NCI Drug Development Workshop:
How to Advance A Therapeutic Candidate from Bench to Bedside

Session V. Development of Biological Products

PROCESS DEVELOPMENT: Navigating the UP/DOWNstreams

Rachelle Salomon, Ph.D.

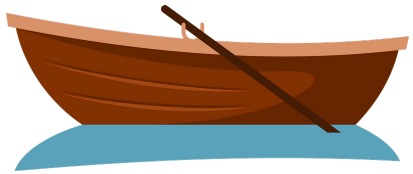
NIH NATIONAL CANCER INSTITUTE

Process Development for Biologics

- Manufacturing a biologic product involves a **process** that uses living cells or their components.

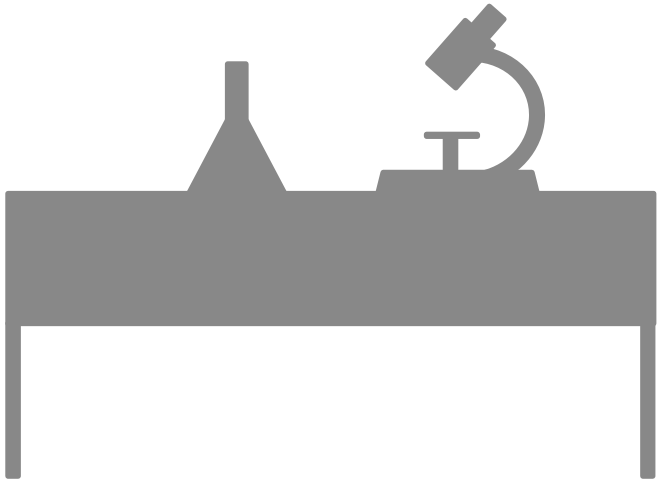
- The process is typically carried out in a bioreactor which allows for the highest control of the environment.

- This presentation will cover:
  - Goals of Process Development (PD)
  - How cell line selection drives the PD
  - Considerations for Upstream PD
  - Navigating Downstream PD



Drug development requires paradigm shift to advance drug from bench to clinic

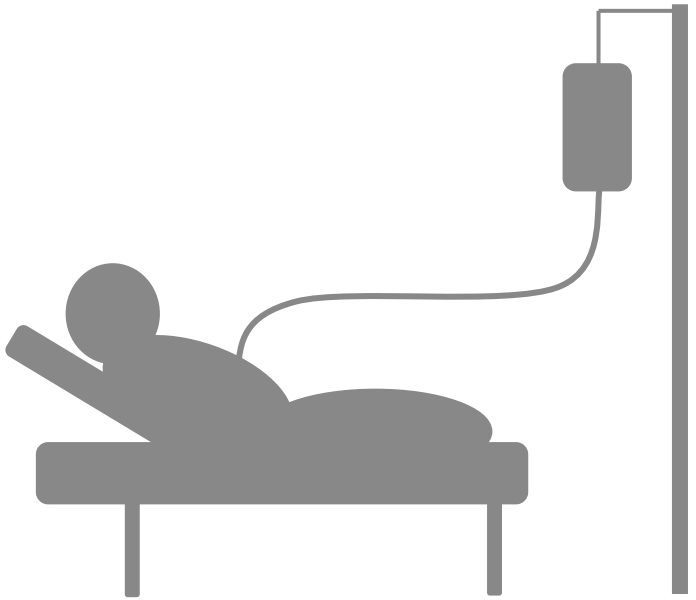
Basic Research



Hypothesis-driven paradigm

'expand knowledge'

Drug Development



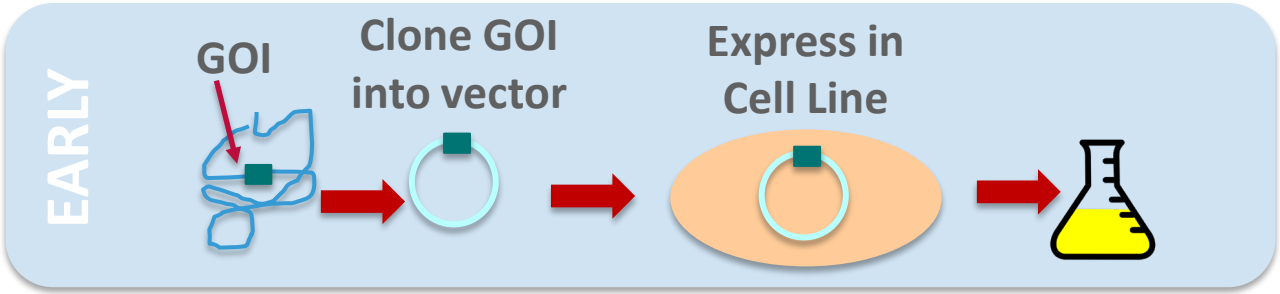
Engineering paradigm

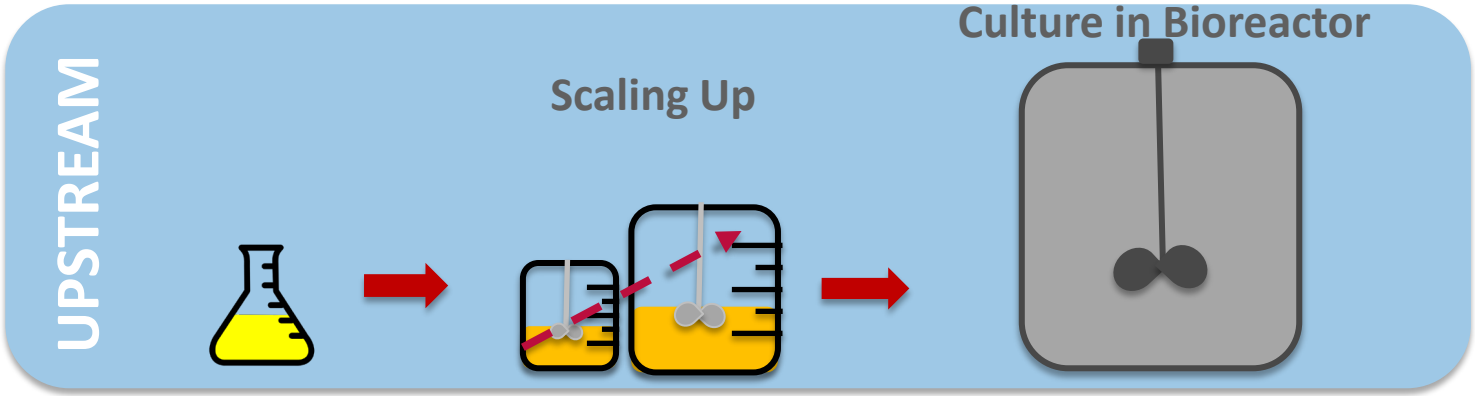
'keep the END in mind'

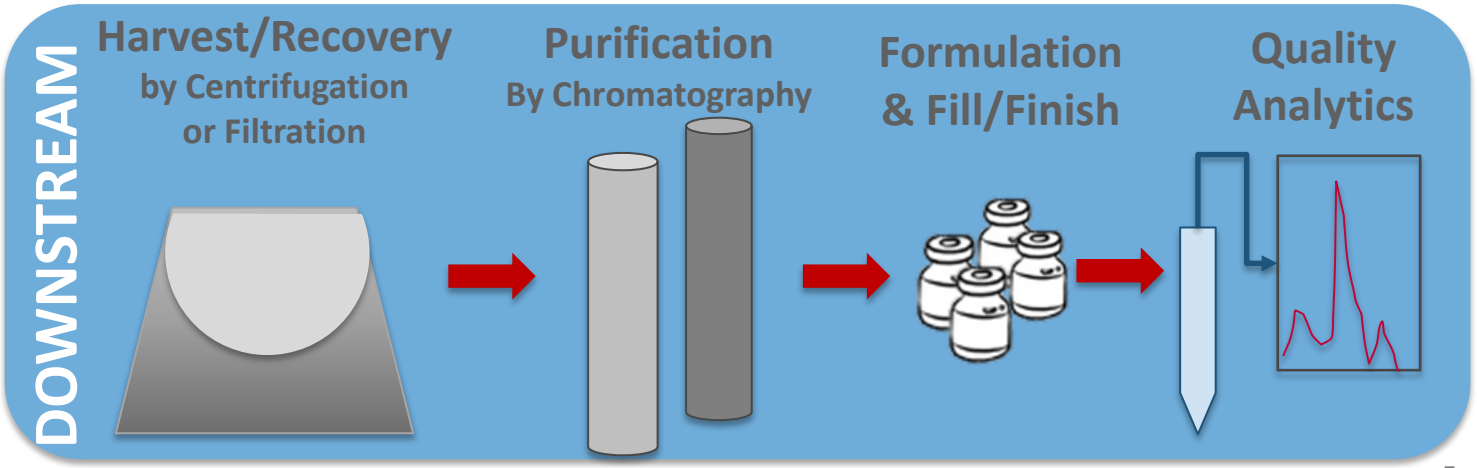
Process Development

- The sequence of operations and parameters required to ensure that manufacturing process produces a product within specification.
- The objective of process development is to develop a robust, scalable, reproducible, and cost-effective process that results in safe and efficacious biopharmaceuticals.
- Process development is the means to the **END**.

Phases of Process Development





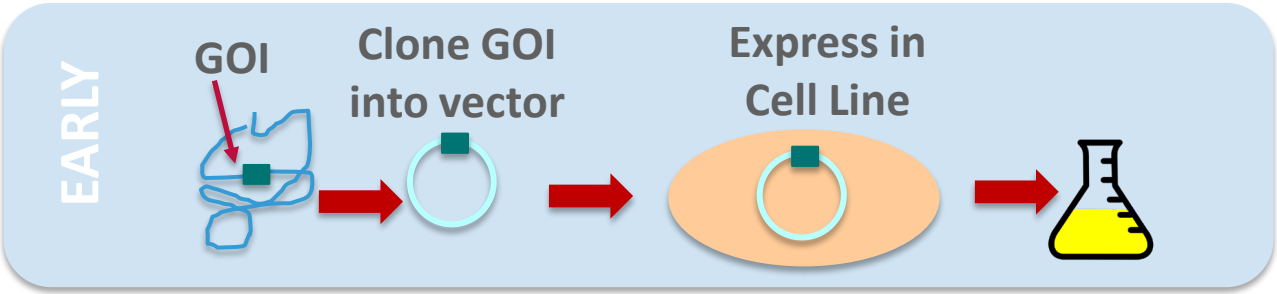


Process Development Links Tech Transfer & cGMP Manufacturing

Technology Transfer → Process Development → Engineering Lot → Clinical Lot

Often may be used to perform IND enabling Toxicology and Safety Studies

Early Process Development



- The early phase of process development involves selection of the cells or components that will produce the biologic.
- Highly characterized cells are required to ensure quality and safety of biologics.
- Regulatory authorities require information about the cells including:

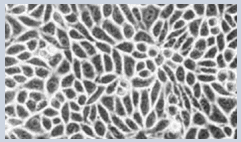
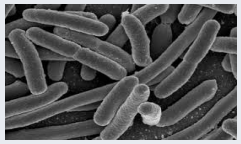
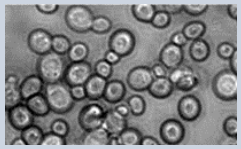
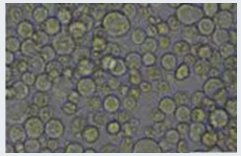
Derivation
- Characteristics – species, strain, genotype, phenotype, generation, pathogenicity, toxins, purity, ect.
- Data - published, historical, and experimental
- Procedures used to generate the cells – e.g. media requirements

Stability
- Consistent production and recovery of product
- Stability during storage and cultivation

Testing
- Consistency of the coding sequence
- Other traits (e.g growth characteristics, biochemical/immunological markers, productivity)

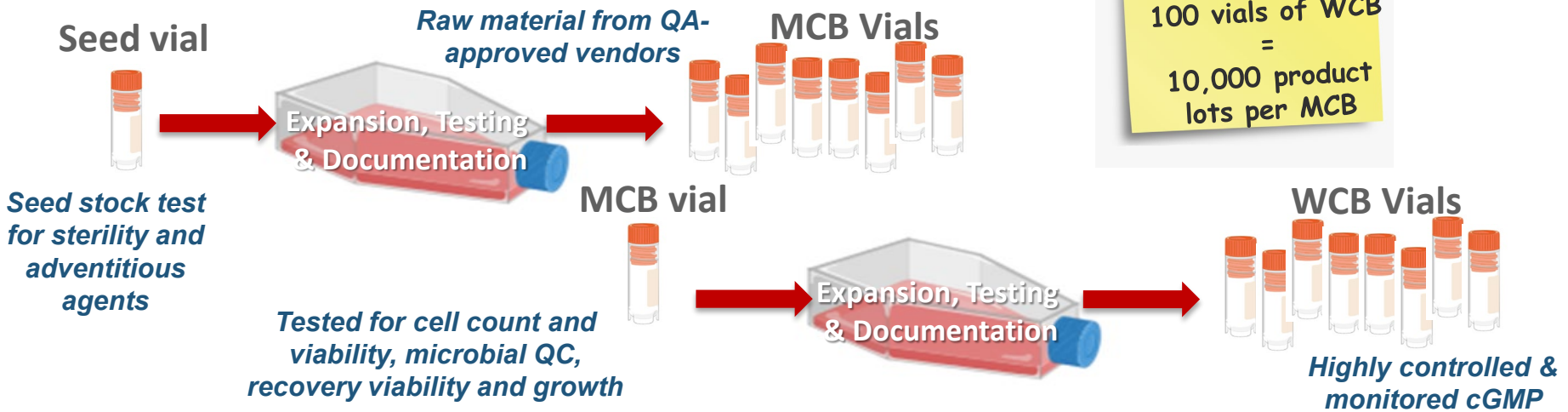
Remember that the sequence, vector & cell line used at the research bench may not be the same as those used in cGMP PD

Choosing An Expression System

System	Advantages	Challenges	Examples
Mammalian 	• Highest-level protein processing • Transient or stable	• High yields (grams/liter) may require suspension cultures • Complex culture conditions	• Human Embryonic Kidney 293 (HEK293) • Chinese hamster ovary (CHO) cells
Bacterial 	• Scalable • Low cost • Simple culture conditions	• Protein solubility • May require protein specific optimization	• Escherichia coli (*E. coli*)
Yeast 	• Eukaryotic protein processing • Scalable • Simple media	• Fermentation required • Growth conditions may require optimization	• Saccharomyces cerevisiae (*S. cerevisiae*) • Pichia pastoris (*P. pastoris*)
Insect 	• Protein processing like to mammalian	• Culture conditions more complex than prokaryotics • Production of recombinant baculovirus vectors are time consuming	• Baculovirus expression vector system (BEVS)

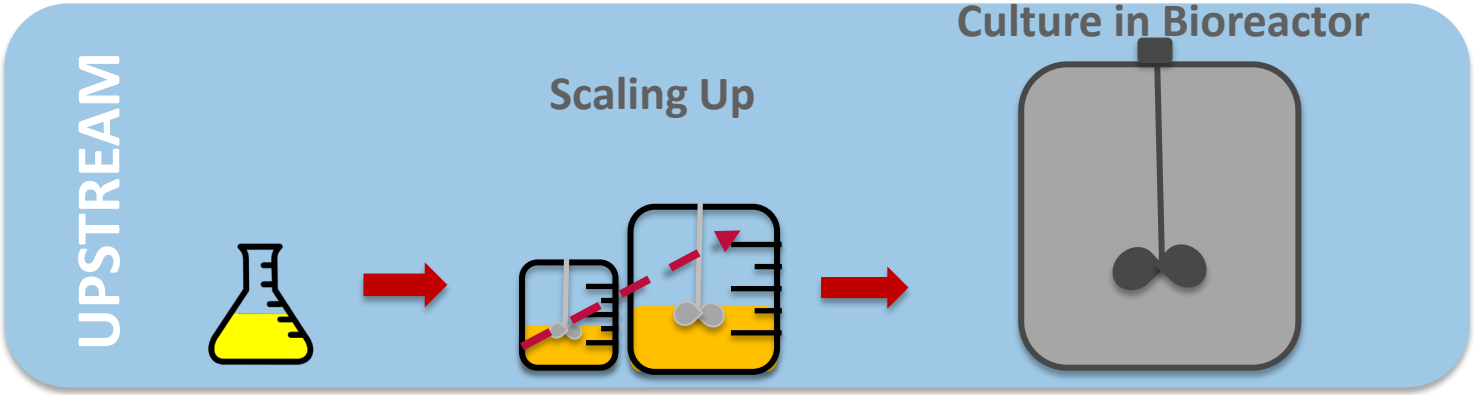
Establishing a Cell Bank System

- Establishing a cGMP cell bank system is early development step for biopharmaceutical production.
- Cell bank systems decrease risks of maintaining a cell line *(e.g. microbial contamination, loss of desired characteristics, and genetic drift)*.
- A **master cell bank (MCB)** – aliquots of a uniform cells derived from a single source – are used to manufacture a **working cell bank (WCB)**. The WCB is used for production.
- The source and process for generating MCBs and WCBs must be documented and traceable.



Cell Banking Math:
100 vials of MCB
X
100 vials of WCB
=
10,000 product lots per MCB

Upstream Process Development



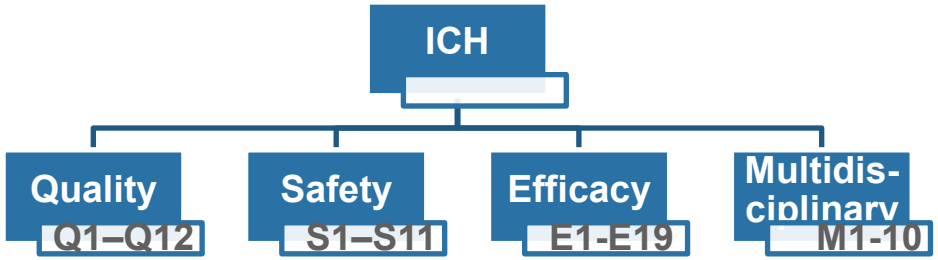
- Upstream processes are the steps in which cells are grown in bioreactors.

- Key upstream processes include:
  - Inoculum development
  - Culture medium generation
  - Optimization of growth kinetics

Remember that the conditions and processes used at the research bench may not be the same as those used in cGMP PD

ICH for Biologics

- The International Council for Harmonization (ICH) documents technical requirements for pharmaceuticals for human use.
- Joint initiative involving both regulatory authorities and pharmaceutical industry representatives of the US, EU, and Japan.




INTERNATIONAL CONFERENCE ON HARMONISATION OF TECHNICAL REQUIREMENTS FOR REGISTRATION OF PHARMACEUTICALS FOR HUMAN USE

ICH HARMONISED TRIPARTITE GUIDELINE

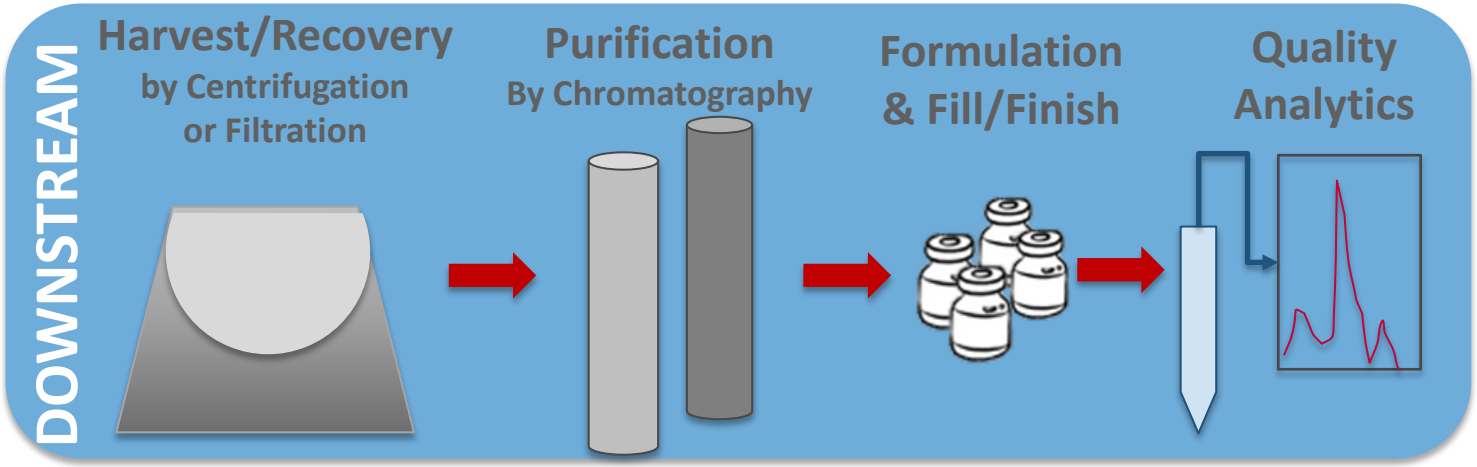
STABILITY TESTING OF NEW DRUG SUBSTANCES AND PRODUCTS Q1A(R2)

Current Step 4 version dated 6 February 2003

This Guideline has been developed by the appropriate ICH Expert Working Group and has been subject to consultation by the regulatory parties, in accordance with the ICH Process. At Step 4 of the Process the final draft is recommended for adoption to the regulatory bodies of the European Union, Japan and USA.

- Q5 A: Viral Safety
- Q5 B: Genetic Stability
- Q5 C: Product Stability
- Q5 D: Cell Substrates
- Q5 E: Comparability
- Q6 B: Specification
- Q7: GMP for APIs
- Q8: Pharmaceutical development
- Q9: Quality Risk Management
- Q10: Pharmaceutical quality system
- Q11: Development and Manufacture of Drug Substances

Downstream Process Development



- Downstream processes are the steps to recover and purify the biologic product.
- Key downstream processes include:
  - Cell disruption if the biologic product is intracellular
  - Separation of the biomass/cells from product
  - Concentration of medium if the product is extracellular
  - Purification of the product
  - Impurities Removal – viral inactivation, endotoxin & host cell impurities removal
  - Protein modification – refolding, PEGylation, bioconjugation, enzymatic processing
  - Formulation & Fill/Finish
  - Quality Analytics – acceptance criteria

Downstream PD Considerations

Precipitation and centrifugation methods

- Salting out
- Isoelectric point precipitation
- Precipitation with miscible solvents
- Non-ionic hydrophilic polymers
- Polyvalent metallic ions
- Centrifugation
- Gradient centrifugation

Filtration Processes

- Microfiltration
- Depth filtration
- DF (diafiltration)
- UF (ultrafiltration)
- TFF (tangential flow filtration)
- CFF (crossflow filtration)

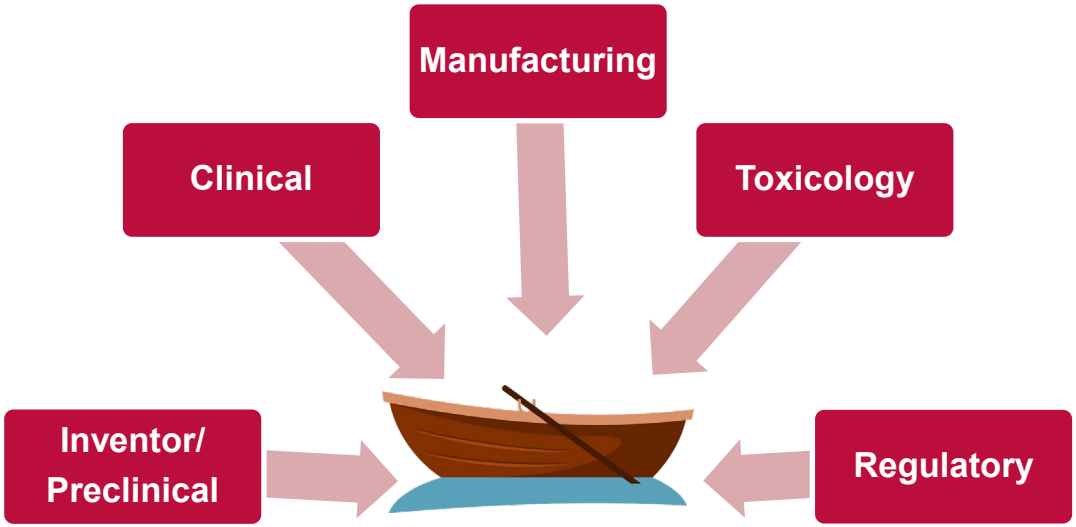
Viral Inactivation & Impurity Removal Processes

- Low/High pH
- Solvent/Detergent
- Heat/Pasteurization/Irradiation/Sterilization
- High-energy light
- Chemical inactivation
- Chromatography
- Filtration

Chromatography Processes

- Column chromatography
  - Ion exchange
  - HIC (Hydrophobic interaction)
  - Anion and cation exchange
  - Size exclusion
  - Immunoaffinity chromatography
  - Normal phase chromatography
  - Reverse phase chromatography
  - Expanded bed absorption
- Membrane chromatography
- HPLC (High performance liquid chromatography)

Comprehensive Product Development Team Is Essential To Navigate PD



- For biologics the **Product is the Process** – effective process development for manufacturing is central to product quality and the path to clinical trials.
- Drug development paradigm shift requires **comprehensive development team** to guide process with **END in mind**.
- Cell line selection defines the upstream & downstream process.

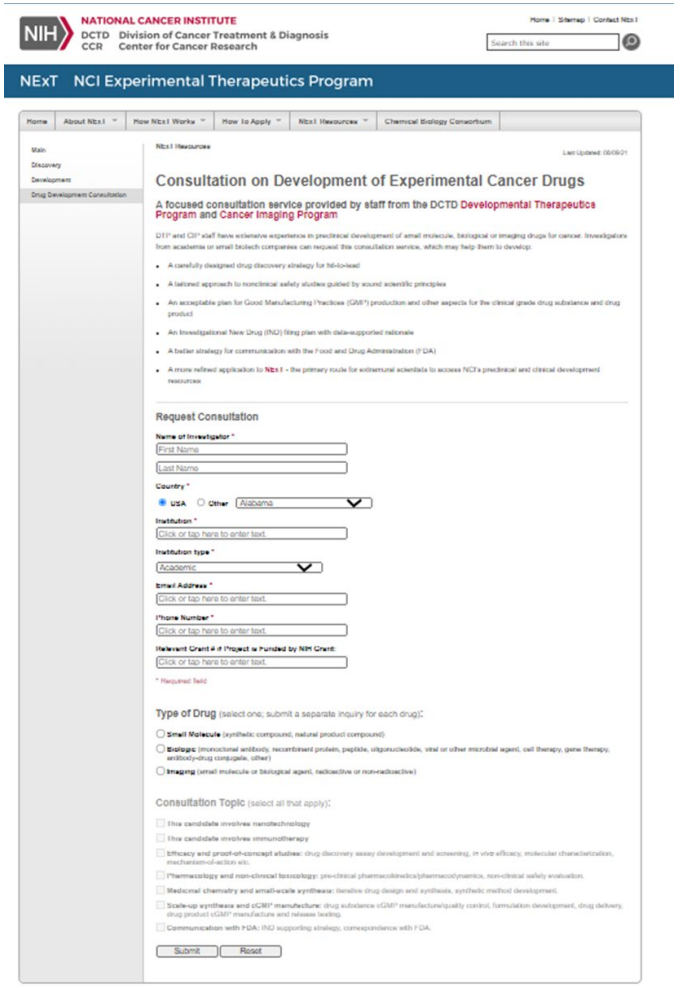
Let's Talk About Developing Your Biologic

Biological Resources Branch (BRB)

Name	Title	Email
Jason Yovandich, PhD, RAC	Branch Chief / COR III	yovandij@mail.nih.gov
Ray Harris, PhD	Program Director / COR III	raymond.harris@nih.gov
Rachelle Salomon, PhD	Program Director / COR III	rachelle.salomon@nih.gov
Anthony Welch, PhD	Program Director / COR III	welcha@mail.nih.gov

https://dtp.cancer.gov/organization/brb/default.htm

DCTD Consultation



https://next.cancer.gov/experimentalTherapeutics/form.htm