



Nanoengineering of Therapeutic and Diagnostic Antibodies

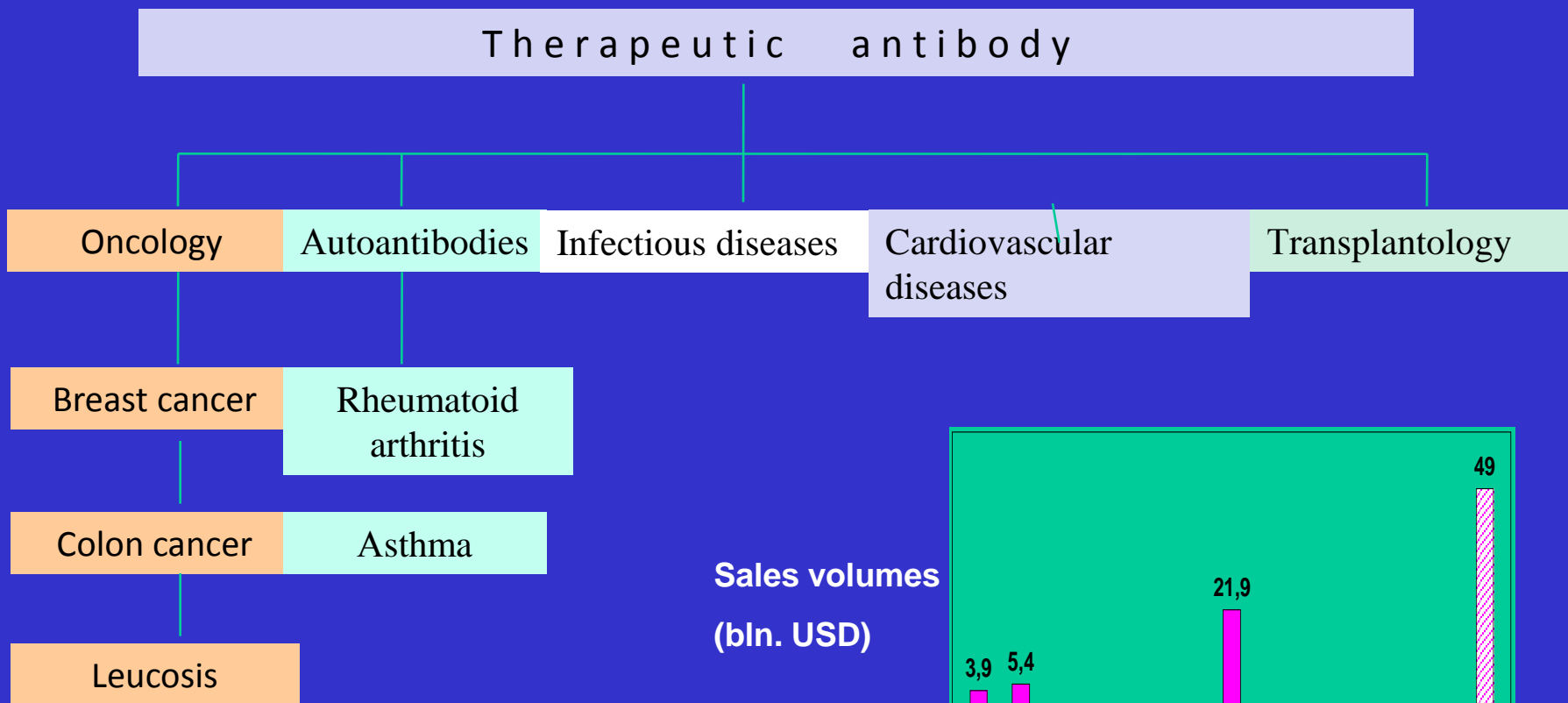
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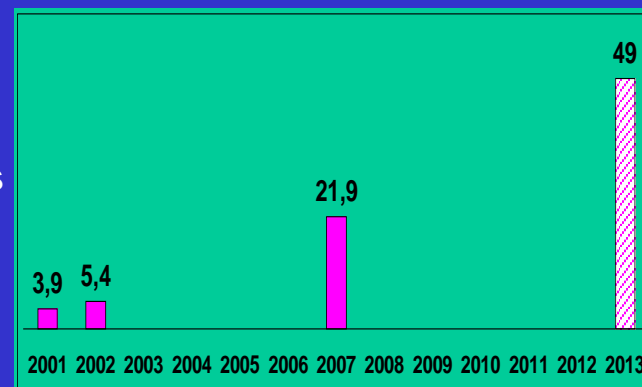
**Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry Russian Academy
of Sciences**

Research Center of Molecular Diagnostics and Therapy

Therapeutic antibodies – new generation of drugs

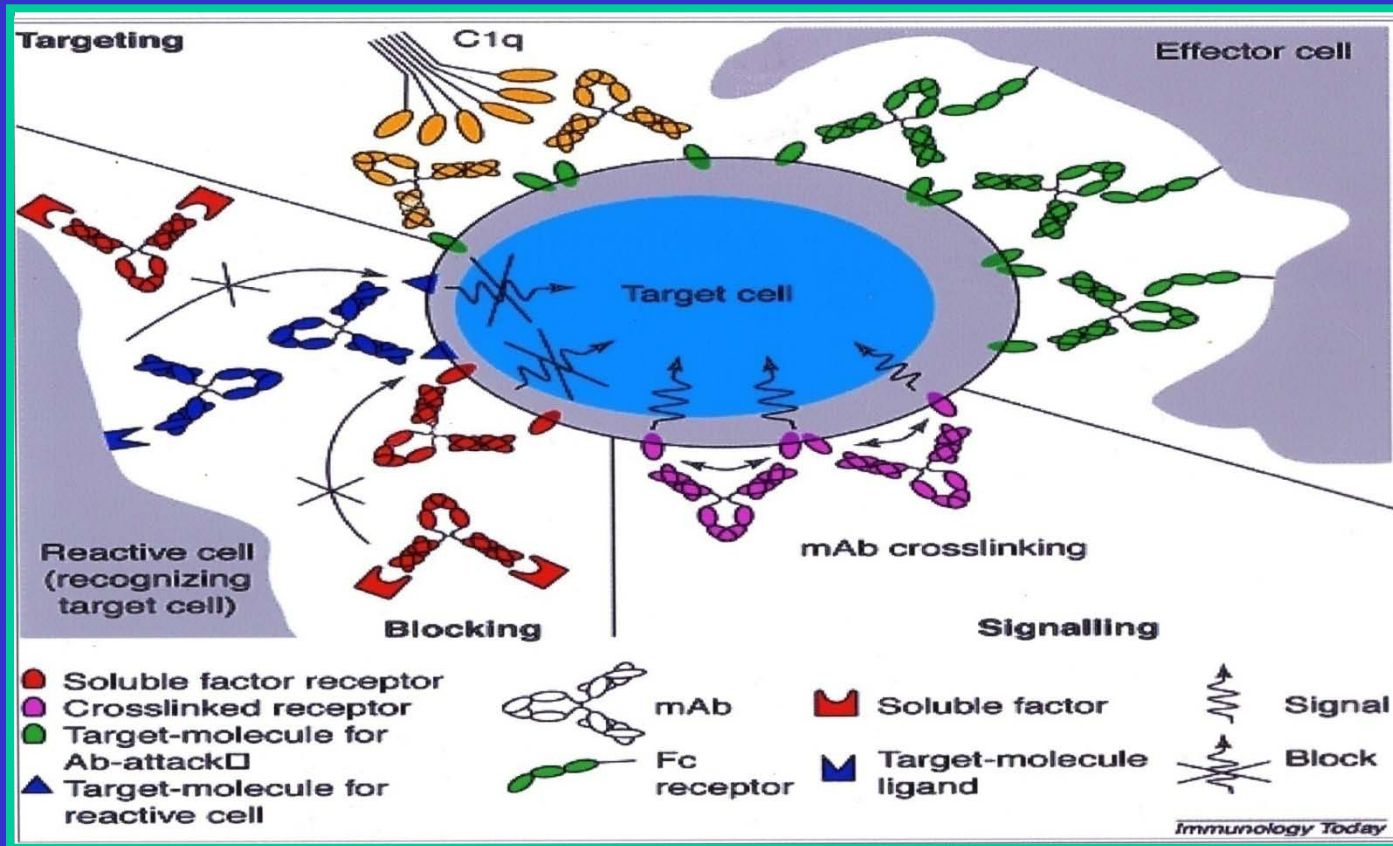


Sales volumes
(bln. USD)



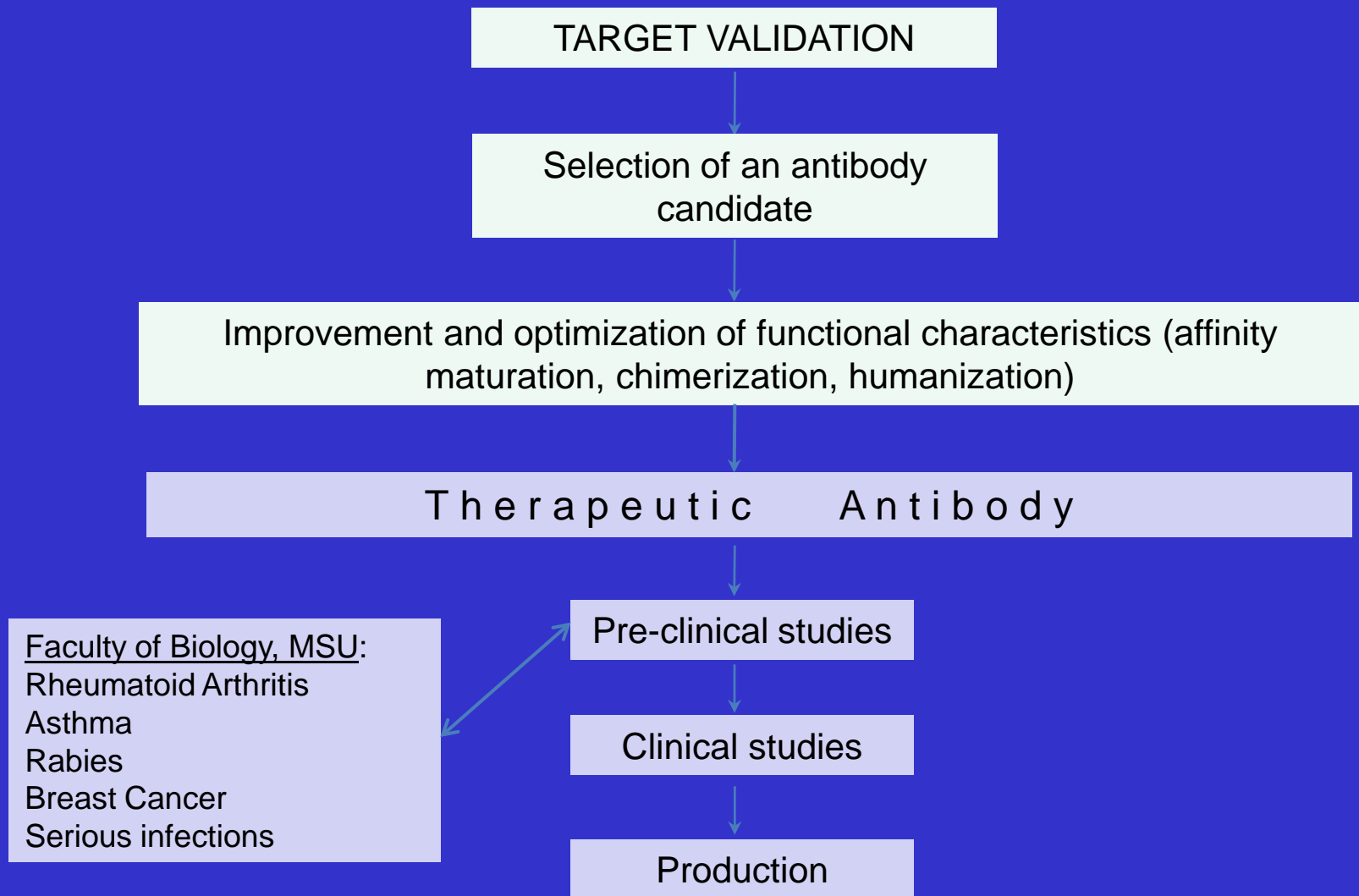
Source: Global Information, Inc. 07/2008

Therapeutic antibodies: properties & principles of action

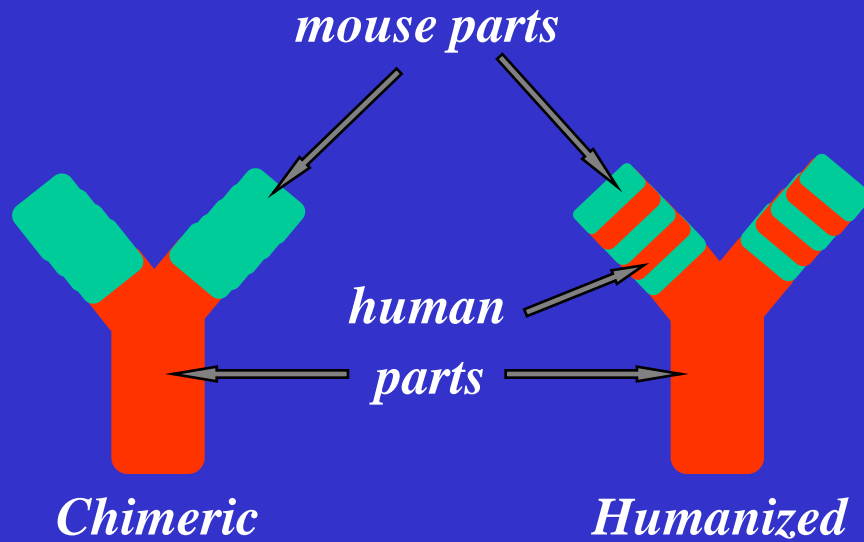
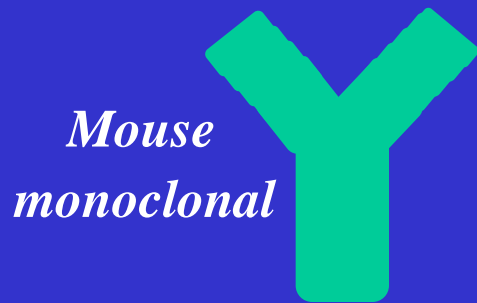


- prolonged therapeutic effect
- low incidence of side effects
- high selectivity of action on the target proteins and cells
- capability for the activation of self-defense mechanisms in patients

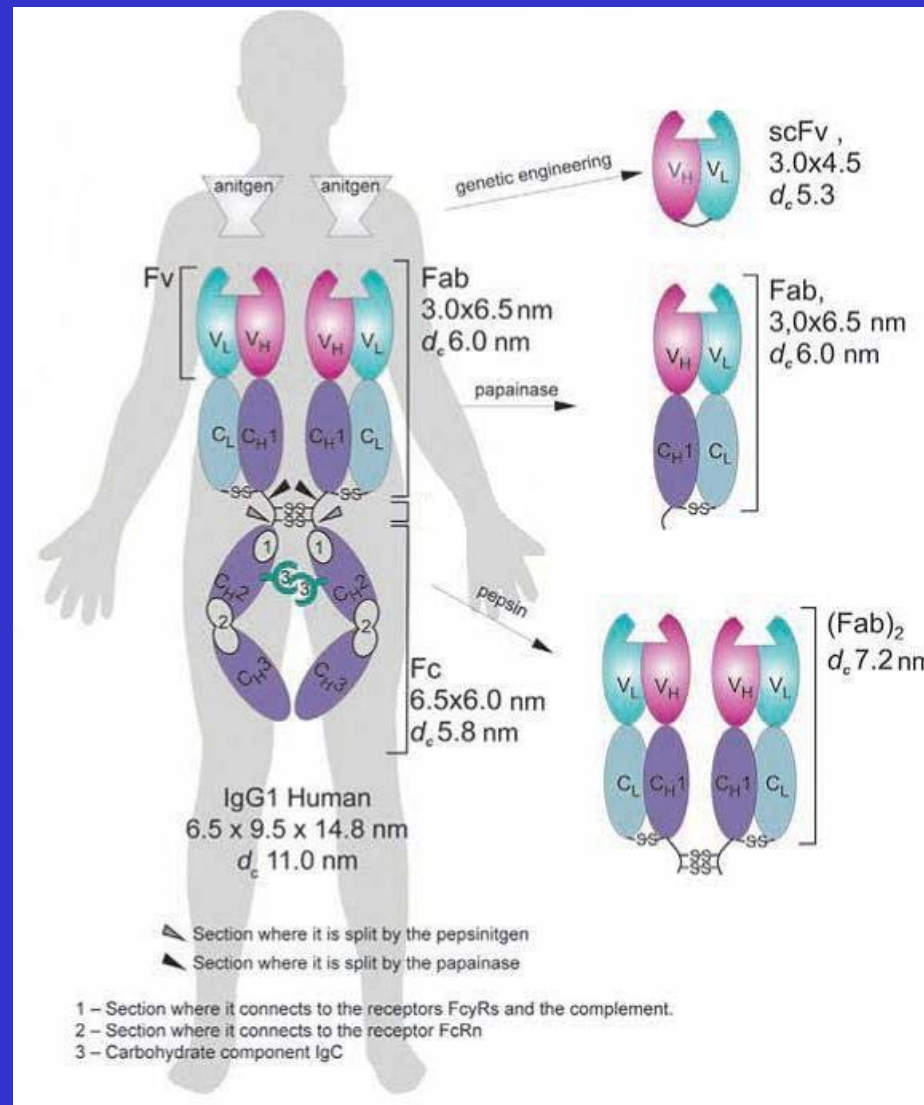
Design and Production of Therapeutic Antibodies



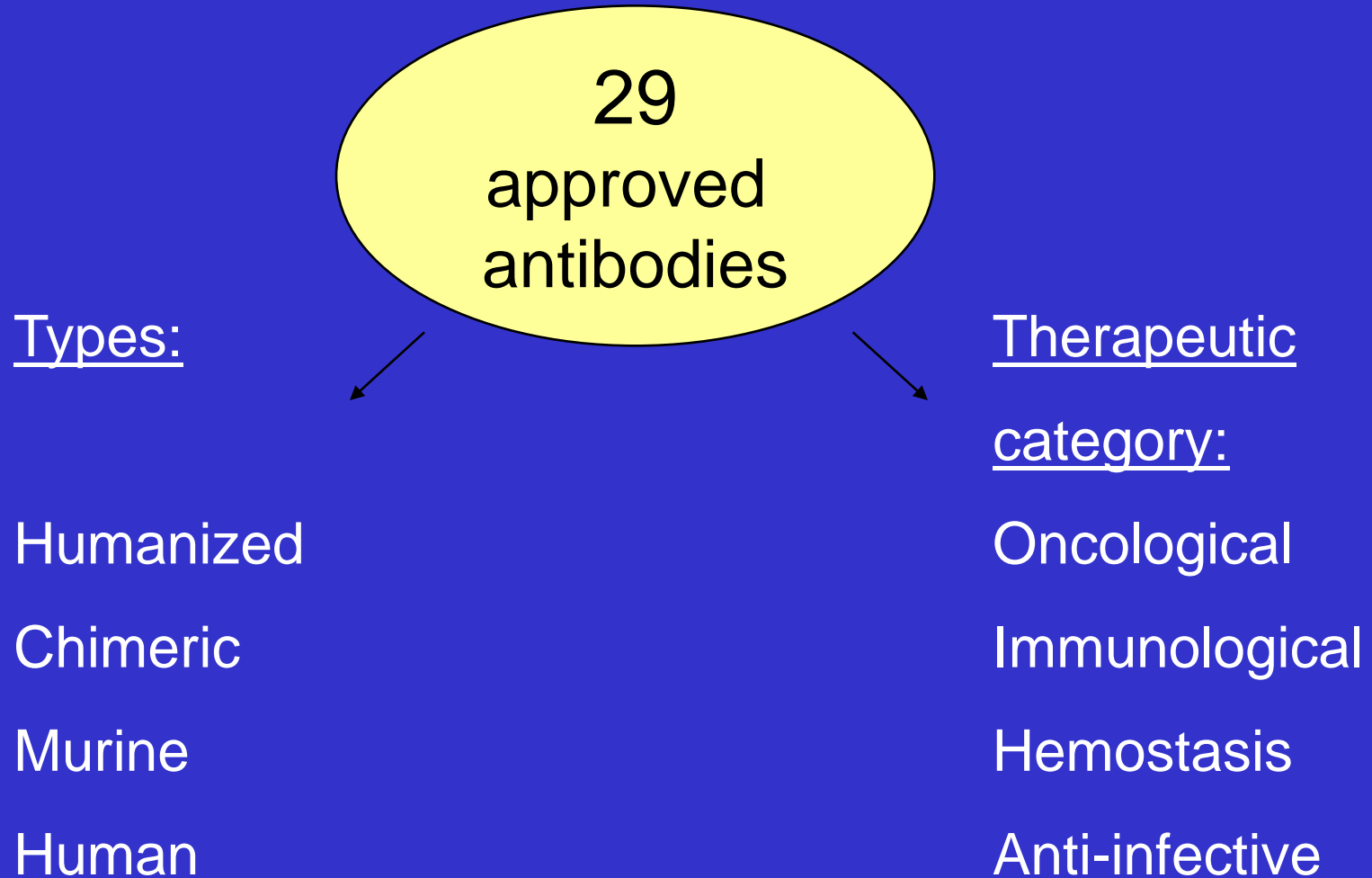
Types of therapeutic antibodies



Nanoengineering of antibodies



Monoclonal antibody market



Antigens

TNF

Smallpox
Vaccinia virus



Recombinant antibodies

Single-chain Fv's
Fab fragments
Full-size **chimeric** and
humanized

Full-size **human**

Types of recombinant antibodies that we can produce

Chimeric mouse-human

Humanized mouse-human

Human

Forms of recombinant antibody molecules and hosts for their production

Single-chain mini-antibodies



E. coli cells



Yeast cells

Fab fragments

Full-size antibodies



Mammalian cell lines

**Mammalian expression
vs.
other expression systems**

Benefits

Correct folding

Correct post-translational modification

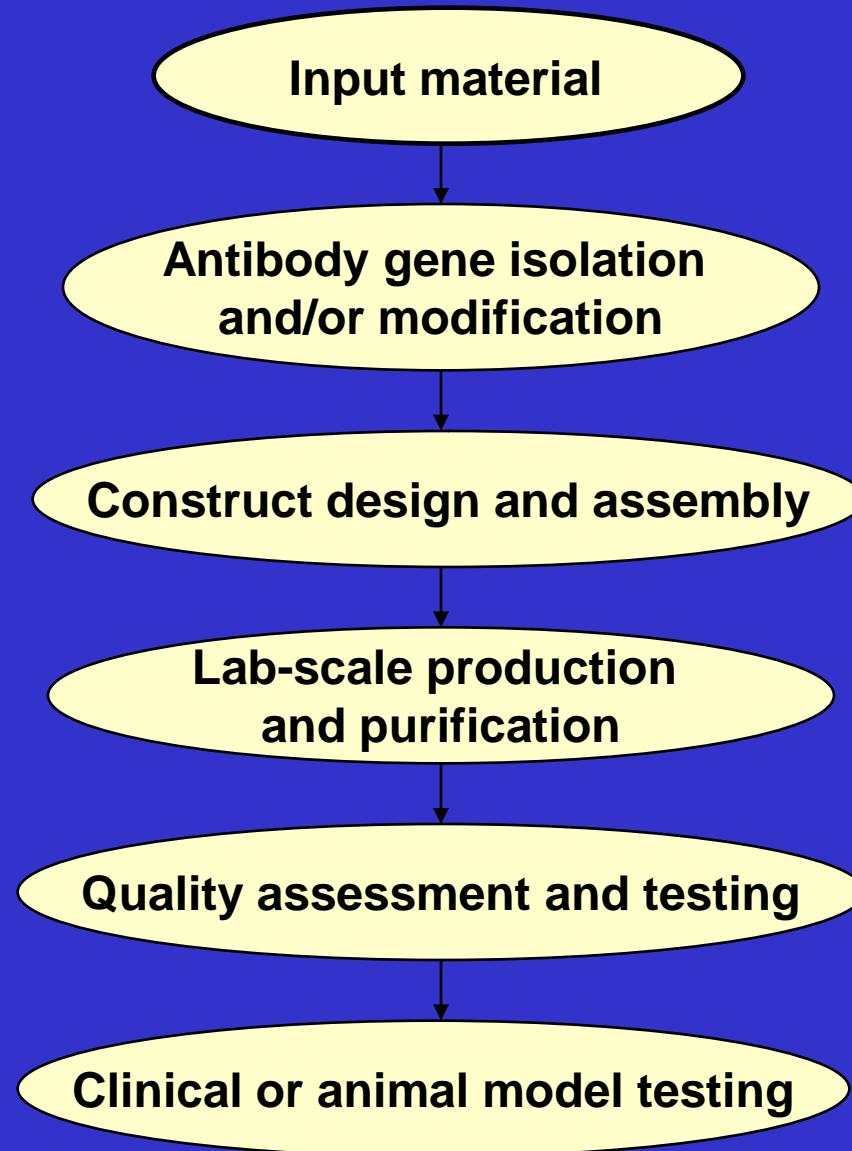
Challenges

Lower production yields

Higher costs

More time to pass from genetic construct to protein

Flow-chart diagram of antibody production



Key elements of antibody production process

Antibody gene structure

Vector design
Constant regions

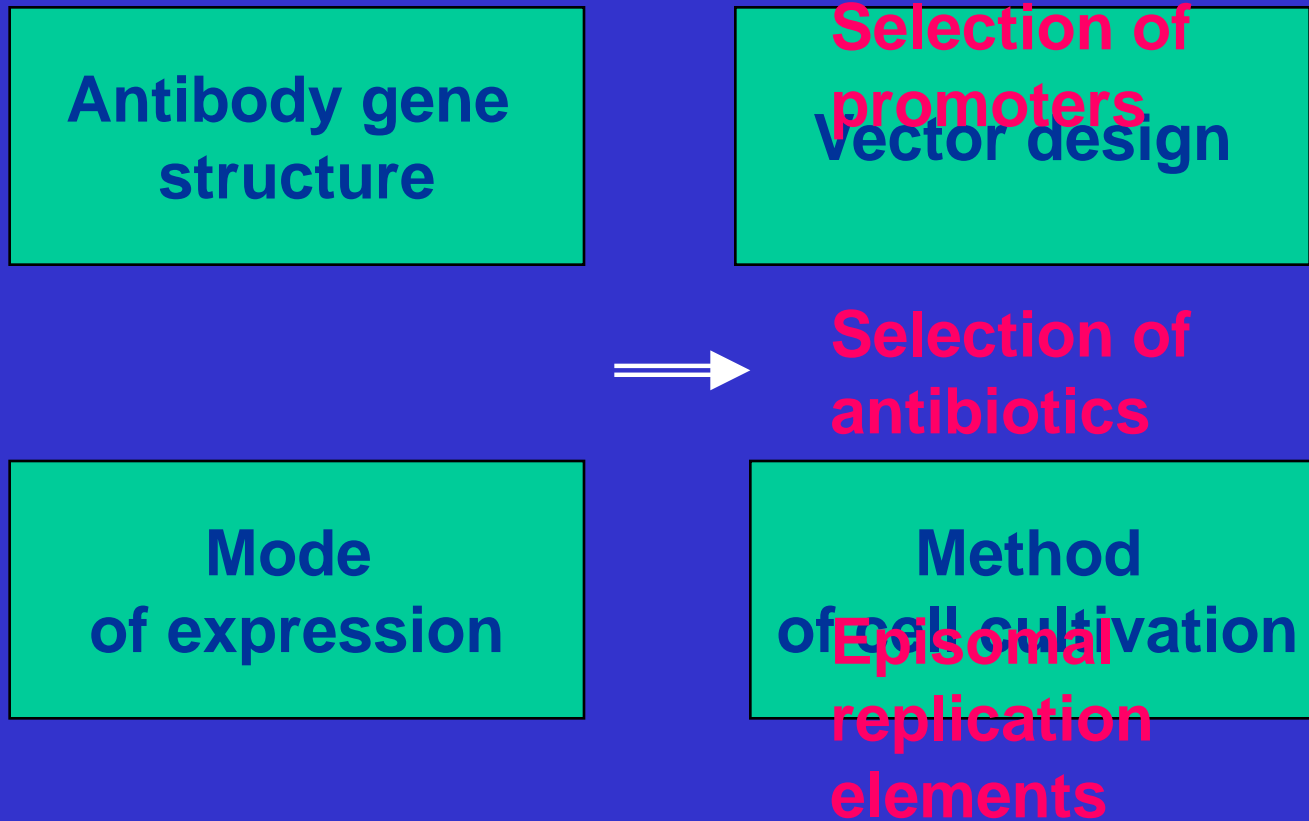


Leader sequences

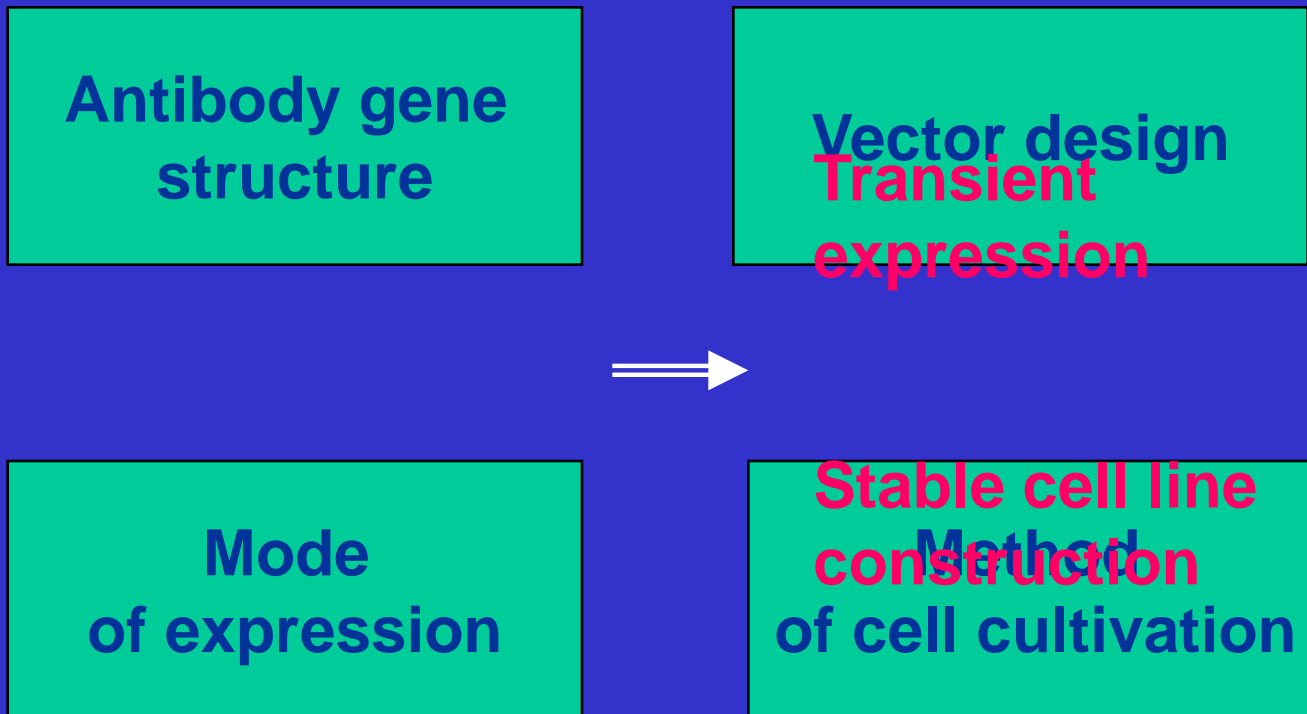
Mode of expression

Method of cell cultivation
Introns and 3' UTR's

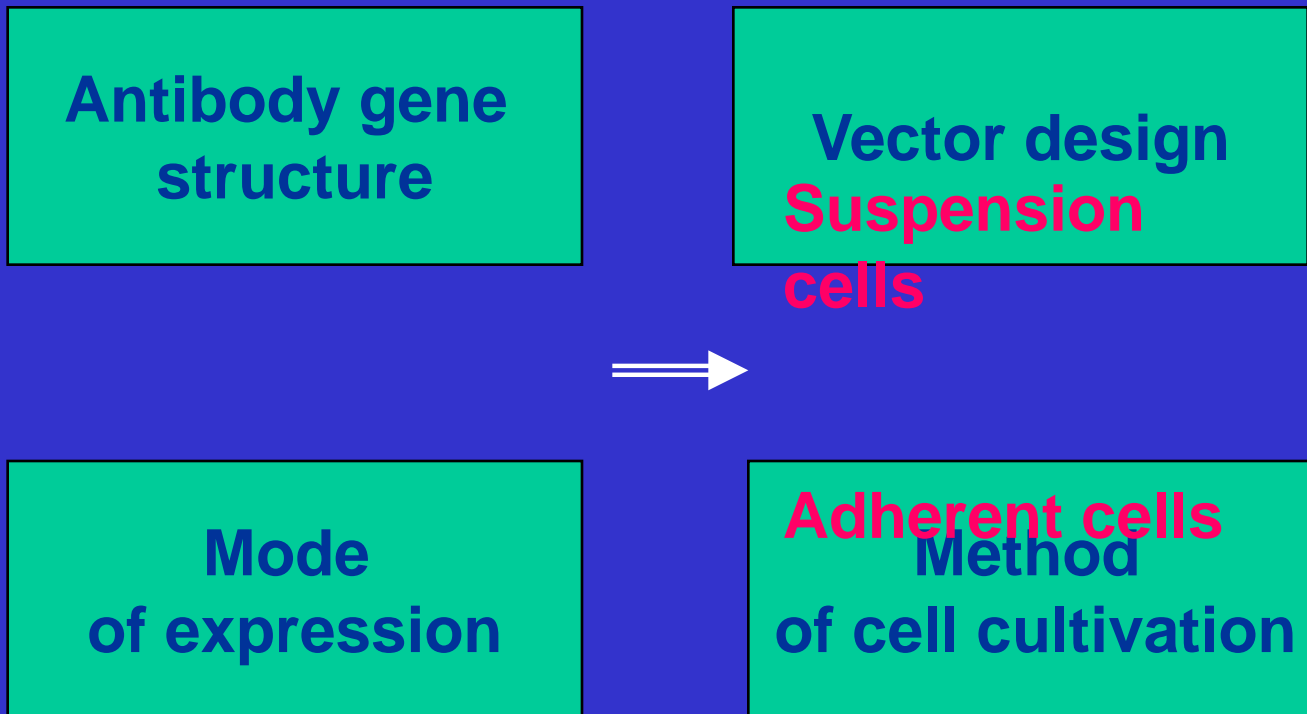
Key elements of antibody production process



Key elements of antibody production process

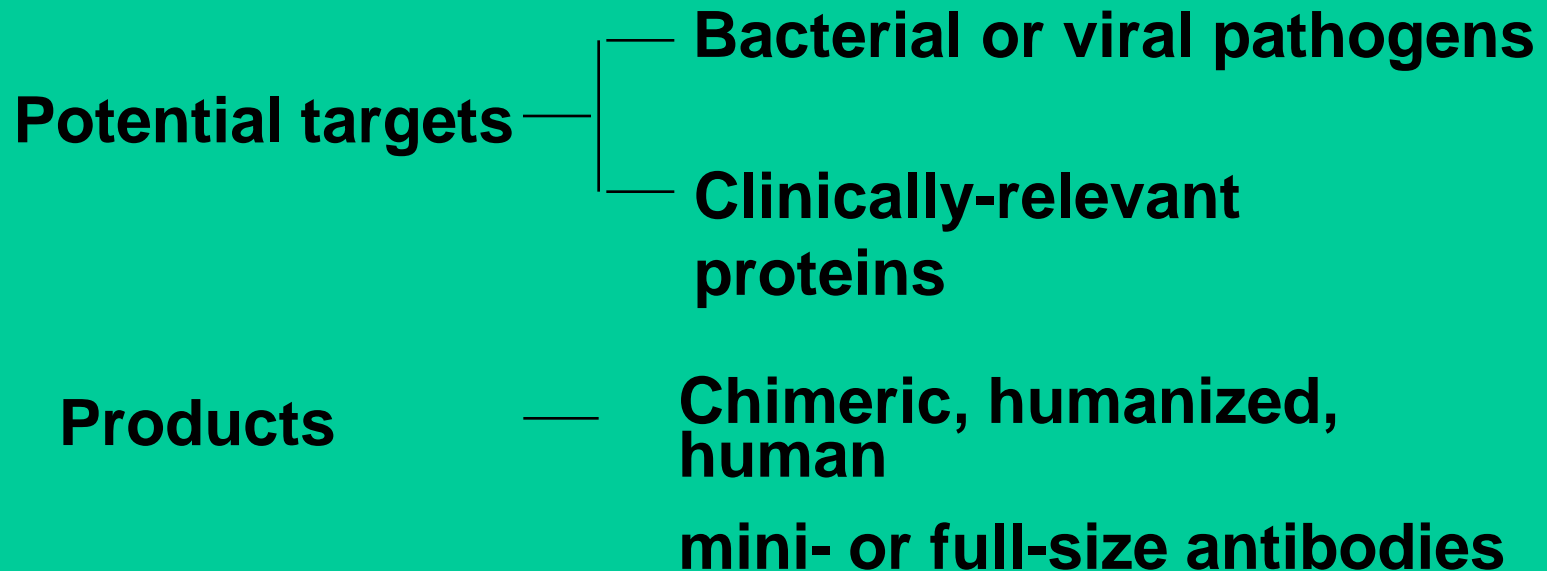


Key elements of antibody production process



Research projects

Development and production of recombinant antibodies for diagnostics and treatment



**Potential targets
for recombinant antibody development**

Microbial agents

Viruses

Toxins

Enzymes

Cytokines

Hormones

Microbial agents

Bacillus anthracis

Borrelia burgdorferi

Brucella abortus

Candida albicans

Chlamydia trachomatis

Dermatophagoides farinae

Echinococcus granulosus

E. Coli H7:O157

Erysipelothrix rhusiopathiae

Francisella tularensis

Helicobacter pylori

Legionella sp.

Listeria monocytigenes

Mycobacterium tuberculosis

Peptostreptococcus magnus

Plasmodium sp.

Staphylococcus aureus

Toxoplasma gondii

Yersinia pestis

Viruses

Adenovirus

Astrovirus

Hepatitis A

Hepatitis B

Hepatitis C

Herpes simplex

Equine herpes

Infectious salmon anemia

Rotavirus

Tick-borne encephalitis

Influenza virus A

Influenza virus A (H1N1)

Influenza virus B

Rabies virus

Papilloma virus,
human , type 16

Papilloma virus,
human, type 18

Rubella virus

Vaccinia virus

Toxins

Aflatoxins

Carbendazim

Clostridium Botulinum toxins

Fumonisin B1

Ochratoxins

Ricinus communis toxins

Staphylococcus aureus enterotoxins

Zearalenone

Enzymes and Proteins

Lactoferrin

Lysozyme

*Matrix metalloproteinase-3
(MMP-3)*

Myelin basic protein

Myosin, heavy chain

Myosin, light chain

*Neurone specific enolase
(NSE)*

*Prostate specific antigen
(PSA)*

*Proteinkinase, cAMP-
dependent regulatory
subunit*

Renin

tPA

Snap

*Superoxide dismutase,
Cu/Zn*

TIMP2

Transferrin

Troponin T, cardiac

Cytokines and Cell Signalling

Biotin

Folic acid

Cyclosporin A

Dopamine

FK506 (Tacrolimus)

Histamine

Interferon-alpha

Interferon-gamma

Interferon- beta

Interleukin-2

Interleukin-3

Interleukin-4

Leukotrienes

Prostaglandins

*Tumour Necrosis Factor-
alpha (TNF- α)*

Vitamin B12

Hormones

Cortisol

Corticosterone

Dexamethasone

17 beta-Estradiol

Estriol

17alpha-OH-Progesterone

Testosterone

Corticoliberin (CRF)

Ghrelin

Growth hormone (hGH)

Insulin

Parathormone (PTH)

Calcitonin

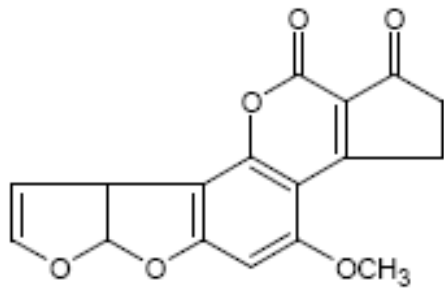
Triiodothyronine

*Epidermal growth factor
(EGF)*

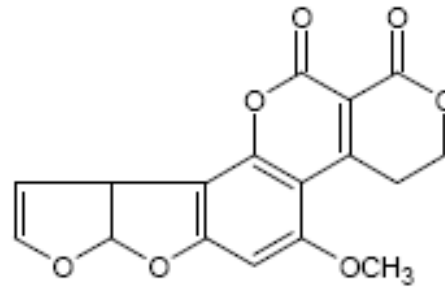
*Adenocorticotrophic hormone
(ACDG)*

Angiotensinogen

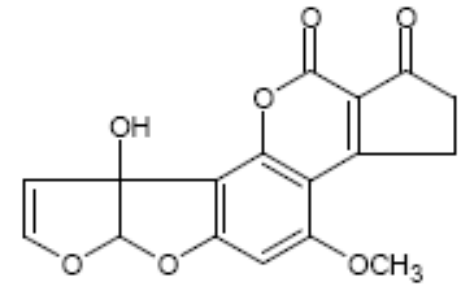
Aflatoxins – toxins from *Aspergillus* molds



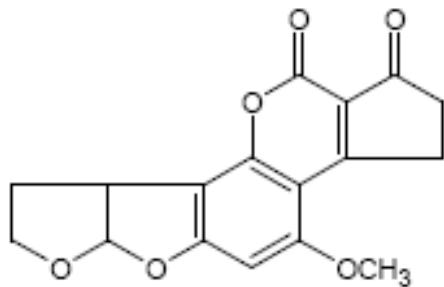
Aflatoxin B₁



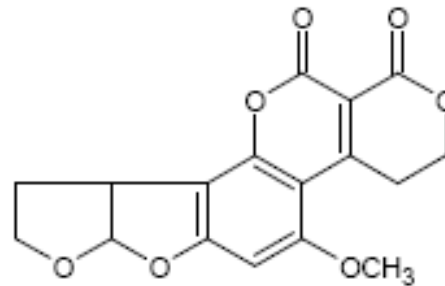
Aflatoxin G₁



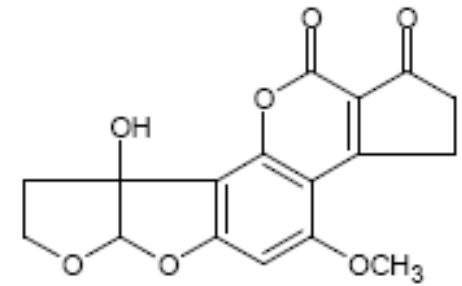
Aflatoxin M₁



Aflatoxin B₂



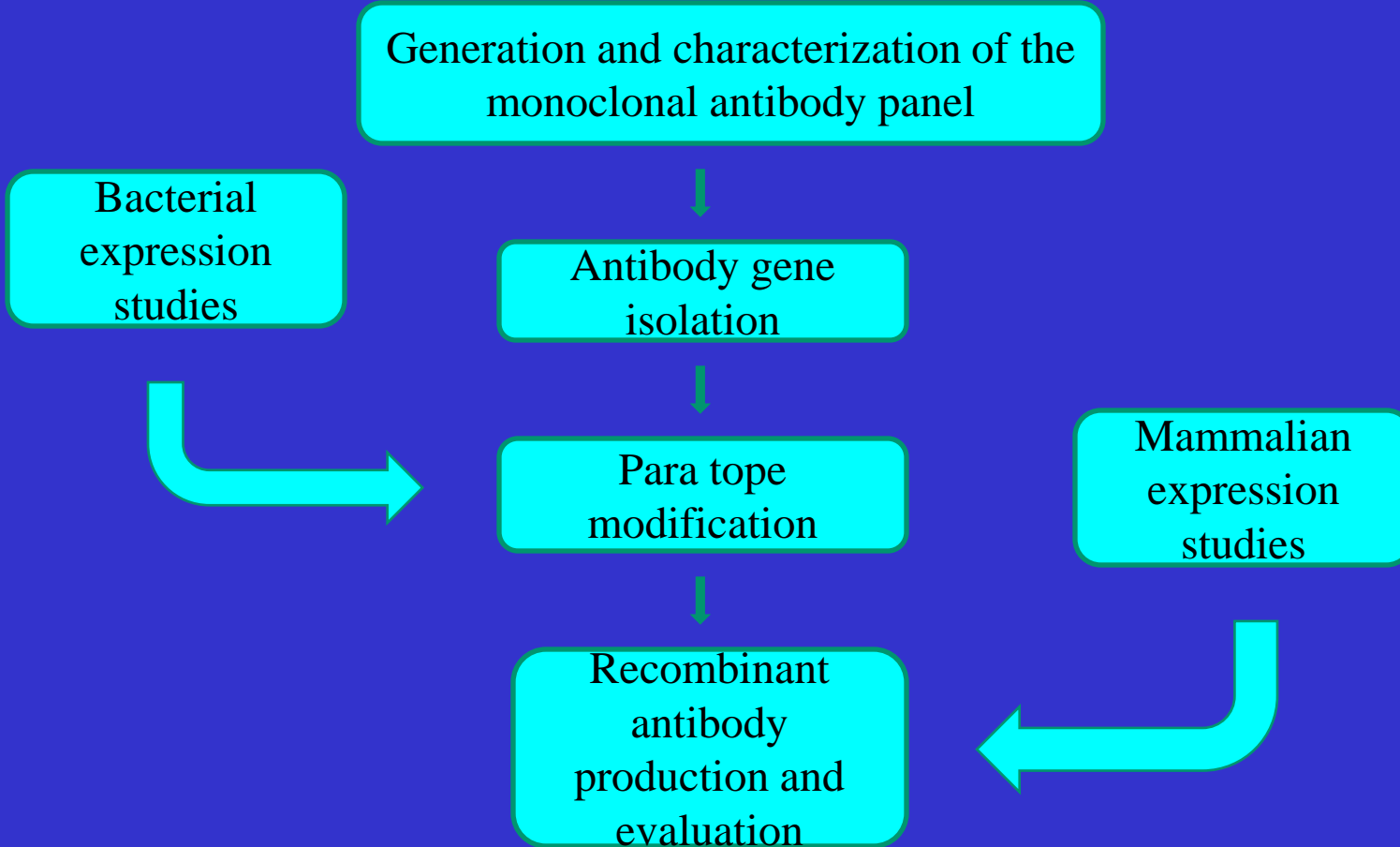
Aflatoxin G₂



Aflatoxin M₂

Toxicity: B₁ > G₁ > B₂ > G₂

Scope of activities



Specificity (% cross-reactivity) / Relative affinity

K_d of K33.1E7 vs. Aflatoxin G1 (2 mM) is chosen as 1

Fab'	Aflatoxin B1	Aflatoxin B2	Aflatoxin G1	Aflatoxin G2
K15.458	100 245	33 80	35 80	12 27
K15.200	100 620	4.4 27	25 153	0.65 4
K15.22	100 394	2.7 10	19 80	0.56 2
K15.727	100 13	71 10	50 7	13.3 2
K33.1E7	32 12	100 37	2.7 1	7.7 3
K33.3D2	62 200	100 325	24 80	18 60

K_d of K15.200 vs. Aflatoxin B1 = 3 nM

Construction of expression plasmids for the production of anti-aflatoxin Fab fragments

- **Isolation and cloning of mouse light chain κ and λ , and heavy chain IgG1, IgG2a, and IgG2b constant domain genes**
- **SOE-PCR generation of V_L-C_L and V_H-C_H gene fragments for each particular Fab fragment**
- **Assembly of bicistron plasmids with corresponding expression control elements**

Bacterially expressed Fab fragments

Original		Chain shuffling	
H-chain	L-chain	H-chain	L-chain
	K15.458	K15.458	K33.3D2
	K15.200	K33.1E7	K15.458
	K15.226	K33.3D2	K15.458
	K33.3D2		
	K33.1E7 (in 3 isoforms)		



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