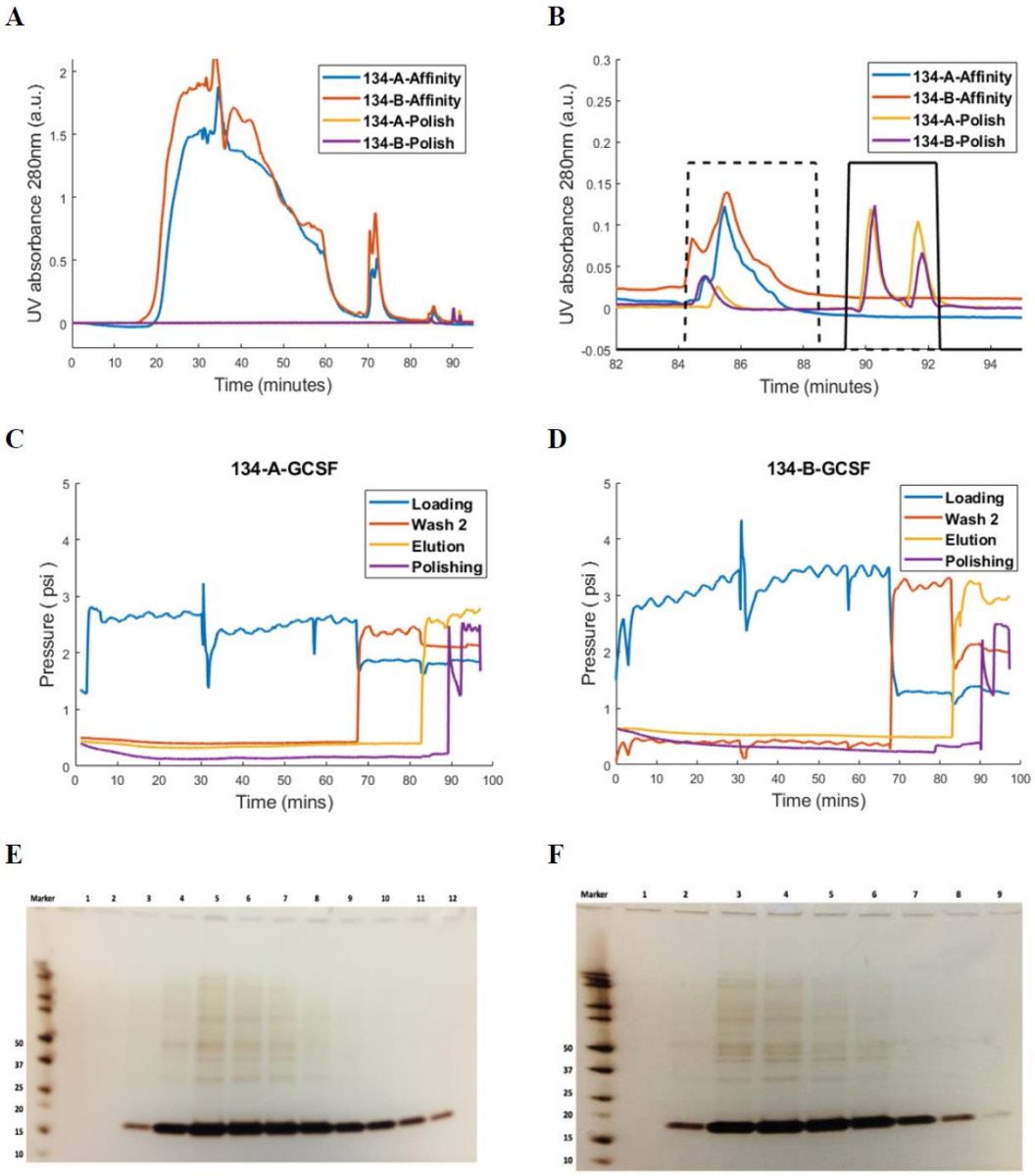
Real-time UV (280 nm)and pressure traces of the affinity column purification of various His-tagged proteins



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EMERGING INFECTIOUS DISEASES - ZIKA

Mosquito born Zika outbreak is rapidly spreading in Americas…

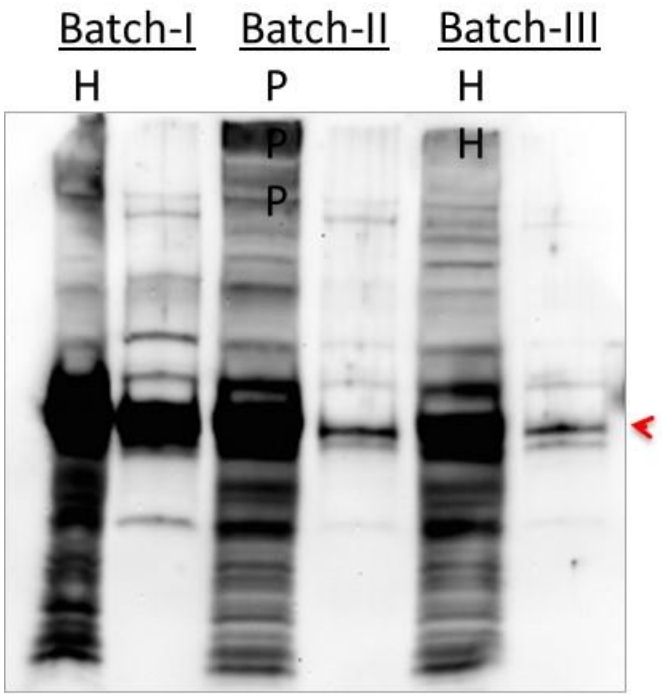
(CDC, National Center on Birth Defects and Developmental Disabilities) - Microcephaly is a birth defect where a baby's head is smaller than expected when compared to babies of the same sex and age.

A quote from news:

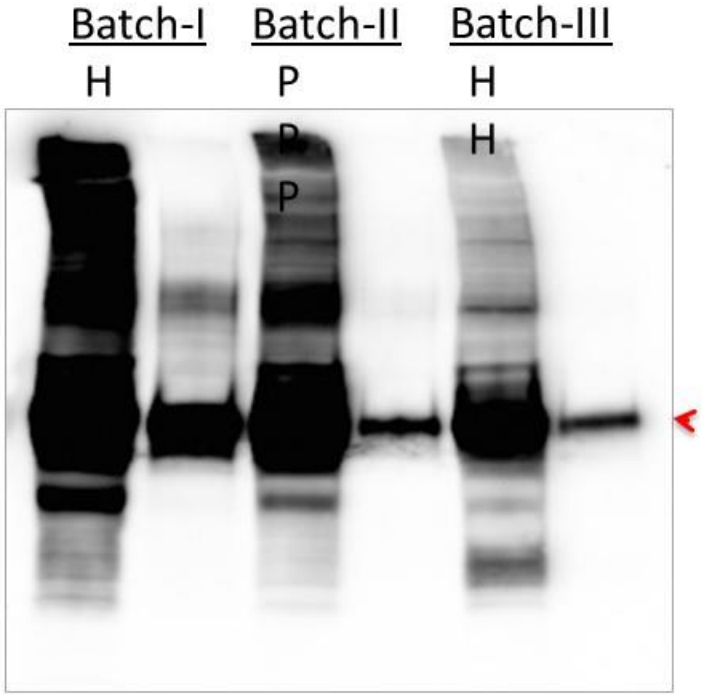
" The Centers for Disease Control in Atlanta, Georgia, warned last week there now are 107 travel-related cases of the Zika virus in 24 states and the District of Columbia.
It issued a travel advisory for pregnant women and others who are planning to attend the 2016 Summer Olympic Games in Rio de Janeiro, Brazil, from Aug. 5 to 21."

3 independent batches of Zika protein expression and purification

(Western blot results for Anti-Zika vs Anti-Flavi virus antibodies)



W.B.: Anti-Flavi virus Ab (Absolute Antibodies)



W.B.: Anti-Zika GP (IBT Bioservices)

H – harvest
P - purified

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nature biomedical engineering

ARTICLES

https://doi.org/10.1038/s41551-018-0259-1

Point-of-care production of therapeutic proteins of good-manufacturing-practice quality

Rajani Adiga[1], Mustafa Al-adhami[1,2], Abhay Andar[1], Shayan Borhani[1,3], Sheniqua Brown[1,3], David Burgenson[1,3], Merideth A. Cooper[4], Sevda Deldari[1,3], Douglas D. Frey[1,3], Xudong Ge[1,3], Hui Guo[1,3], Chandrasekhar Gurramkonda[1], Penny Jensen[5], Yordan Kostov[1,3], William LaCourse[6], Yang Liu[3], Antonio Moreira[1,3], KarunaSri Mupparapu[1], Chariz Peñalber-Johnstone[1], Manohar Pilli[1], Benjamin Punshon-Smith[7], Aniruddha Rao[1,6], Govind Rao[1,3*], Priyanka Rauniyar[8], Sergei Snovida[5], Kanika Taurani[1], Dagmawi Tilahun[1], Leah Tolosa[1,3], Michael Tolosa[1], Kevin Tran[1], Krishna Vattem[5], Sudha Veeraraghavan[9,10], Brandon Wagner[1], Joshua Wilhide[8], David W. Wood[4] and Adil Zuber[3]

https://rdcu.be/2Jk8

SCIENTIFIC REPORTS

OPEN

Rapid recombinant protein expression in cell-free extracts from human blood

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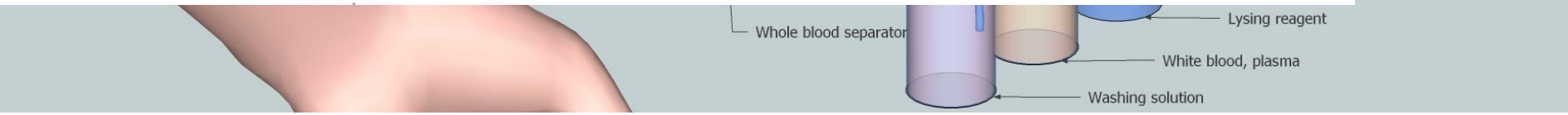
David Burgenson, Chandrasekhar Gurramkonda, Manohar Pilli, Xudong Ge, Abhay Andar, Yordan Kostov, Leah Tolosa & Govind Rao

Several groups have recently reported on the utility of cell-free expression systems to make therapeutic proteins, most of them employing CHO or *E. coli* cell-free extracts. Here, we propose an alternative that uses human blood derived leukocyte cell extracts for the expression of recombinant proteins. We demonstrate expression of nano luciferase (Nluc), Granulocyte-colony stimulating factor (G-CSF) and Erythropoietin (EPO) in cell-free leukocyte extracts within two hours. Human blood is readily available from donors and blood banks and leukocyte rich fractions are easy to obtain. The method described here demonstrates the ability to rapidly express recombinant proteins from human cell extracts that could provide the research community with a facile technology to make their target protein. Eventually, we envision that any recombinant protein can be produced from patient-supplied leukocytes, which can then be injected back into the patient. This approach could lead to an alternative model for personalized medicines and vaccines.



CELL-BASED THERAPIES

- Novel technologies using cells as living, replicating biological structures

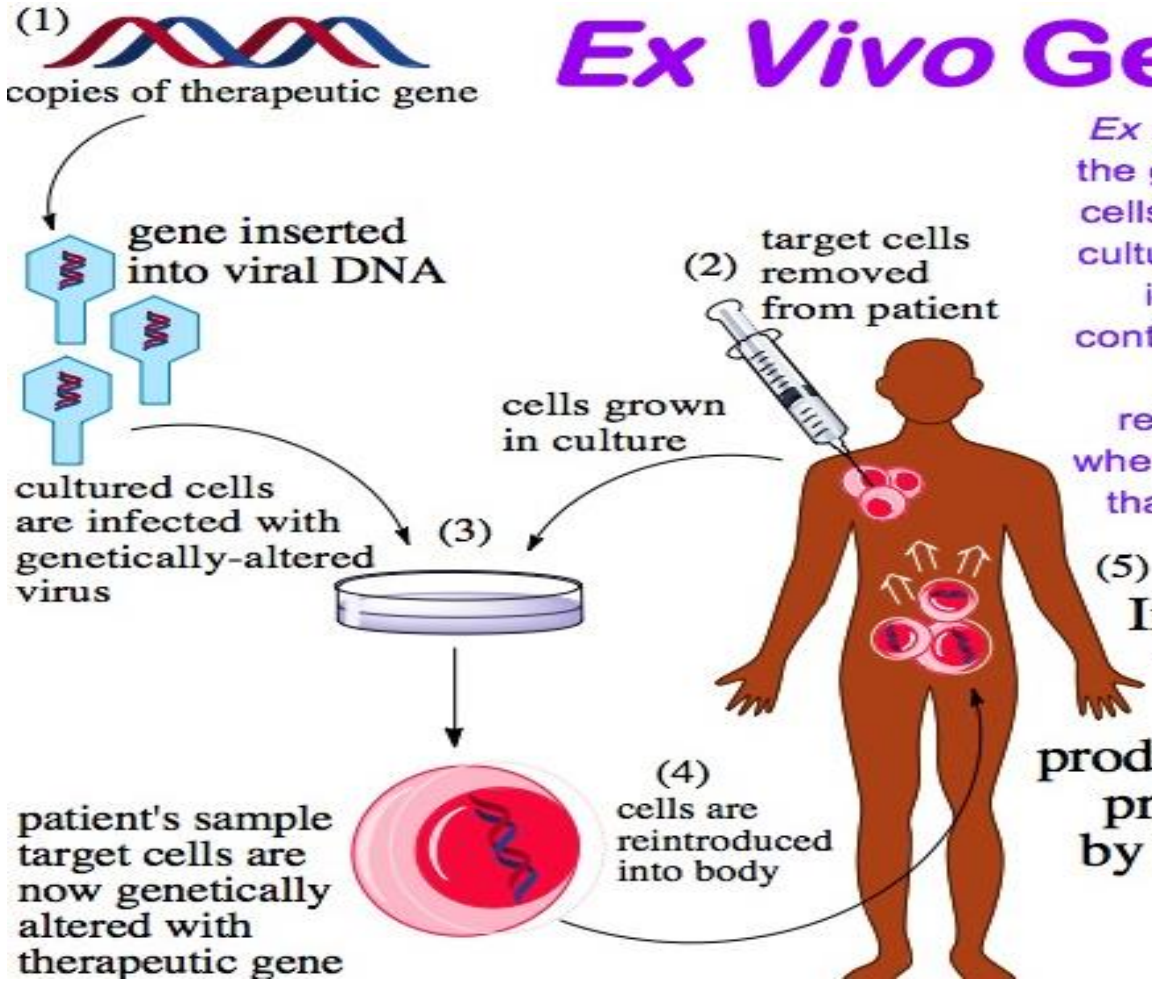
- Include cells from the human donor that are minimally modified as well as cells that are modified genetically prior to delivery back to the patient

- These technologies represent a major change from current biopharmaceuticals that are non-living biological products such as monoclonal antibodies, cytokines and vaccines.

What is Gene Therapy

- Definition: an experimental technique for correcting defective genes that are responsible for disease development
- The most common form of gene therapy involves inserting a normal gene to replace an abnormal gene
- Other approaches used:
  - Replacing a mutated gene that causes disease with a healthy copy of the gene.
  - Inactivating, or "knocking out," a mutated gene that is functioning improperly.
  - Introducing a new gene into the body to help fight a disease.

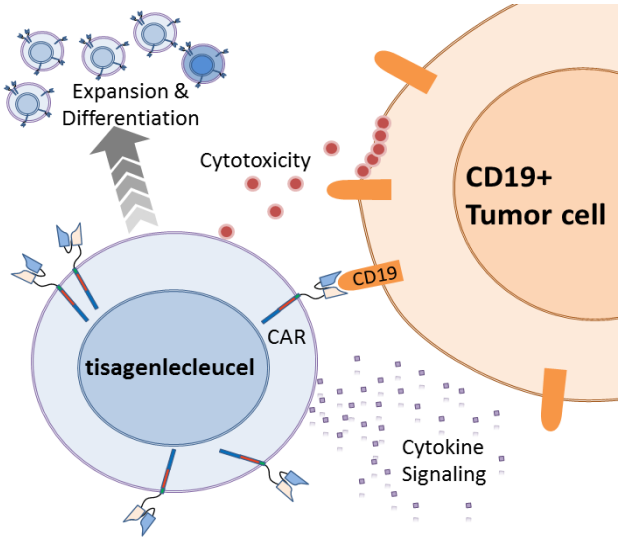
Ex Vivo Gene Therapy



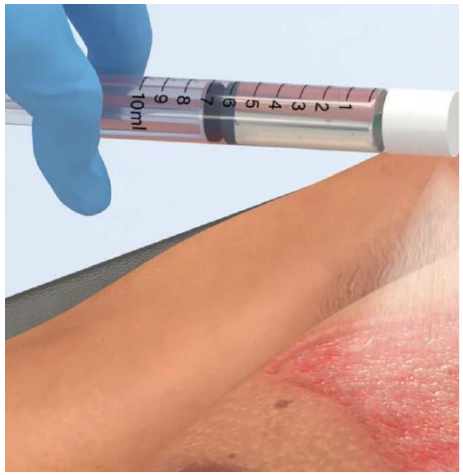
Ex vivo gene therapy is performed with the genetic alterations of patient's target cells happening outside of the body in a culture. Target cells from the patient are infected with a recombinant virus containing the desired therapeutic gene. These modified cells are then reintroduced into the patient's body, where they produce the needed proteins that correspond to the inserted gene.

RECENTLY APPROVED GENE THERAPY PRODUCTS

- **Luxturna (Voretigene Neparvovec):** Adeno-associated virus vector-based gene therapy for treatment of patients with confirmed biallelic RPE65 mutation-associated retinal dystrophy
- **Yescarta (Axicabtagene Ciloleucel):** CD19-directed genetically modified autologous T cell immunotherapy for treatment of adult patients with relapsed or refractory large B-cell lymphoma (DLBCL), primary mediastinal large B-cell lymphoma, high grade B-cell lymphoma, and DLBCL arising from follicular lymphoma
- **Kymriah (Tisagenlecleucel):** CD19-directed genetically modified autologous T cell immunotherapy for treatment of patients up to 25 years of age with B-cell precursor acute lymphoblastic leukemia (ALL) that is refractory or in second or later relapse



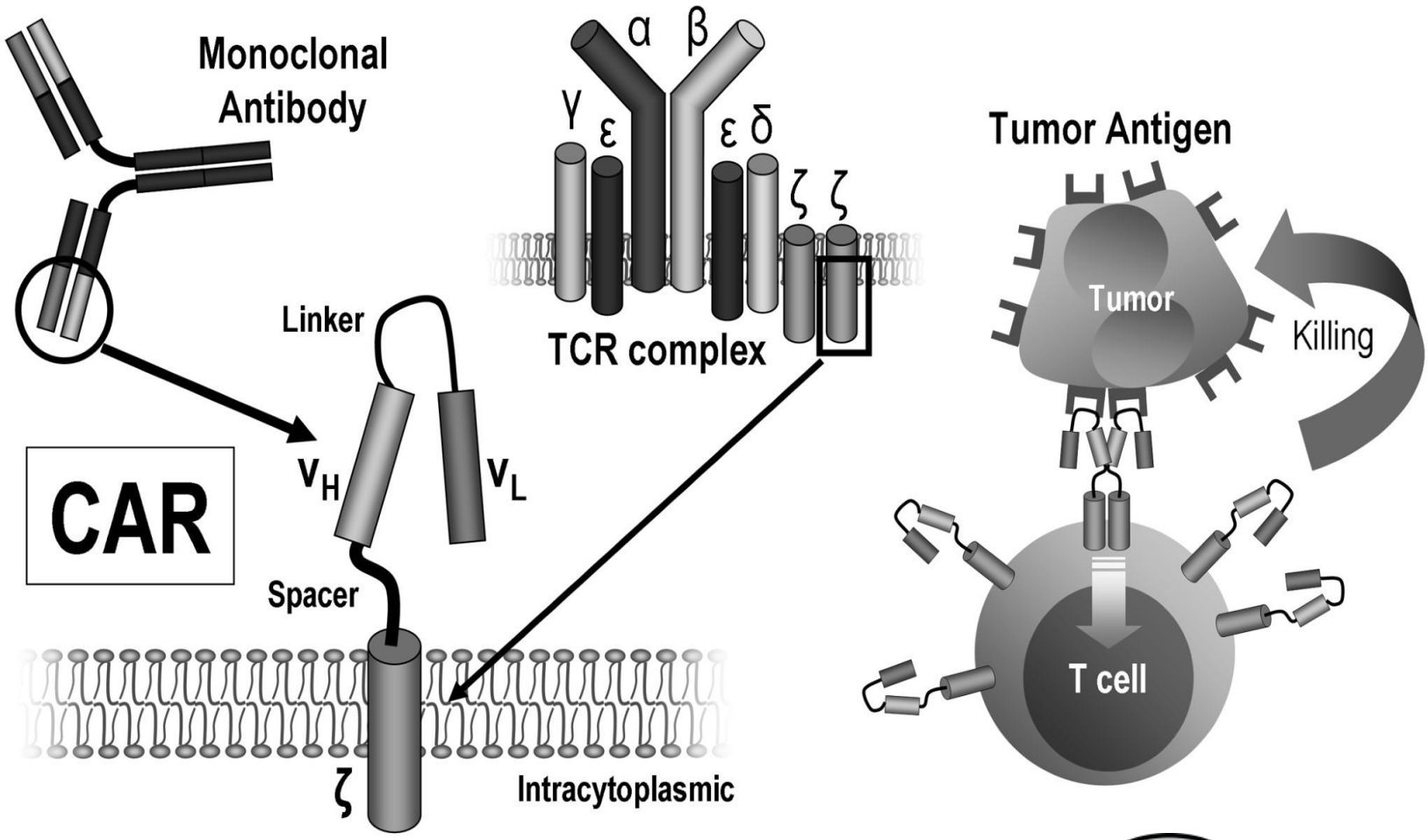
CBER APPROVED CELL THERAPY PRODUCTS



- **RECELL Autologous Cell Harvesting Device:** For treatment of acute thermal burn wounds in adult patients. Used at the patient's point-of-care to prepare autologous Regenerative Epidermal Suspension (RES™) for direct application to acute partial-thickness thermal burn wounds or application in combination with meshed autografting for acute full-thickness thermal burn wounds.
- **MACI (Autologous Cultured Chondrocytes on a porcine collagen membrane):** Autologous cellularized scaffold product for repair of single or multiple symtomatic, full-thickness cartilage defects of the knee with or without bone involvement in adults.
- **GINTUIT (Allogeneic Cultured Keratinocytes and Fibroblasts in bovine collagen):** For topical (non-submerged) application to a surgically created vascular wound bed in the treatment of mucogingival conditions in adults.
- **Provenge (sipuleucel-T):** Autologous cellular immunotherapy for treatment of asymptomatic or minimally symptomatic metastatic castrate resistant (hormone refractory) prostate cancer

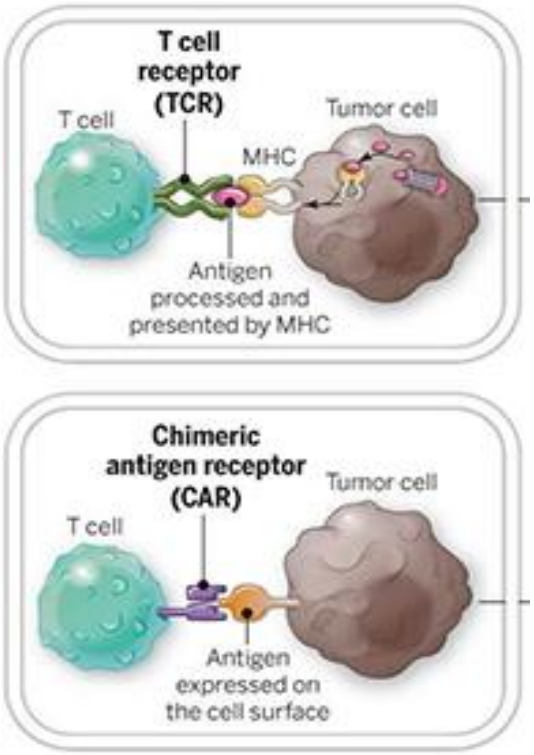
CHIMERIC ANTIGEN RECEPTOR (CAR)-T CELLS: PERSONALIZED THERAPY

THE BASIC STRUCTURE OF A MONOCLONAL ANTIBODY (MAB)-DERIVED CHIMERIC ANTIGEN RECEPTOR (CAR)



WHAT IS CAR-T CELL?

- Genetically engineered T cell immunotherapy
  - Targets antigen on the tumor cell surface
  - Retains endogenous TCRs; can be removed by genome editing
- “Living Drug”
  - Dynamic cell population during manufacturing process
  - Expands and differentiates after administration
- Control of the manufacturing process is essential for consistency

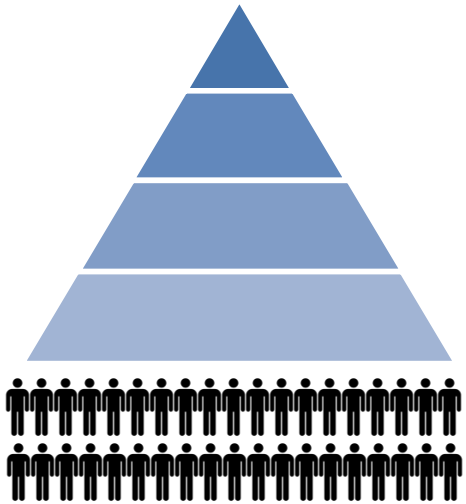


www.cancer.gov

PERSONALIZED MEDICINE:
A DIFFERENT MANUFACTURING PARADIGM

Conventional Drug/Biologic

1 product lot



Many patients

Raw materials

cGMP Manufacturing

In Process and Lot Release Testing

Distribution

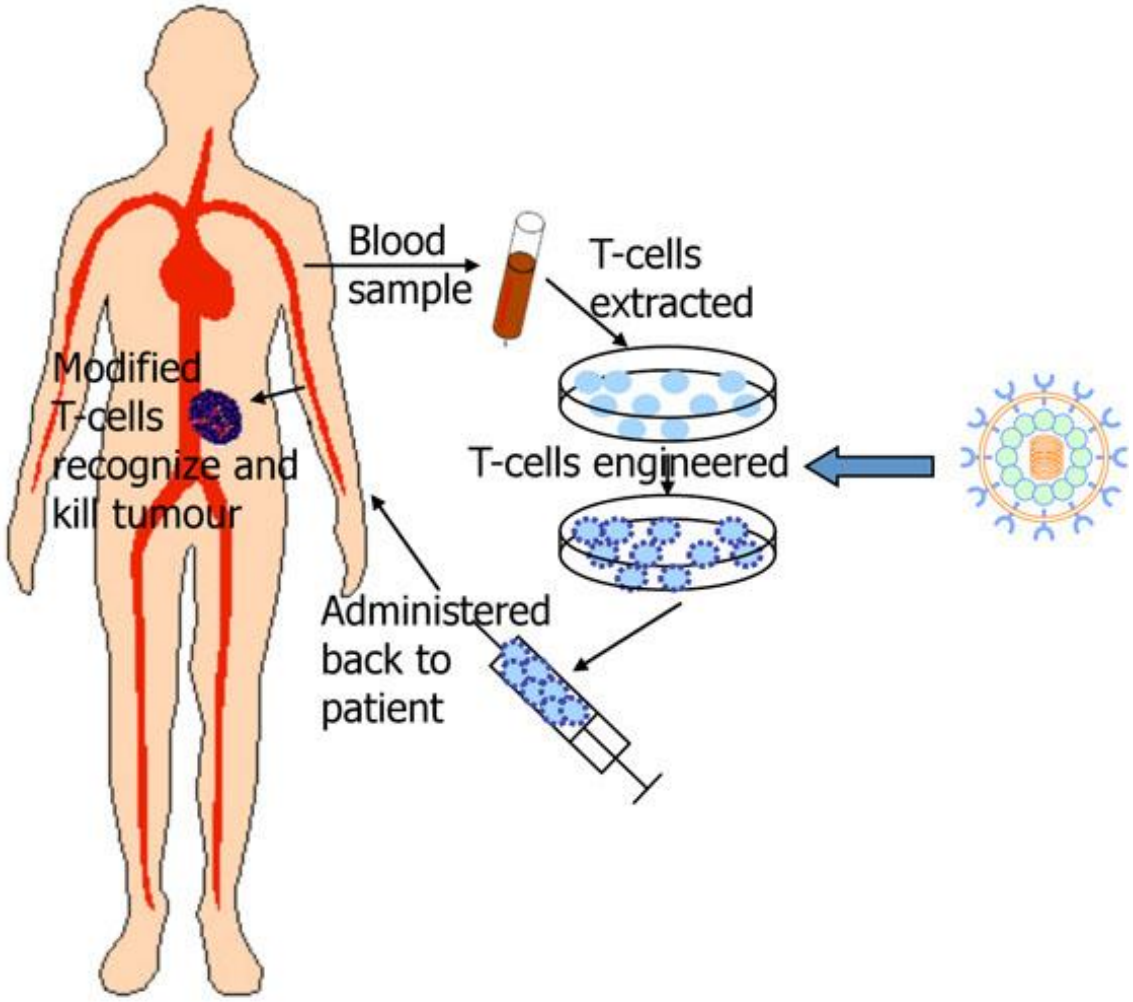
Autologous cellular product (ex: CAR T cells)

1 product lot

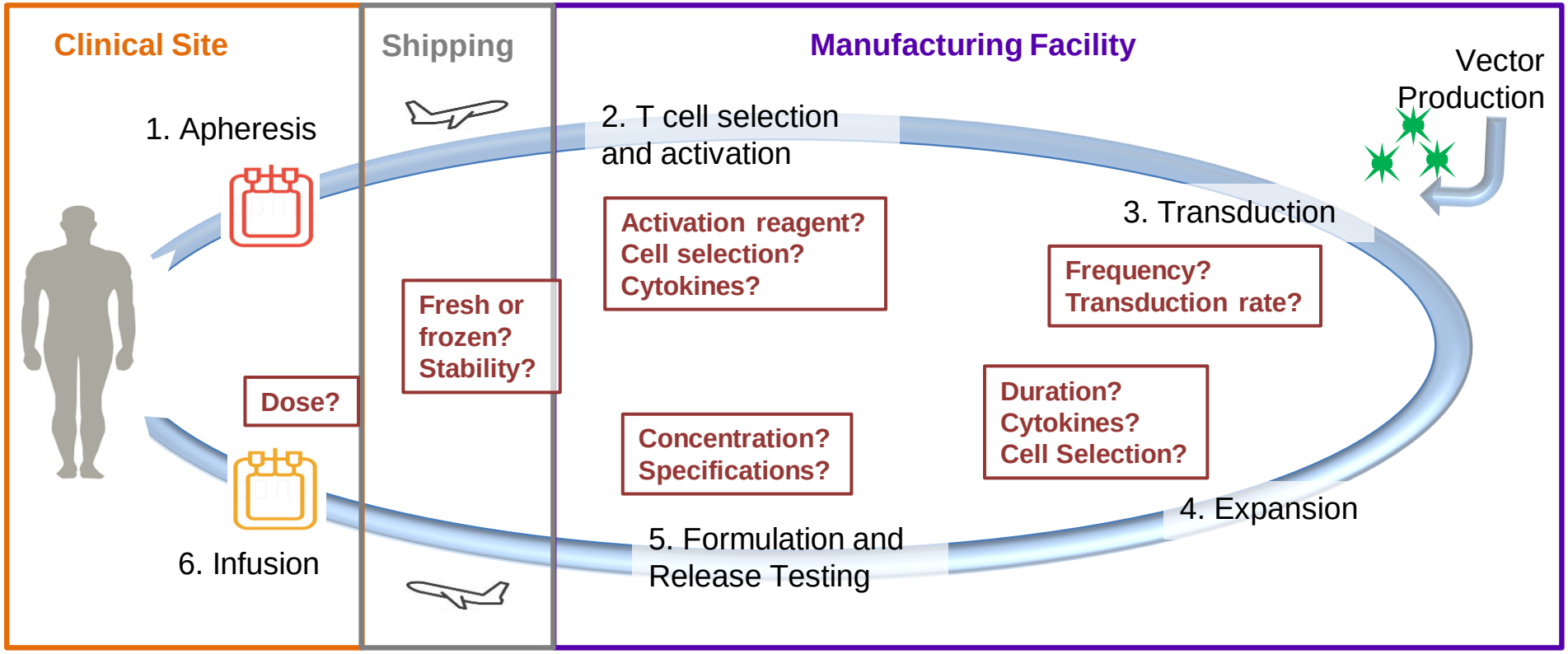
One patient

Kymriah® Manufacturing Process

- **Leukapheresis**
  - T cells within white blood cells extracted from patient through blood filtration process and then crypreserved
- **Reprogramming**
  - T cells are genetically encoded to recognize tumor antigen expressed on cancer cells using inactive viral vector
- **Expansion**
  - The newly created CAR-t cells are expanded
- **Returned to patient**
  - After QC testing the product is released and shipped to the patient who has received lymphodepleting chemotherapy which aims to deplete the level of white blood cells



CAR-T: COMPLICATED MANUFACTURING PROCESS



Each step can affect the final product

MANUFACTURING CHALLENGES

- Production of cells for larger patient populations
- Fully closed sterile manufacturing process required
- Cell characteristics are strongly influenced by the environment
- Cells are fragile, relatively unstable, damaged easily
- Cryopreservation required for storage and back up
- Cold chains required for cell delivery from and to the patient
- Defining and testing the quality of cell therapy manufacturing challenging

ADDITIONAL CHALLENGES (I)

- Many factors influence CQAs, such as: cell source (autologous cell therapies are specific for each patient), growth media variability, random events inside the cells, etc.
- Monitoring needed to ensure that the CQAs are maintained throughout the cold chain
- Final cells product can be neurotoxic/immunogenic, cause graft-versus-host disease, or lead to cytokine release syndrome (dangerously high fevers, precipitous drop in blood pressure and potentially resulting in organ failure)
- Deaths have occurred in some clinical trials

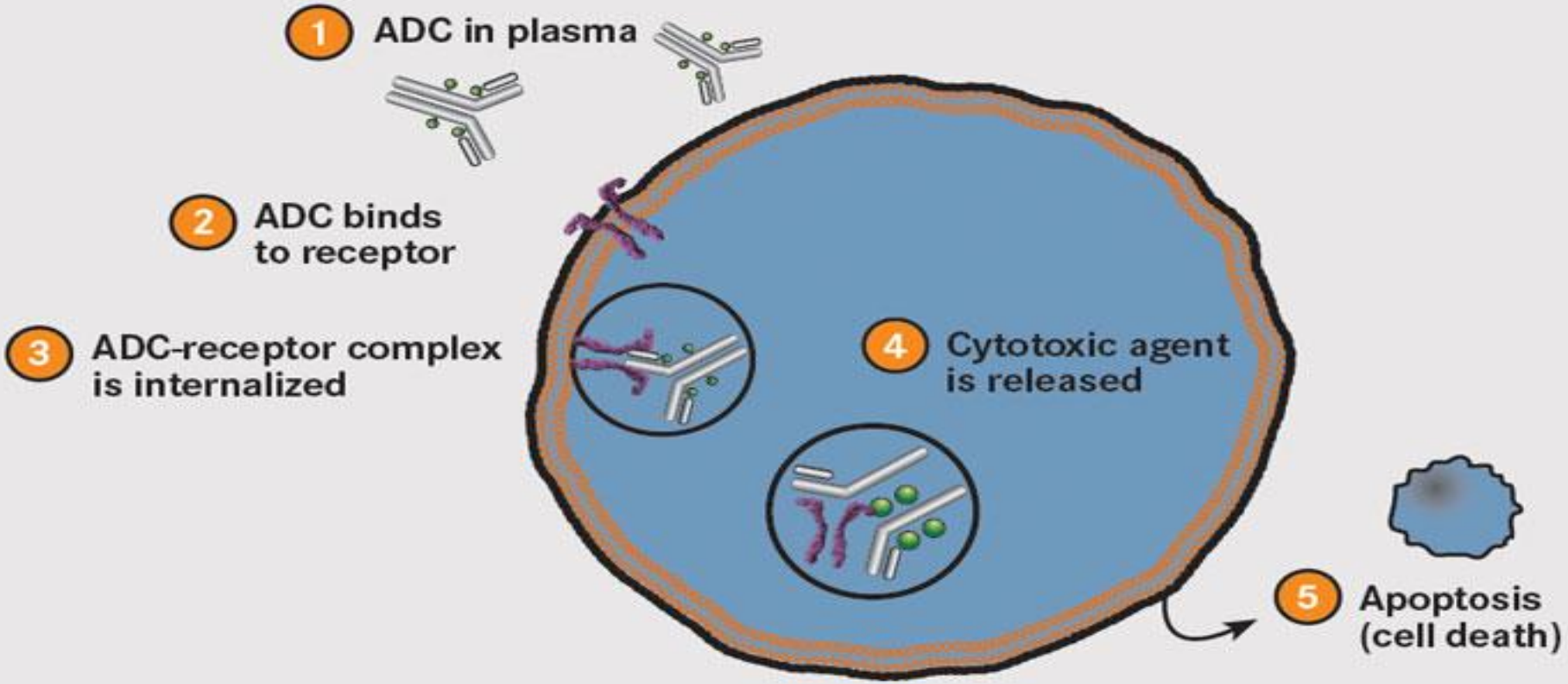
ADDITIONAL CHALLENGES (II)

- Development path and timelines for cell and gene therapies can vary widely
- Identification of optimal drug delivery mechanism on the critical path
- For gene therapies based on nucleic acids is is difficult to stabilize the molecules long enough for them to take effect
- Viral vectors present a risk of immune response or other unwanted side effects
- Environmental health and safety issues
- Need strategy for adventitious agents control
- Technology for viral vector production lacking. Limited CMO options for viral vectors can lead to delays in development programs and unpredictable manufacturing costs

WHY MAKE ANTIBODY-DRUG CONJUGATES (ADCS)?

- MAbs have exquisite selectivity and affinity for their target antigens
- They are not always effective as standalone therapies, but are excellent targeting modalities
- MAbs may thus serve as delivery vehicles to selectively deliver agents (toxins, radionuclides, imaging agents) to specific tissues
- Much current interest in delivering cytotoxics to cancer tissue via targeting tumor-selective antigens

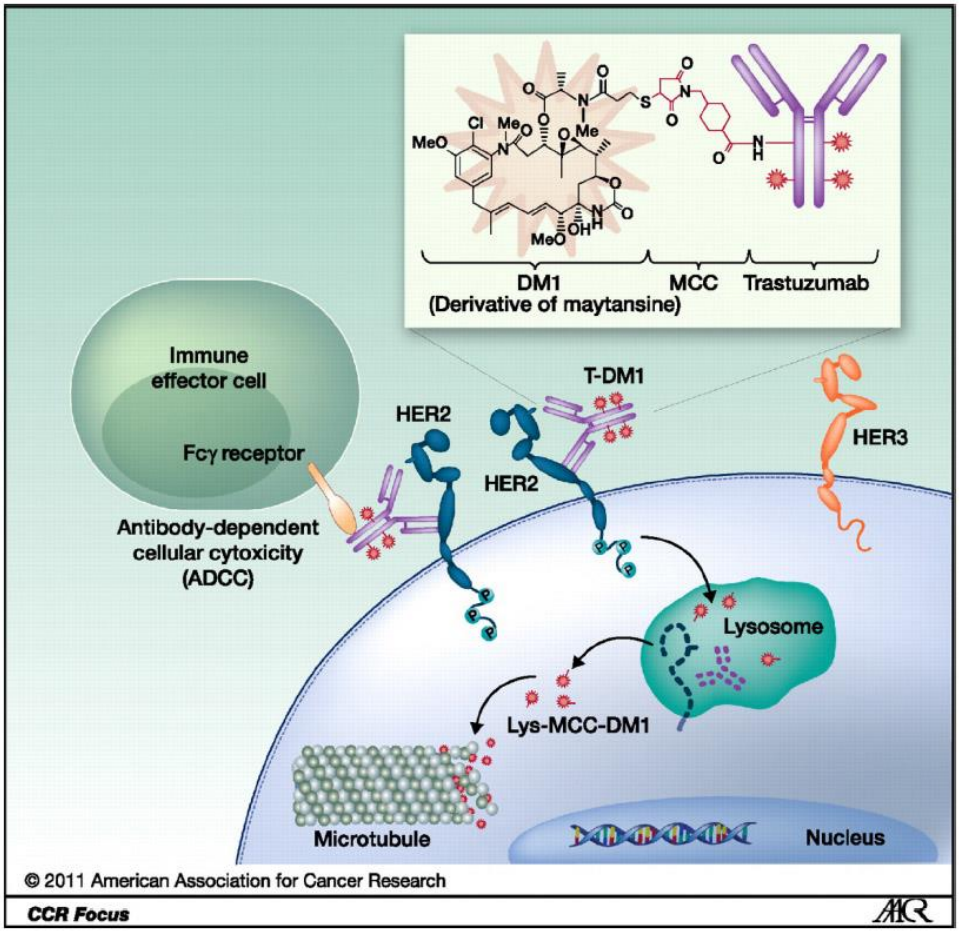
Primary Mechanism of Action of ADCs: Targeted Delivery of a Cytotoxic Agent



Reference: Carter PJ et al. *Cancer J*. 2008;14(3):154-169.
Source: Antibody-drug conjugates (ADCs): empowering monoclonal antibodies to fight cancer. Seattle Genetics website. http://www.seagen.com. Published June 2011. Accessed May 29, 2012. Reprinted with permission.

“Cellular View” of Kadcyla® (TDM1-Trastuzumab) Mechanism of Action

- Binds to the breast cancer cell HER2 receptor via the Fab portion of the trastuzumab antibody thus preventing receptor dimerization which slows down tumor growth
  - Potential **downregulation of HER2/neu** leading to disruption of HER2 receptor dimerization
  - Activation of **ADCC** method of cancer cell killing
  - Arrest of tumor cells at G1 phase
- The toxic DM1 portion of the drug is deposited into the lysosome of the cell via endocytosis and then released intracellularly where it interferes with microtubule formation and hence tumor cell division.



CHALLENGES IN DEVELOPMENT OF ANTIBODY DRUG CONJUGATES

Toxic molecule or toxin must stay attached to Mab while in circulatory system but be released after introduction into the cell.Must be released from the Mab, internalized, and then achieve a sufficient intracellular concentration to promote cell death.

The potency of the released toxin must be high enough to kill tumor cells at low concentrations.

Conjugated Mab must retain the high affinity for the tumor cell antigen.

Free toxin may be extremely dangerous due to high level of potency

Difficult to work with in manufacturing and QC operations

Special production facilities may be needed

Design of linker, stability and conjugation scheme may be difficult to achieve.

COMBINATION PRODUCT

- Combination of two or more constituent parts:

  biologic + device

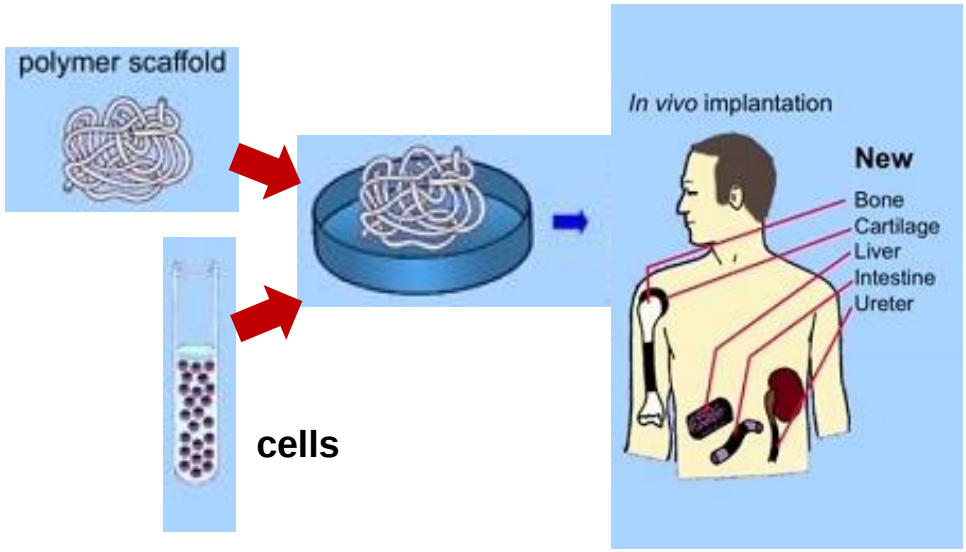
  biologic + drug

  drug + device

  biologic + device + drug

- Constituent parts are typically developed under disparate manufacturing and regulatory approaches before combining

- Consider both constituent parts and the final combination product



Adapted from: https://www.solveforx.com/moonshots/biomaterials-for-the-21st-century

Q/C/C WELCOME

ACKNOWLEDGEMENT

Some slides excerpted from presentation by

Dr. Steven Oh, Deputy Director

Division of Cellular and Gene Therapies

Office of Tissues and Advanced Therapies

Center for Biologics Evaluation and Research

Food and Drug Administration

Obrigado!

Perguntas?

moreira@umbc.edu