

Animal Cell Culture



Animal Cells

- Produce important **therapeutic proteins**
- Possess the machinery to do complex **posttranslational modifications** to proteins
- Currently, more than 50% of new therapeutic proteins are being produced using mammalian cell culture.
- The development of **stem cell technology** opens the possibility that the cells themselves will become the product of interest.

Biopharmaceuticals (Biologics)

Small
Molecule
Drug

Biopharmaceuticals

Size	<p>Aspirin 21 atoms</p> 	<p>hGH ~ 3000 atoms</p> 	<p>IgG Antibody ~ 25,000 atoms</p> 
Complexity	<p>Bike ~ 20 lbs</p> 	<p>Car ~ 3000 lbs</p> 	<p>Business Jet ~ 30,000 lbs (without fuel)</p> 

Small Molecules vs. Biologics

2013 Rank	2012 Rank	Drug	Company	Small Molecule/ Biologic
1	1	Humira	AbbVie	Biologic
2	7	Remicade	Johnson & Johnson & Merck & Co.	Biologic
3	3	Rituxan/MabThera	Roche (Genentech) & Biogen Idec	Biologic
4	2	Advair/Seretide	GlaxoSmithKline	Small Molecule
5	11	Enbrel	Amgen & Pfizer	Biologic
6	4	Lantus	Sanofi	Biologic
7	8	Avastin	Roche	Biologic
8	5	Herceptin	Roche	Biologic
9	6	Crestor	AstraZeneca & Shionogi	Small Molecule
10	--	Abilify	Otsuka Pharmaceutical & Bristol-Myers Squibb	Small Molecule

Small Molecules vs. Biologics

Table 1. Top 10 Global Prescription Drugs in 2016 in Terms of Sales²

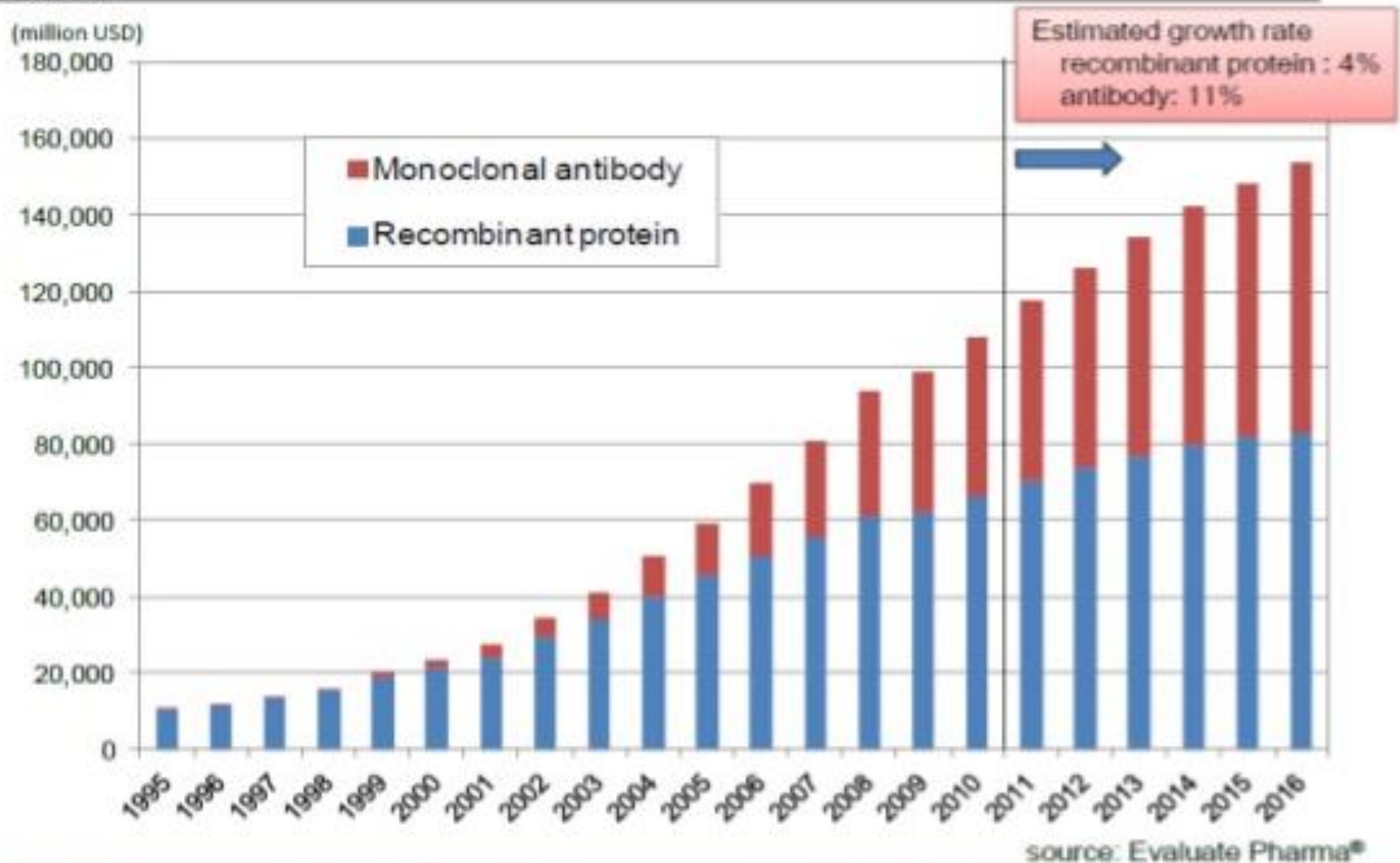
rank	product	sales (US \$BN)
1	Humira	16.078
2	Harvoni	9.081
3	Enbrel	8.874
4	Rituxan	8.583
5	Remicade	7.829
6	Revimid	6.974
7	Avastin	6.752
8	Herceptin	6.751
9	Lantus	6.054
10	Prevnar	5.718

2016 witnessed 9 out of the top 10 pharmaceutical products being biologics!



PHARMAWIN

Therapeutic mAbs drive BioPharma Market



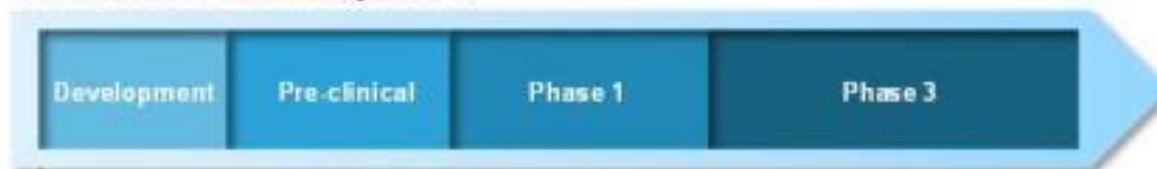
Biopharmaceutical Development Procedure

- Average time: 12 years
- Average cost: \$2 billion

Reference Biologic Development



Biosimilar Development



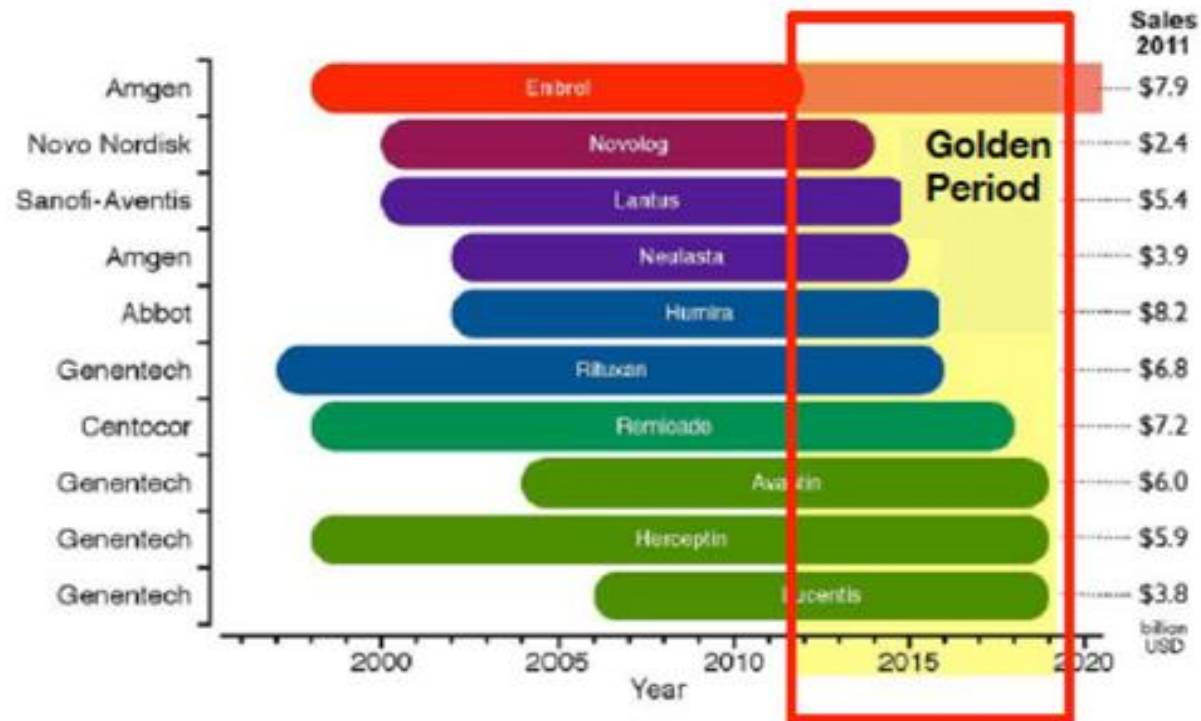
(Total Costs: \$100-\$200 MM)



Golden Period for Biosimilar

- 2012 – 2019

Top 10 Selling Biologic's Patent Cliff



Structure and Biochemistry of Animal Cells

- No cell wall but surrounded by a thin and fragile plasma membrane
 - Results in significant **shear sensitivity**
- Plasma membrane
 - Composed of lipid, protein, and carbohydrate
 - Composition is not uniform and varies in different regions of the membrane.
- In some cells, a portion of the membrane is modified to form a number of projections called **microvilli**, which increase the surface area and mass transfer across the membrane.

Structure and Biochemistry of Animal Cells

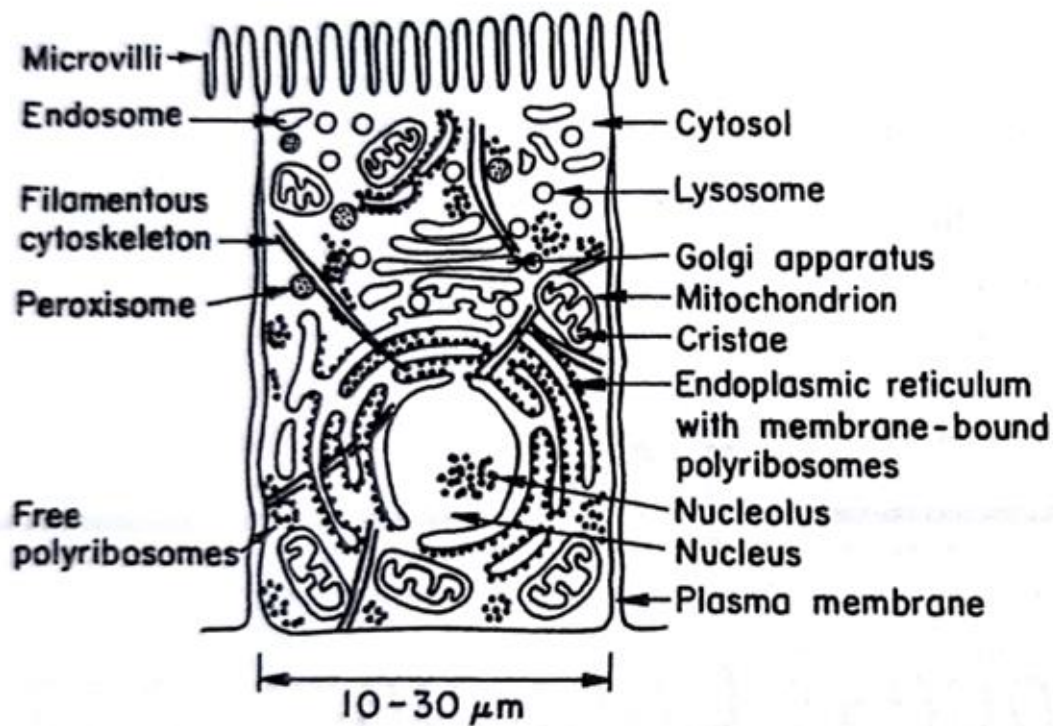


Figure 12.1. Generalized animal cell. The cytosol, endoplasmic reticulum, Golgi apparatus, endosome, nucleus, lysosome, mitochondrion, and peroxisome are distinct compartments isolated from the rest of the cell by selectively permeable membranes.

Structure and Biochemistry of Animal Cells

- Cell surface: negatively charged
 - Cells tends to grow on positively charged surfaces, such as Sephadex or collagen (anchorage-dependent cells)
- Many cells possess specific cell receptors that adhere to ligands on the surface.
 - Higher adhesiveness

Animal Cell Culture

- Typical laboratory growth medium
 - Glucose, glutamine, nonessential and essential amino acids, serum (calf or horse), and mineral salts
 - e.g. Dulbecco's modified Eagle's media (DMEM)
- Waste products of metabolism is a major problem in high-cell-density culture.
 - lactate, ammonia
 - Toxic to cell primarily because they alter intracellular and lysosomal pH

Animal Cell Culture

- Primary culture
 - Cells directly derived from excised tissues
- Secondary culture
 - Cell line obtained from the primary culture
 - Most differentiated mammalian cell lines are **mortal**.
 - These cell lines undergo **senescence**.
 - Cell division only for a limited number of generations
 - Cells that can be propagated indefinitely are called **continuous**, **immortal**, or **transformed** cell lines.
 - Cancer cells are naturally immortal.

Normal and Transformed Cells

TABLE 12.1. Comparison of Normal and Transformed Cells

Normal	Transformed
Anchorage dependent (except blood cells)	Nonanchorage dependent (i.e., suspension culture possible)
Mortal; finite number of divisions	Immortal or continuous cell lines
Contact inhibition; monolayer culture	No contact inhibition; multilayer cultures
Dependent on external growth factor signals for proliferation	May not need an external source of growth factors
Greater retention of differentiated cellular function	Typically, loss of differentiated cellular function
Display typical cell surface receptors	Cell surface receptor display may be altered

Attachment

- Anchorage-dependent cells
 - Monolayer culture using T-flasks, roller bottles, and microcarriers
- Nonanchorage-dependent cells
 - Transformed cells usually become attachment independent.
 - Suspension culture: highly desirable for large-scale production

Mammalian Cell Culture

