

Pharmville: Pembroza

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Company Goal

- Develop a biosimilar to Keytruda (pembrolizumab)
 - Human antibody (IgG4) used in cancer immunotherapy
 - PD-1 pathway inhibitor
 - Large molecule
- How we reach this goal
 - FDA's Biosimilar Action Plan (BAP)
 - 351(k) Biologics License Applications (BLAs)
 - Merck was approved in Sept. 2014
 - Potential approval by 2026



Target Indications



- Merck and Co (Keytruda)
 - 13 FDA approved indications, the first being melanoma
 - FDA approval in 2017 for any unresectable or metastatic solid tumor with certain genetic anomalies
- Pembroza will initially target 3 indications:
 - Melanoma
 - Non-Small Cell Lung Cancer (NSCLC)
 - Small Cell Lung Cancer (SCLC)
- Upon FDA approval as a biosimilar, Pembroza will receive all 13 approved indications

Treatment Conditions

Melanoma

- Spread and cannot be removed by surgery
- Decrease relapsation

Non small cell lung cancer (NSCLC)

- Use with chemotherapy
- Use as first treatment
- Use for advanced NSCLC



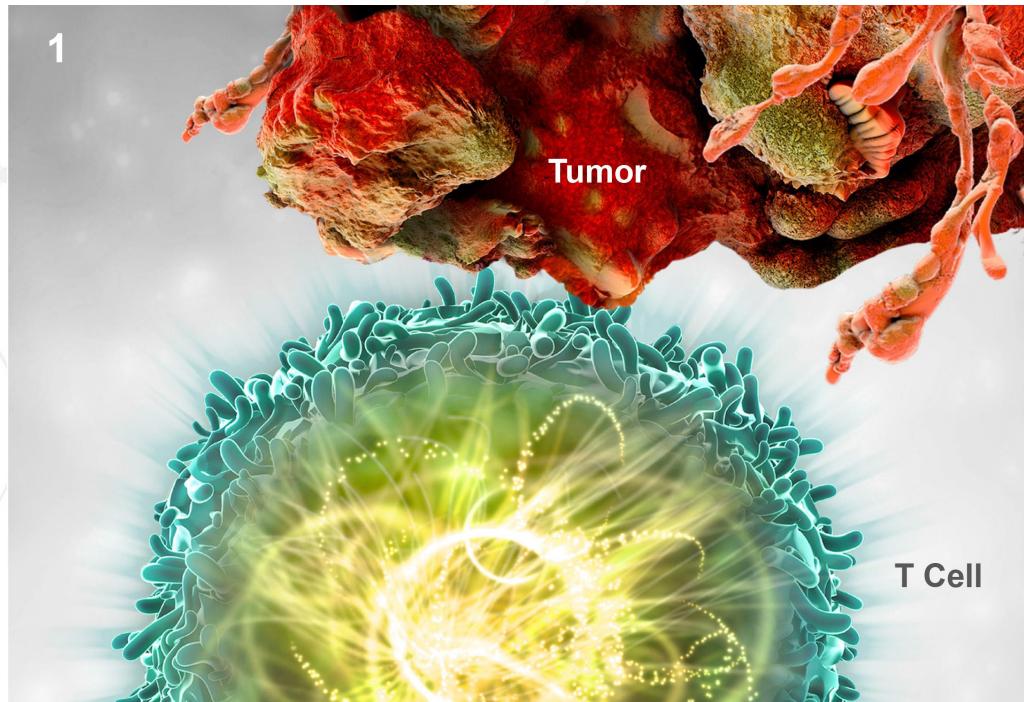
Small Cell Lung Cancer (SCLC)

- Metastatic SCLC with disease progression on or after platinum-based chemotherapy and at least one other prior line of therapy

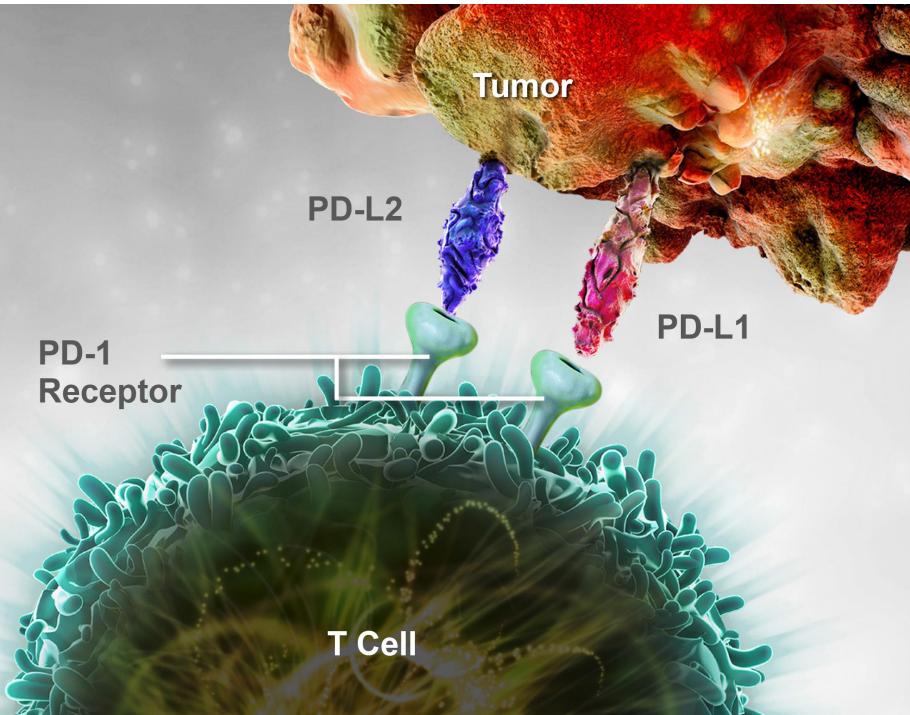
Mechanism of Action

Mechanism of Action

- Properly working T cells can recognize cancerous cells in the body and attack them
- Cancer cells often have antigen proteins on their surface, that can be recognized by antibody proteins of the immune system



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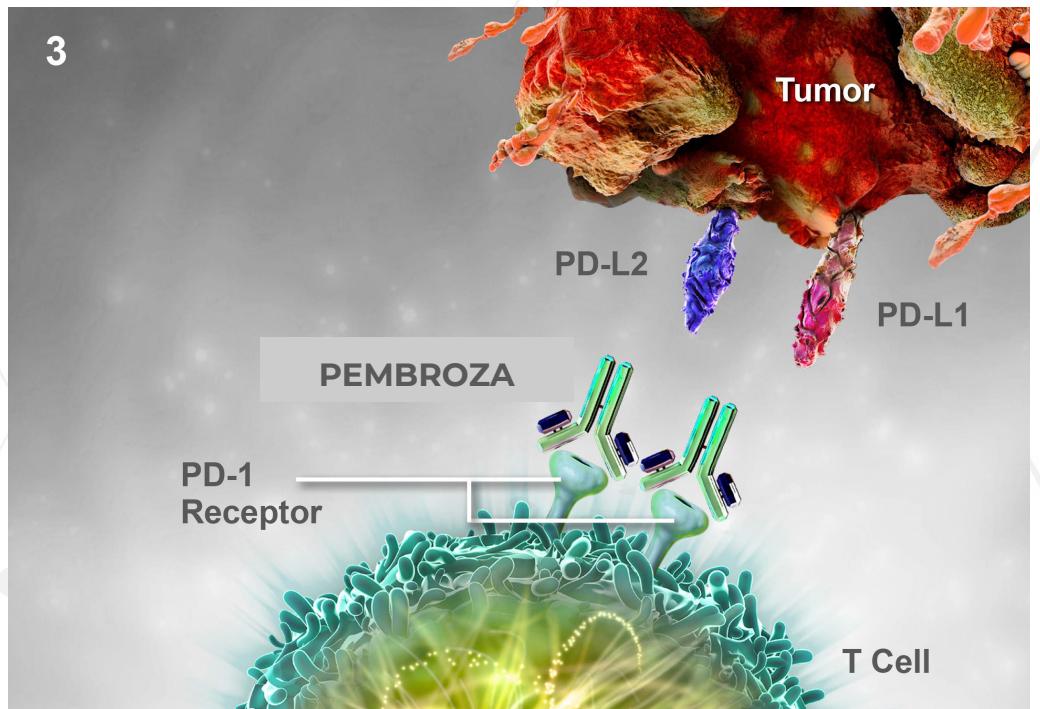


Mechanism of Action

- PD-L1 and PD-L2 are ligands on tumors that can bind to PD-1 receptors on T cells
- T cells with bound ligands become “inactivated” and can no longer recognize cancerous cells

Mechanism of Action

- Pembrolizumab has a high affinity to PD-1 receptors on T cells
- Conformational change to the surface of the PD-1 receptors and blocks interactions with PD-L1 and PD-L2 ligands on the tumor cells
- The T cells can now recognize the tumors



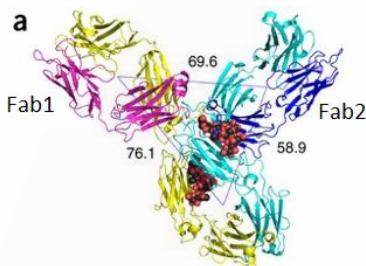
Critical Quality Attributes

Identity, Purity, Safety and Efficacy

Identity



Purposes	Quality Attribute	Method/Test	Specification
Physical and Chemical Properties	PI	CEX-icIEF	6.8-6.9
	Molecular Weight	SDS-PAGE	149 KDa
	Glycosylation	SDS-PAGE	Glycosylated sites of the CH2 domains of each of the chains of the Fc structure.
	Amino Acid Sequence	Peptide mapping	Same amino acid sequence as Keytruda (primary)
	Concentration (per vial)	UV absorbance @ 280 spectrophotometer	25 mg/mL
	Structure	UV circular dichroism	Asymmetrical Y-shape, the Fc domain



Purity



Purposes	Quality Attribute	Method/Test	Specification
Process Related Impurities	Residual HCP	Immunoassay, 2D-LC-MS	<100 ng/mg
	Residual DNA	qPCR	< 10 pg/mg
	Residual Protein A	ELISA	<1 ppm
	Deamidation	LC-MS	<5% Heavy chain: Asn55, Asn384, Asn389
	Aggregates	Size-exclusion chromatography	<2% 32 cysteine residues, 4 disulfide bonds, 9 H-bonds, 3 water-mediated H-bonds, 2 salt bridges
Product Related Impurities	Oxidation	LC-MS	<5% Heavy Chain: Met105, Met252, Met358, Met428
	Charge Variants	Ion Exchange Chromatography	<3% Shallow pH gradient → smaller pH range → higher resolution of charge variants

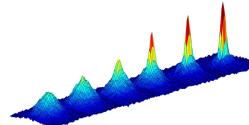


Safety



Purposes	Quality Attribute	Test	Specification
Contaminants	Endotoxin	Kinetic Chromogenic LAL	<2.5 EU/mg
	Mycoplasma	PCR	Negative

Efficacy



Purposes	Quality Attribute	Method/Test	Specification
Bioactivity Attributes	Potency Effecter function	Antigen Binding Assay Flow Cytometry	IC_{50} range from 625 to 700 pM. $>80\%$

QTPP

Quality Target Product Profile

Attribute

Target

Indication

Melanoma Cancer
Non-small cell and small cell lung cancers

Mechanism of Action

PD-L1 and PD-L2 pathway inhibitor. Biosimilar causes a conformational change on PD-1 receptors

Dosage Form

Sterile solution in single-dose vial

Dosage Strength

100 mg/4 mL

Mode of Administration

Intravenous, diluted with isotonic saline

Drug Product Primary Container

5 mL type 1 borosilicate glass vials, fluoro-resin laminated stopper

Attribute	Target
Drug Product Shelf-Life	Minimal claim at submission 36 months at 2 - 8 °C
Compatibility with Application Devices and Stability during Administration	IV bag and application lines compatible (conc. range 0.4-20 mg/mL) Infusion rate 4 mL/h without requirement of inline filter Stable solution for 6 h at room temp., 24 h if refrigerated
Drug Product Quality Requirements	Meets pharmacopoeial requirements for parenteral dosage forms (PhEur, USP, JP)
Degradants and Impurities	Acceptable patient risk due to process-related and product-related impurities in relation to the benefit

FDA Requirements



351(k) application requirement for CDER approval

- Analytical Studies and Clinical Studies

Pharmacovigilance

- Monitor all adverse effects and warnings
 - Biosimilar to Keytruda
- Mini-Sentinel FDA program

Validation

- Individual systems (Autoclaves, oven, centrifuges)
- Methods (SE-HPLC, SDS-PAGE)
- Processes (Cell culture, upstream, downstream)
- Facility (Warehouse)

cGMPs



Clinical Studies

Phase II

	Number of Patients
Melanoma	22
NSCLC	14
SCLC	10
<i>Total</i>	46

- Use immunohistochemistry to test for PD-L1 positive (expression in > 1% of tumor)
 - Except for trial with melanoma
- Dosage: 10 mg/kg every 2 weeks until:
 - 24 months, disease progression, or tolerable toxicity reached
- Tumor is evaluated every 8 weeks for the first 6 months and every 12 weeks thereafter
- Record response on benefits and adverse effect



Clinical Studies

Phase III

	Number of Patients
Melanoma	140
NSCLC	70
SCLC	40
<i>Total</i>	250



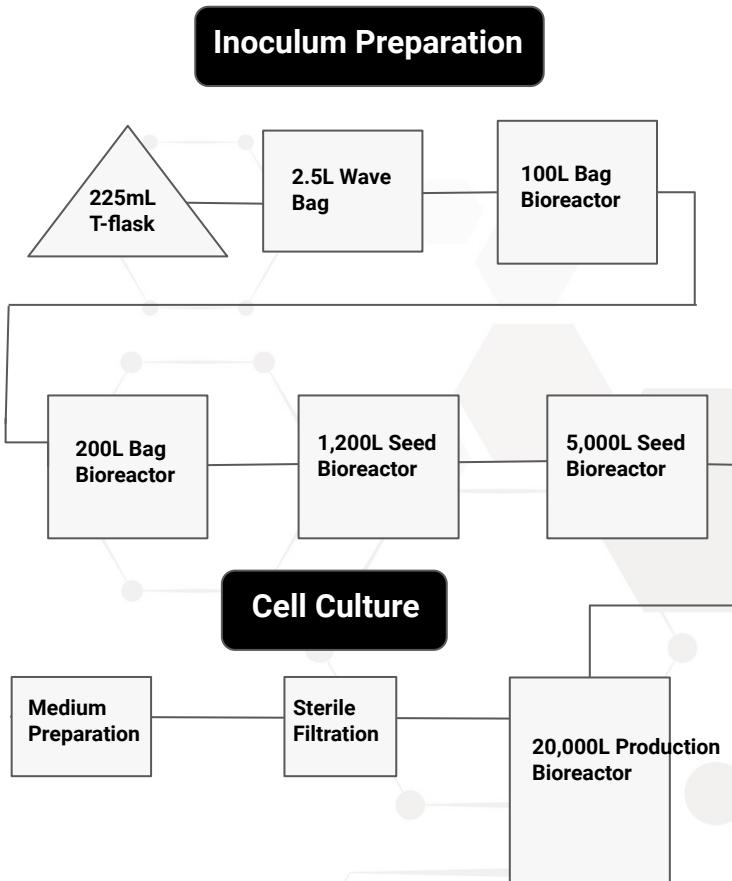
- Advanced Melanoma
 - Dosage: 10 mg/kg every 2 weeks
 - Unresectable stage III or IV melanoma
 - Response check every 6 weeks for 48 weeks and every 12 weeks thereafter for at least 21 months total
- NSCLC and SCLC
 - Dosage: 200mg every 3 weeks - up to 35 cycles
- Subjects are over 18 years old and tested for presence of PD-L1

General Process Manufacturing

Upstream & Downstream Processing

Upstream Processing

Block Flow Diagram



Cells and Medium

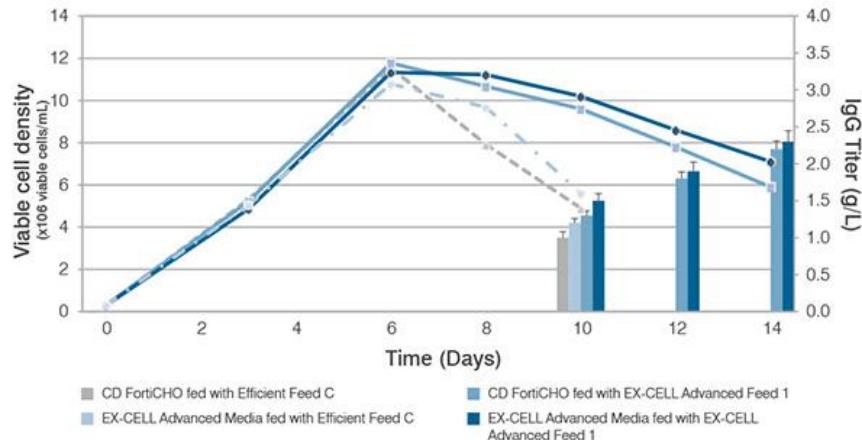


CHO-K1 Cell Line

- Inexpensive cell line for developing therapeutic products
- Well-researched and well-established
- Capable of producing large quantities of recombinant protein

EX-CELL CD CHO Growth Medium

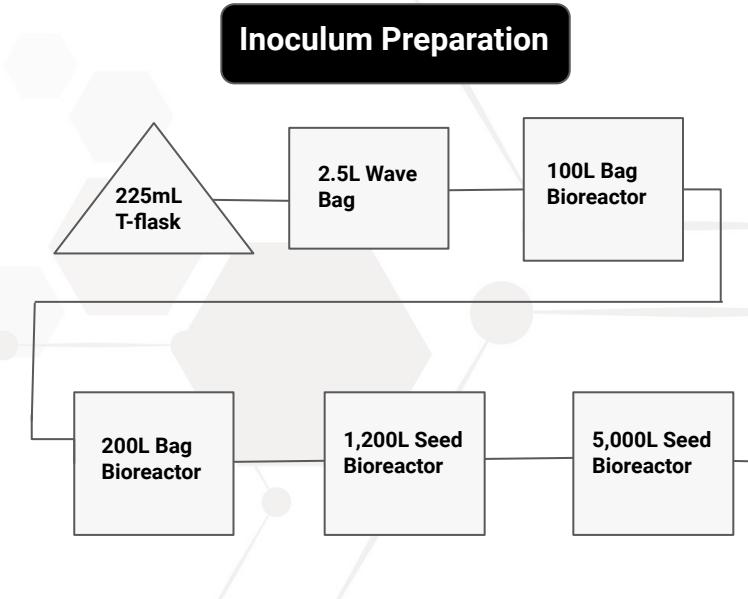
- CHO cell medium designed for antibody production
- Serum-free and chemically defined medium
- Requires addition of L-glutamine



Inoculum Preparation

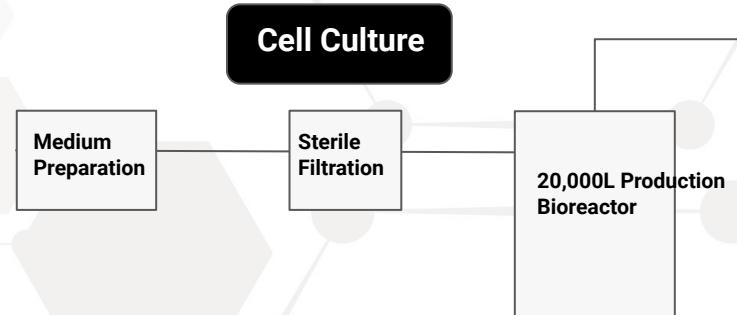
Seed train:

- 18-unit rack of 225mL T-flasks
~3-4 days
- 2.5L wave bag
~3-4 days
- 100L disposable bag bioreactor
~3-4 days
- 200L disposable bag bioreactor
~3-4 days
- 1,200L seed bioreactor
~3-4 days
- 5,000L seed bioreactor
~3-4 days
- Moves into Cell Culture

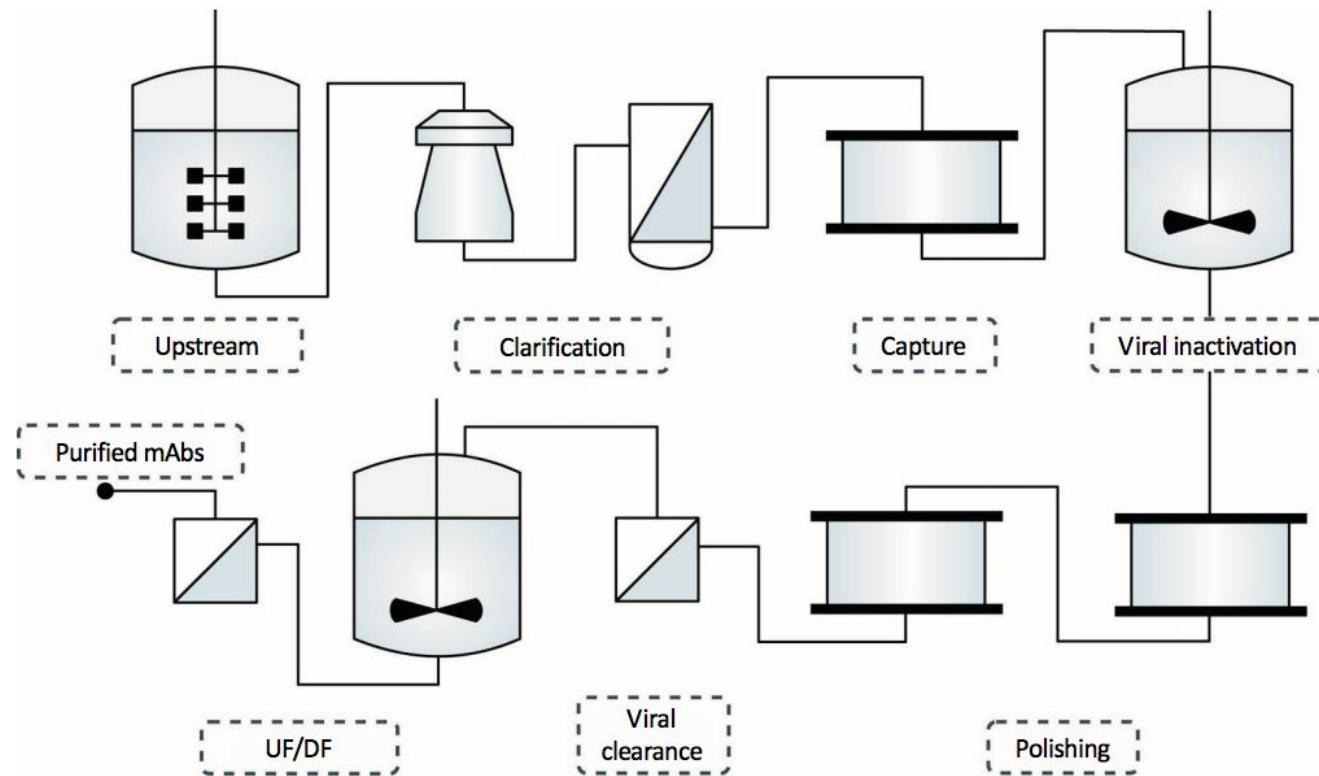


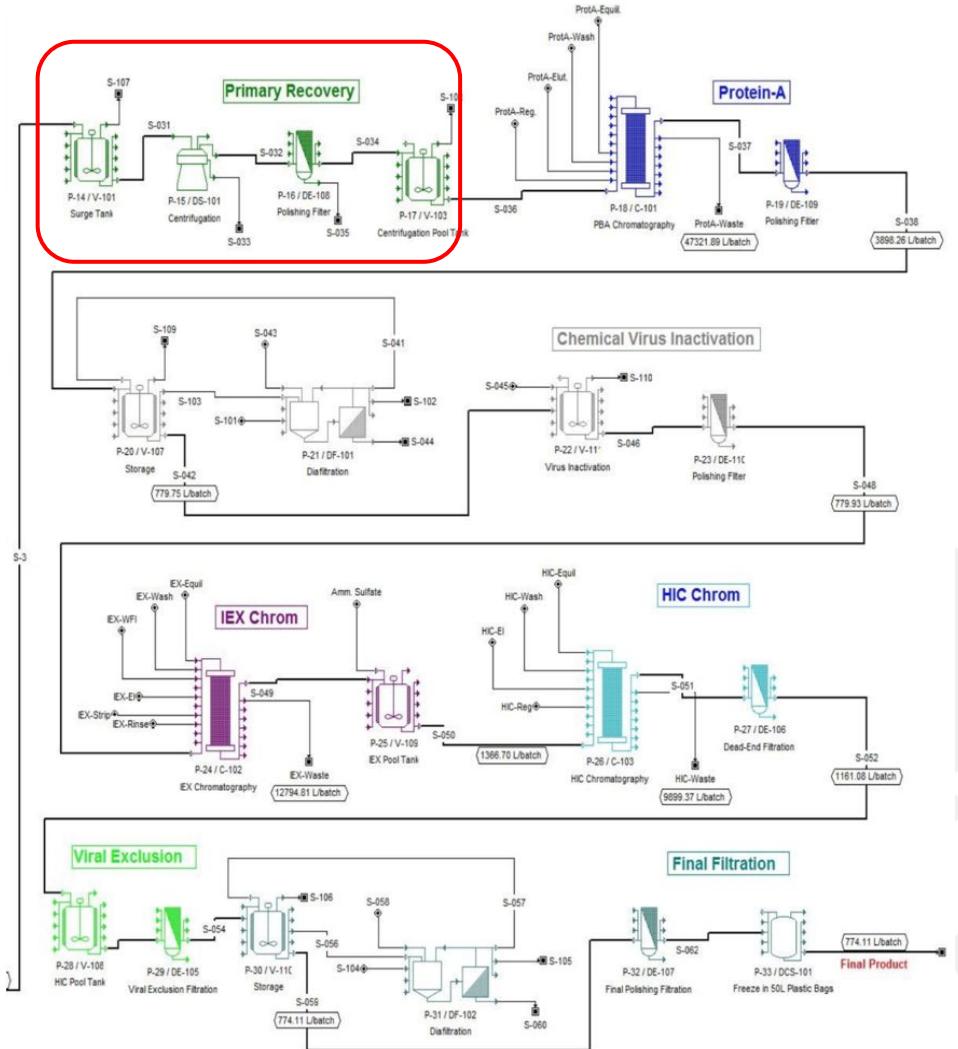
Production Bioreactor

- 1x 20,000L (stainless steel)
- 75% working volume results in 15,000L @ 3g/L
- Bioreactor parameters:
 - Temperature: 37 degrees Celsius
 - pH: 7.0-7.2
 - Impeller speed: 80rpm
 - No manual oxygen level control
- Fed-batch method results in a 18 day process
- 1-2 days for bioreactor cleaning and sterilization
- 15 batches a year in 300 working days
- Can produce a total of 45 kg/batch, 675kg/yr
- ~44 total days to perform upstream tasks



Downstream Processing



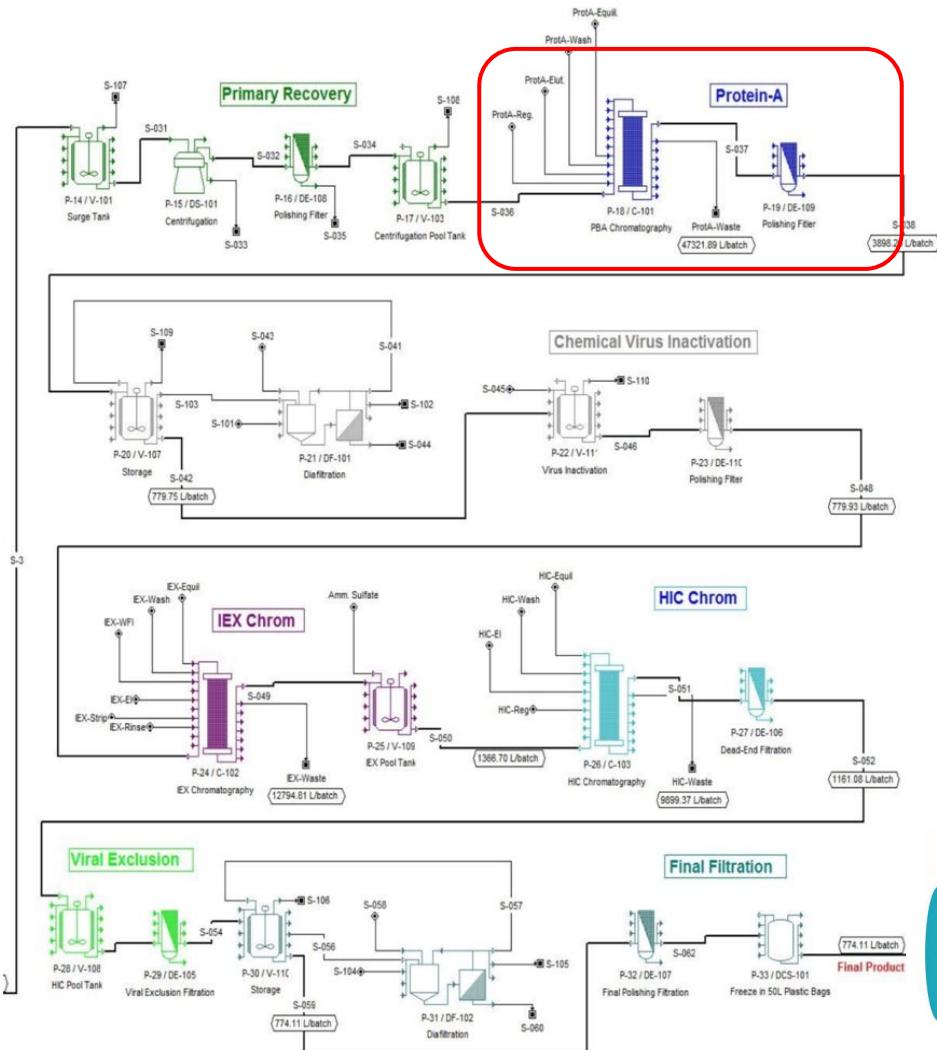


Downstream Processing

Clarification

- Disc-stack centrifugation
 - Removal of biomass (DS-101)
- Depth filtration (x2)
 - Remove cellular debris
 - Size exclusion from 0.6 to 0.2 um

**96% recovery
1-2 days**



Downstream Processing

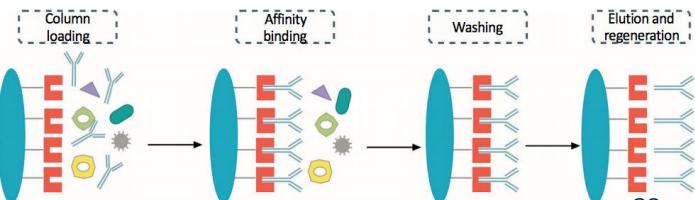
Protein-A

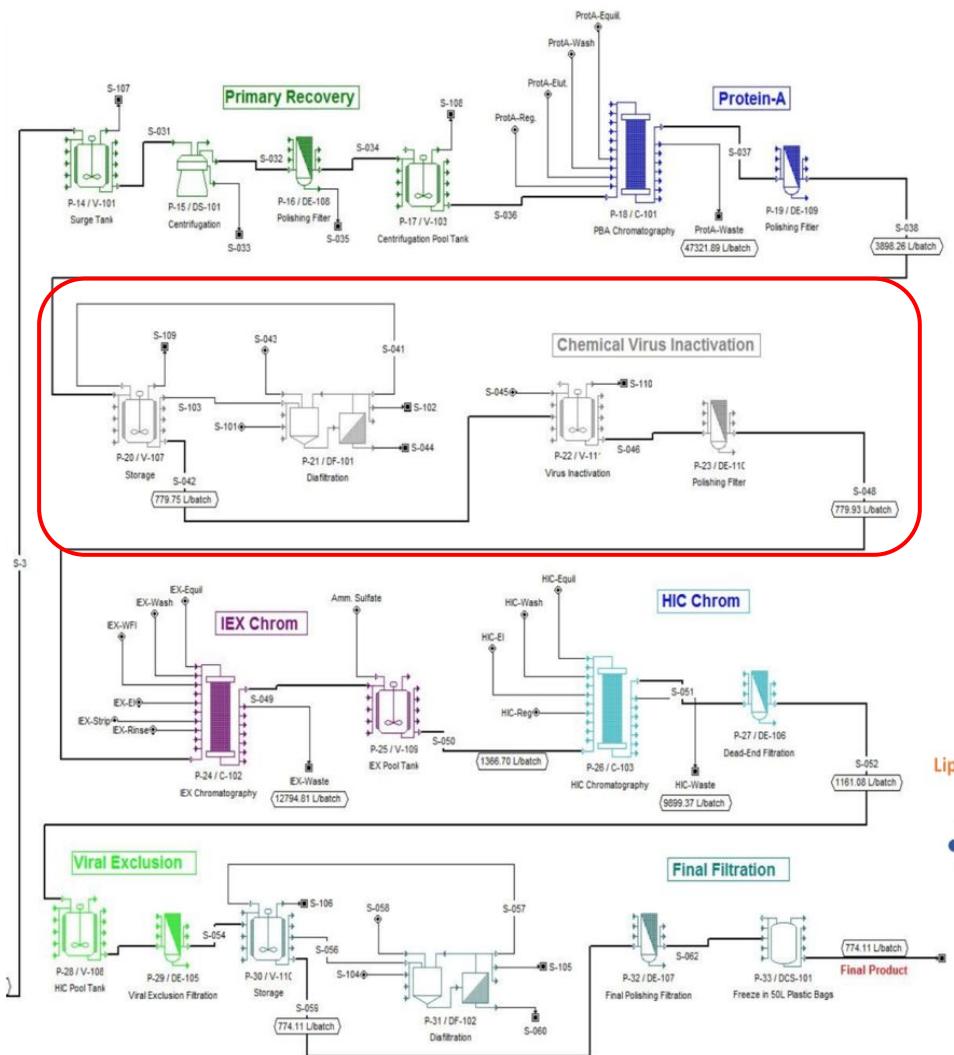
- Bulk of contaminant proteins are removed (C-101)
- Operating assumptions:
 - 15g of product per L of resin

95% recovery
~26 hours

- Concentrated five-fold and diafiltered 2x using WFI as diluant

95% recovery
~5 hours



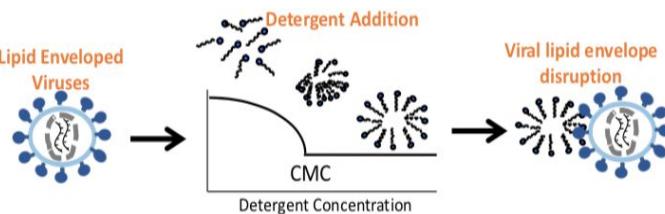


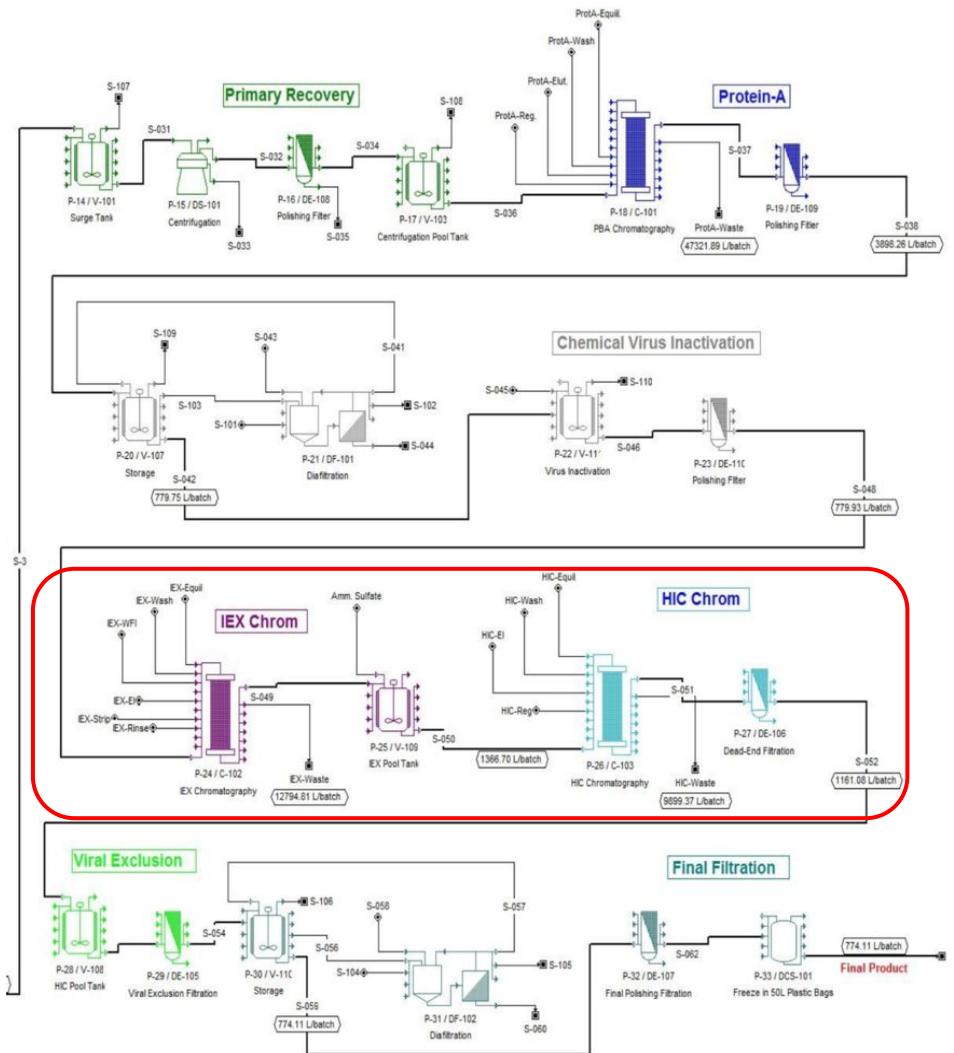
Downstream Processing

Viral Inactivation

- Two orthogonal steps:
 - Low pH treatment
 - Detergent (before Protein A)
- Concentrated protein solution treated with Polysorbate 80

99% recovery
~1.5 hours





Downstream Processing

Ion Exchange Chromatography (IEX)

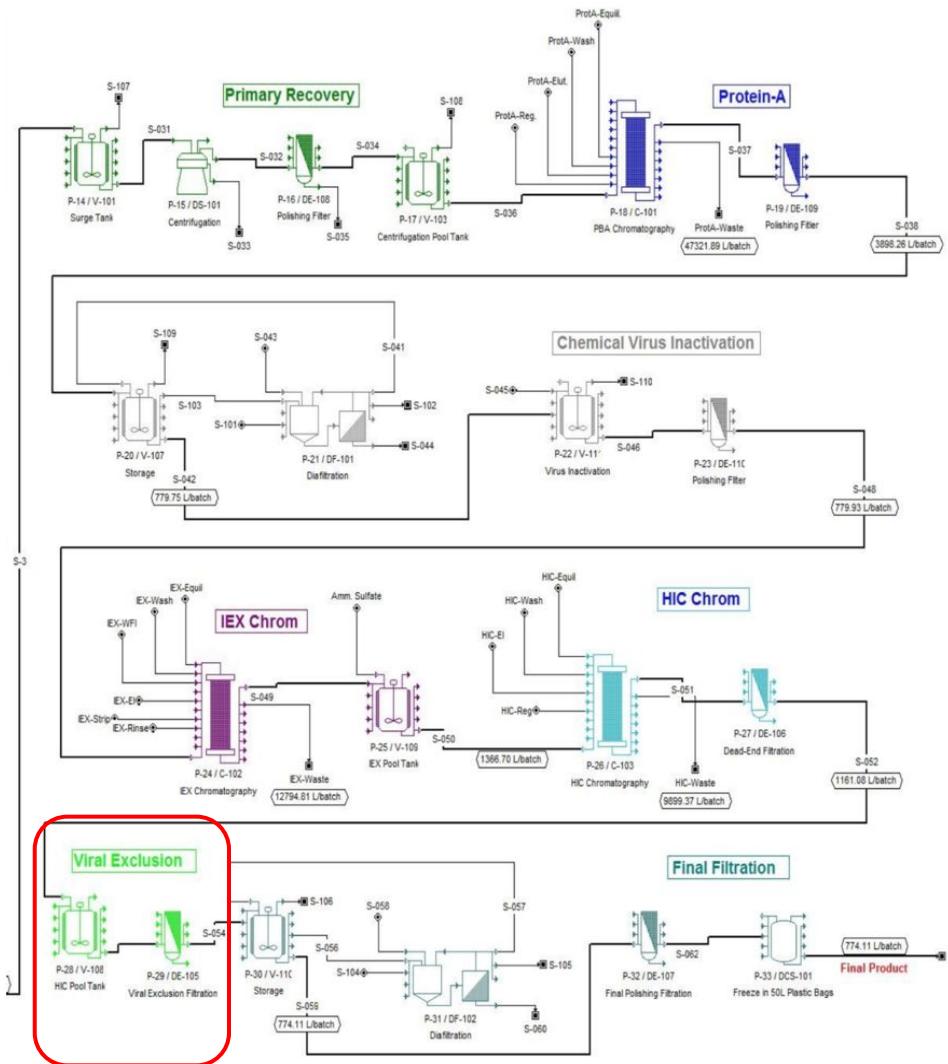
- CEX (Cation Exchange)
 - Uses resin modified with negatively charged functional groups

~95% recovery
~22 hours

Hydrophobic Interaction Chromatography (HIC)

- Add Ammonium sulfate added to increase ionic strength
- Operating Assumptions:
 - 40g of product per L of resin

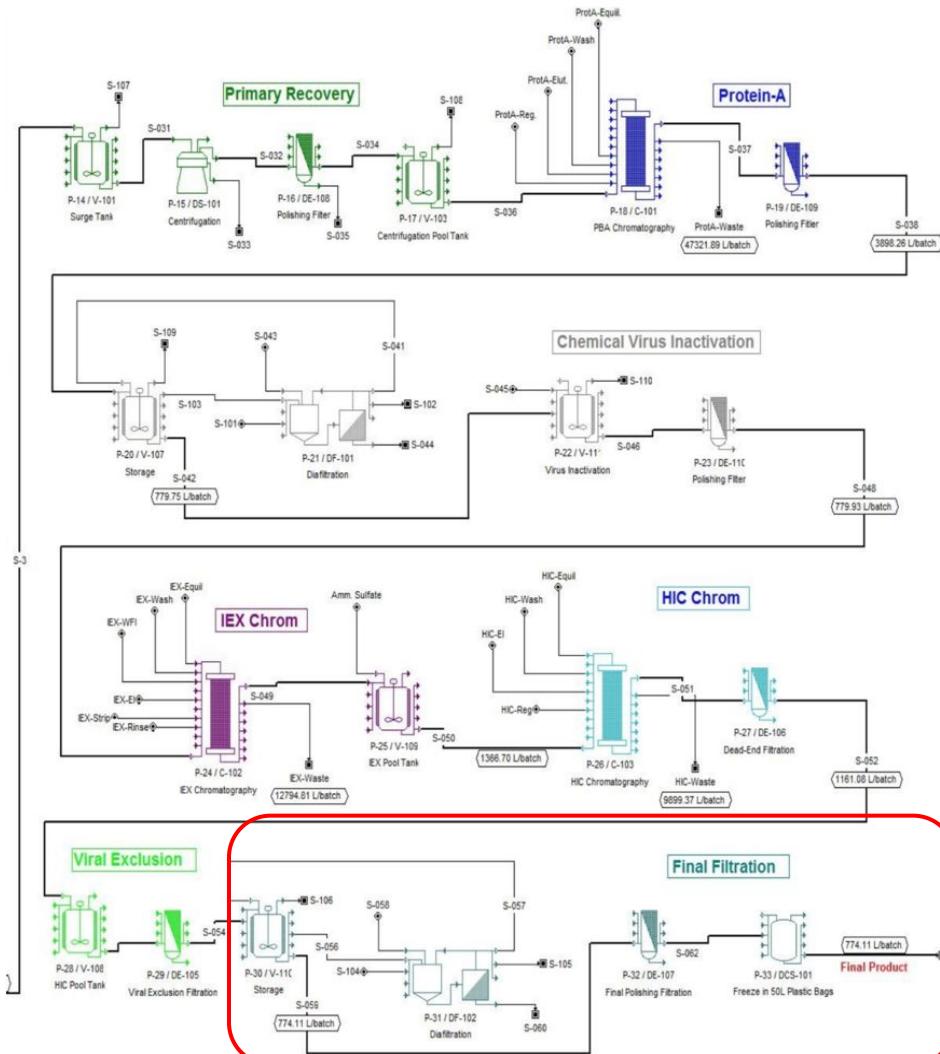
~95% recovery
~13.5 hours



Viral Exclusion

- Dead end filtration nanofilter
 - Pore size: 20 nm

96% recovery
~2.8 hours



Final Filtration

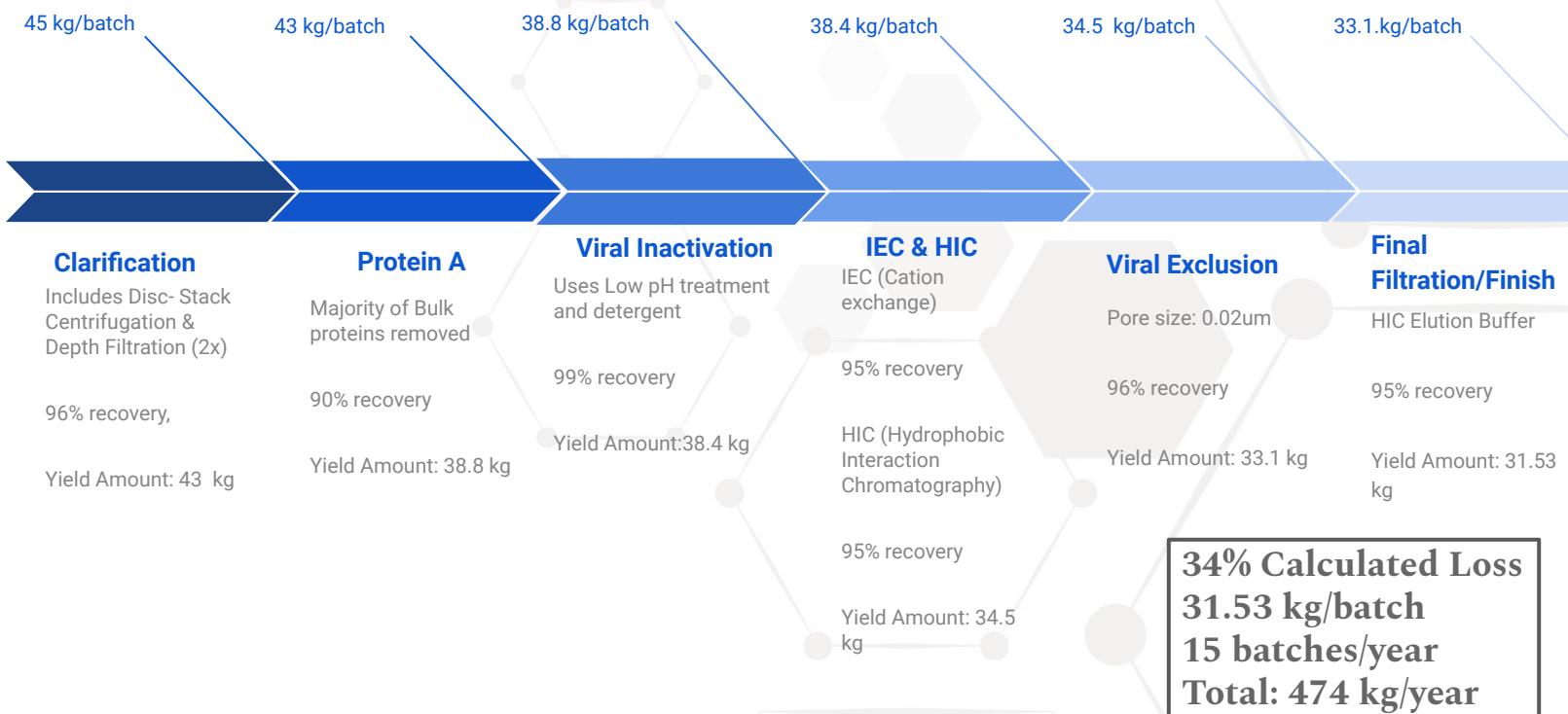
- HIC elution buffer is exchanged for the PBS solution
- Concentrated 1.5-fold in DF-102

Formulation, Fill/Finish, Packaging

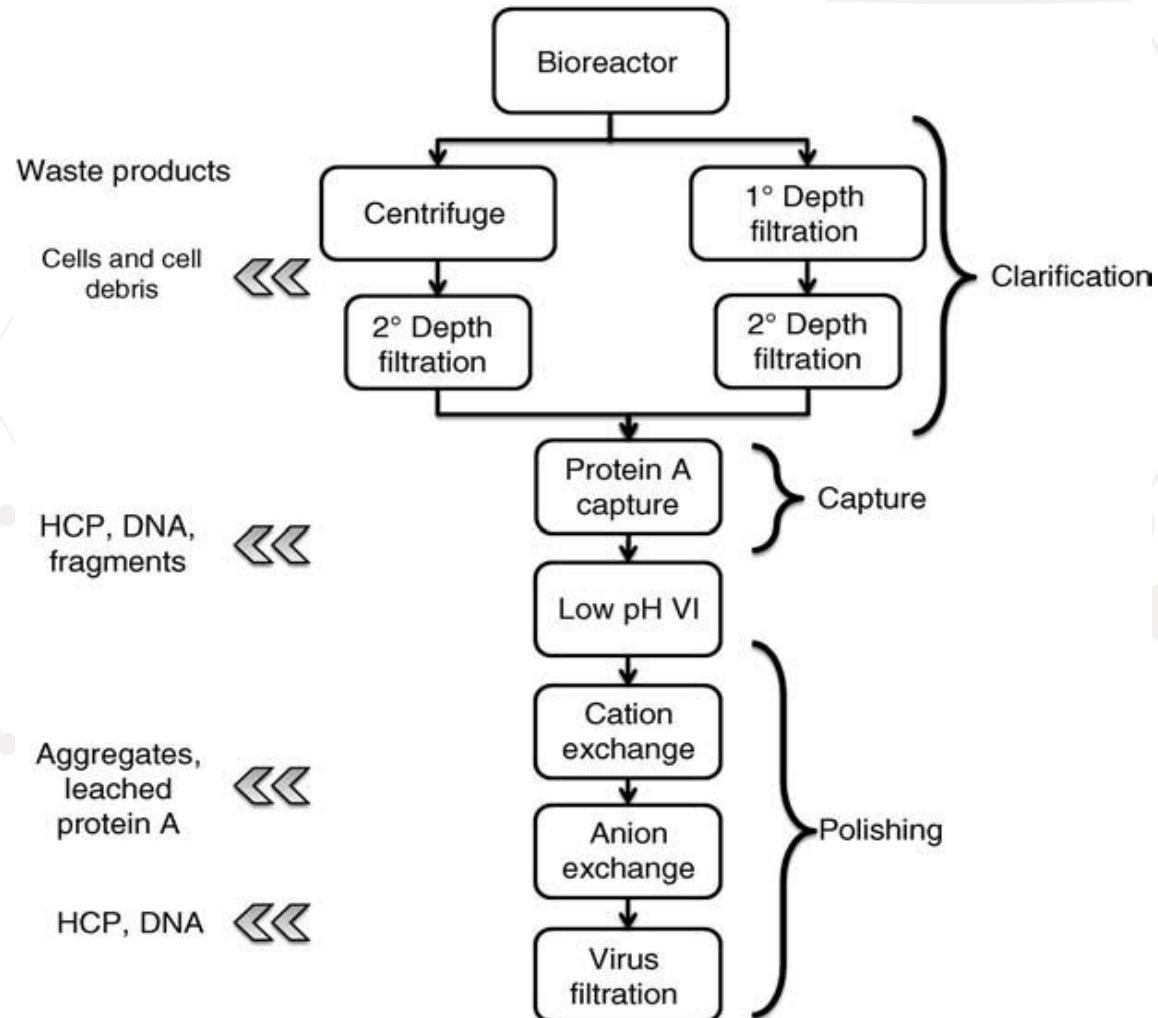
- Freezing → Thawing
- Fill into vials
- Liquid Formulation Packaging
 - Pass through inspection
- Labelling

**95% recovery
~1 day**

Theoretical Yield



Removal of Impurities



cGMPs

Current Good Manufacturing Practice

cGMPs

- A. Organization and Personnel
- B. Buildings and Facilities
- C. Equipment
- D. Control of Components and Drug Product Containers and Closures
- E. **Production and Process Controls**
- F. Packaging & Labeling Control
- G. Holding & Distribution
- H. **Laboratory Controls**
- I. Records & Reports
- J. Returned & Salvaged Drug Product



Organization and Personnel

- Each person responsible for supervising the manufacture, processing, packing, or holding of a drug product shall have the training and experience to perform assigned functions to provide assurance that our drug product has the safety, identity, strength, quality and purity.
- The responsibilities and procedures applicable to the quality control unit (QCU) shall be in writing; such written procedures shall be followed.



Buildings and Facilities



- **ISO 5** (3520 particles/ m³ > 0.5μm)
 - Aseptic-filling of vials, aseptic processing, sterility testing, UF/DF
(hair cover, hood, coverall, beard cover, face mask, shoe cover, boots)
- **ISO 6** (35200/ m³ >0.5μm) - upscale processing, Viral inactivation, harvesting of CHO cells
(hair cover, beard cover, face mask, coverall, shoe covers)
- **ISO 7** (352000 particles/m³ >0.5μm)
 - Buffer preparation, downstream processing (purification)
(hair cover, beard cover, face mask, frock, shoe cover)
- **ISO 8** (3520000 particles/m³ > 0.5μm)
 - Packaging of vials, inspection of vials
(hair cover, beard cover, frock, shoe covers)



Laboratory Control

- Tryptic Soy Agar (TSA)
 - Monitor bacterial growth on surfaces even after cleaning
- SabDex
 - Monitor fungi growth
- MetOne particle counter
 - Non-viable particle in air
- TSA settle and contact plates
 - Monitoring viable air particles through incubation, then identification of microorganism through special software
- Vitek-2 system
 - Microbial identification



Clean Area classification (0.5 μm particles/ ft^3)	ISO Classification	>0.5 μm particles/ m^3	Active Air Action level (cfu/ m^3)	Settle Plates Action Plate level (90mm diam. cfu/ 4 hours)	Inanimate surface (cfu/contact plate)
100	5	3520	0	0	0
1000	6	35200	7	3	3
10000	7	352000	10	5	5
100000	8	3520000	100	50	100
					40

Laboratory control (cont.)

- Monitoring the HVAC-HEPA system and measuring non-viable air particles ensuring it meets standards per ISO class clean rooms
- Environmental monitoring
- Personnel participating in the manufacturing or packaging process are required to wear adequate clothing and PPE, and practice good sanitation and health habits.
- Usage of HVAC-HEPA system, positive pressure for all cleanrooms
- Laminar airflow for all cleanrooms
- Work in unhurried manner
- Entering ISO clean rooms in sequential order (8-7-6-5)
- A dedicated room for testing, validating and calibrating all equipment
- QC department will conduct all chemical analysis for all chemicals
Used in the whole manufacturing processes
- All equipment are validated every 6 months



Production and Process Controls

- Written procedures must ensure drug products have the right identity, purity, quality, and strength according to the claims made.
- Batches must contain 100% of the labeled or established amount of the active ingredient.
- Time limits need to be applied for drug production.
- Microbial contamination has to be addressed in written form.



Investment Plan

Market Size



- ~10,000 annually eligible for Keytruda's Melanoma indication
- ~565,000 are eligible to utilize Keytruda for Non-Small Cell Lung Cancer (NSCLC) and Small Cell Lung Cancer (SCLC)
 - Both indications are in the top 5 most prevalent cancers in the U.S.
- ~300,000 new cases in 2019 (3 indications), ~200,000 are treatable with Keytruda
- Our target market is the current eligible patient population: 575,000
- We want 20%, 115,000 patients, which demands 391kg of pembrolizumab produced per year
 - At 200mg every 3 weeks per patients (3.4g per patient per year)
- Our process can produce a maximum of 474kg of pembrolizumab per year

Competitors

- Keytruda (pembrolizumab)
 - Bladder, cervical, kidney, Merkel cell carcinoma, head and neck, mediastinal B-cell lymphoma, gastric, melanoma, non small and small lung cancer, Hodgkin Lymphoma, and Hepatocellular carcinoma. (\$9,919/200mg dose, \$171,858/per year)
- Bristol Myers - Opido (nivolumab)
 - FDA approval for both small and non small cell lung cancer, kidney, liver, colorectal, melanoma, head and neck squamous cancer, bladder, and Hodgkin Lymphoma
 - Must consider and be aware of their patent U.S. Patent No. 5693761
- AstraZeneca - Imfinzi (durvalumab)
 - FDA approval for non small cell lung cancer
- Roche - Tecentriq (atezolizumab)
 - FDA approval for both small and non small cell lung cancers, triple-negative breast cancer and Urothelial Carcinoma



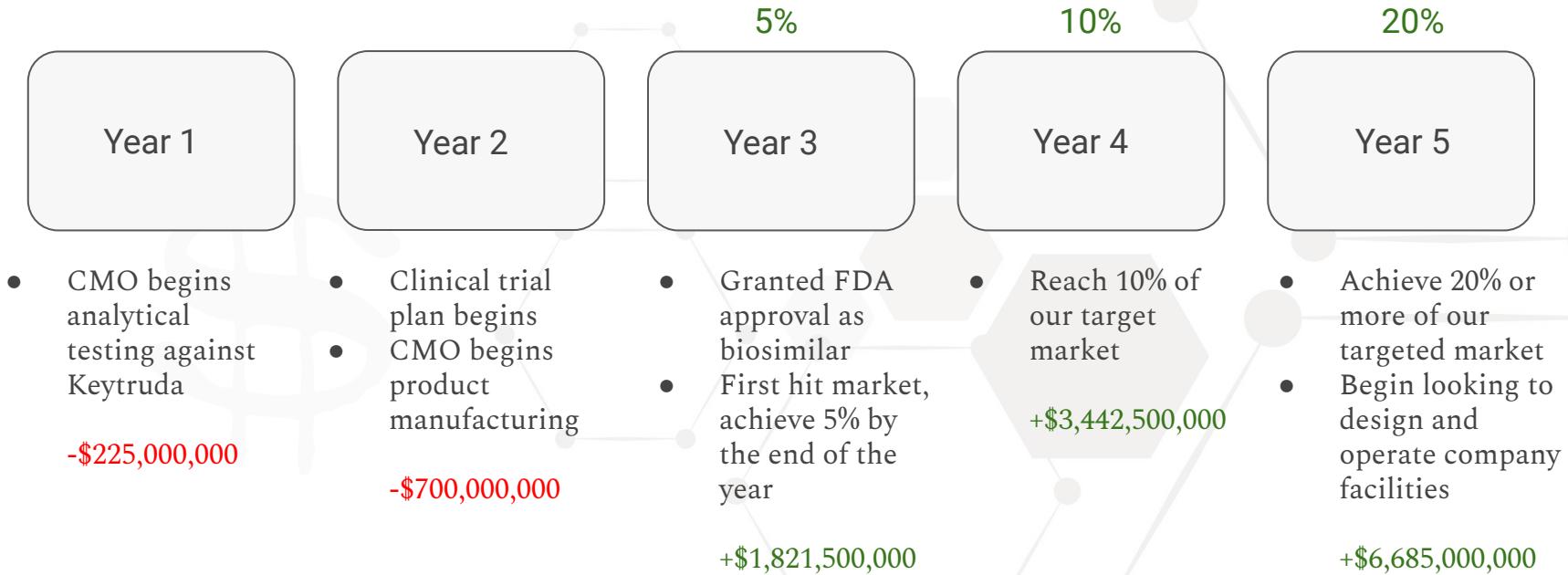
CMO

- Analytical testing and validation
- Stability testing and product storage
- Manufacturing for all stages
- Facilities are cGMP compliant

Lonza



Five Year Plan



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