Biologics, an overview

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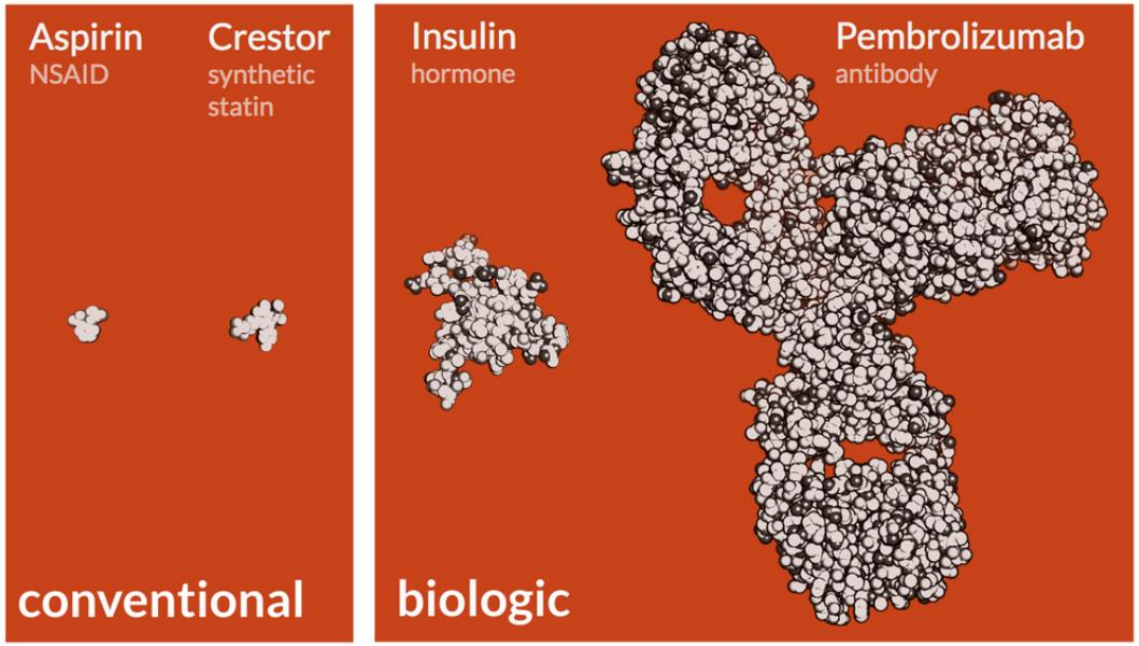

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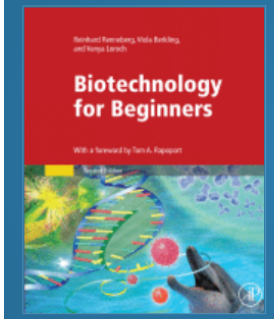
E-book available, see link via studium

Biotechnology for Beginners

Book • Second Edition • 2016



Authors:
Reinhard Renneberg, Viola Berkling and Vanya Loroch

About the book

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By table of contents

Book description

Biotechnology for Beginners, Second Edition, presents the latest information and developments from the field of biotechnology—the applied science of using living organisms and thei ... read full description

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Immunology and biotechnology

beskriva, förklara och jämföra metoder för framställning av låg- och högmolekylära läkemedelssubstanser såsom antibiotika, vacciner, och proteiner såsom antikroppar med hjälp av bioteknologi

beskriva och förklara för- och nackdelar med olika produktionssystem, såsom bakterier och växt- och djurceller, för bioteknologiska läkemedel

redogöra för antikroppar som läkemedelsmolekyler, och strategier för immunoterapi
redogöra för olika typer av överkänslighetsreaktioner och uppkomst av olika typer av autoimmunitet, inklusive underliggande mekanismer som driver autoimmuna sjukdomar och som utgör eller kan utgöra mål för läkemedelsbehandling med biologiska läkemedel

beskriva den immunologiska bakgrunden till avstötningsreaktioner vid transplantation, och hur risken för avstötningsreaktioner kan minimeras, inklusive strategier för immunosuppression med hjälp av biologiska läkemedel

redogöra för den immunologiska bakgrunden till transfusionsreaktioner och andra blodgruppsrelaterade immunreaktioner, samt hur sådana kan förhindras och förebyggas

redogöra för tumörimmunologi och olika strategier för immunoterapi vid cancer, inklusive monoklonala antikroppar och cellbaserade terapier

redogöra för olika immunbristsjukdomar, och förutsäga immunbrist orsakad av olika genetiska faktorer eller miljöfaktorer

redogöra för och analysera utvecklingen av bioteknologin (till exempel biologiska läkemedel, avancerade terapier, stamcellsforskning), och de bioteknologiska metoder som idag används i farmaceutisk forskning och industri
diskutera och värdera bioteknologins roll i samhället

i skrift visa förmåga att informera specialisten och en allmänhet genom PM och poster

Definition

A medicine who's active substance is made by a living organism

•Biological medicines contain active substances produced or extracted from a biological source, such as living cells, organisms, tissue
cannot be characterised by traditional chemical-pharmaceutical tests of finished product

•Most biological medicines in current clinical use active substances made of proteins

differ in size and structural complexity simple proteins like insulin or growth hormone to more complex ones such as coagulation factors or monoclonal antibodies

lakemedelsverket.se; www.ema.europa.eu

Examples of biologics

Blood products plasma-derived products

Immunological products
-vaccines, antisera

Advanced therapy medicinal products
-cell- and gene therapies (CAR T cells), tissue engineering

Therapeutic proteins produced by recombinant DNA technology (biotech products)
-hormones, cytokines, monoclonal antibodies, coagulation factors

Products of human or animal origin
-hormones, heparins, enzymes

Other biologicals
-allergens

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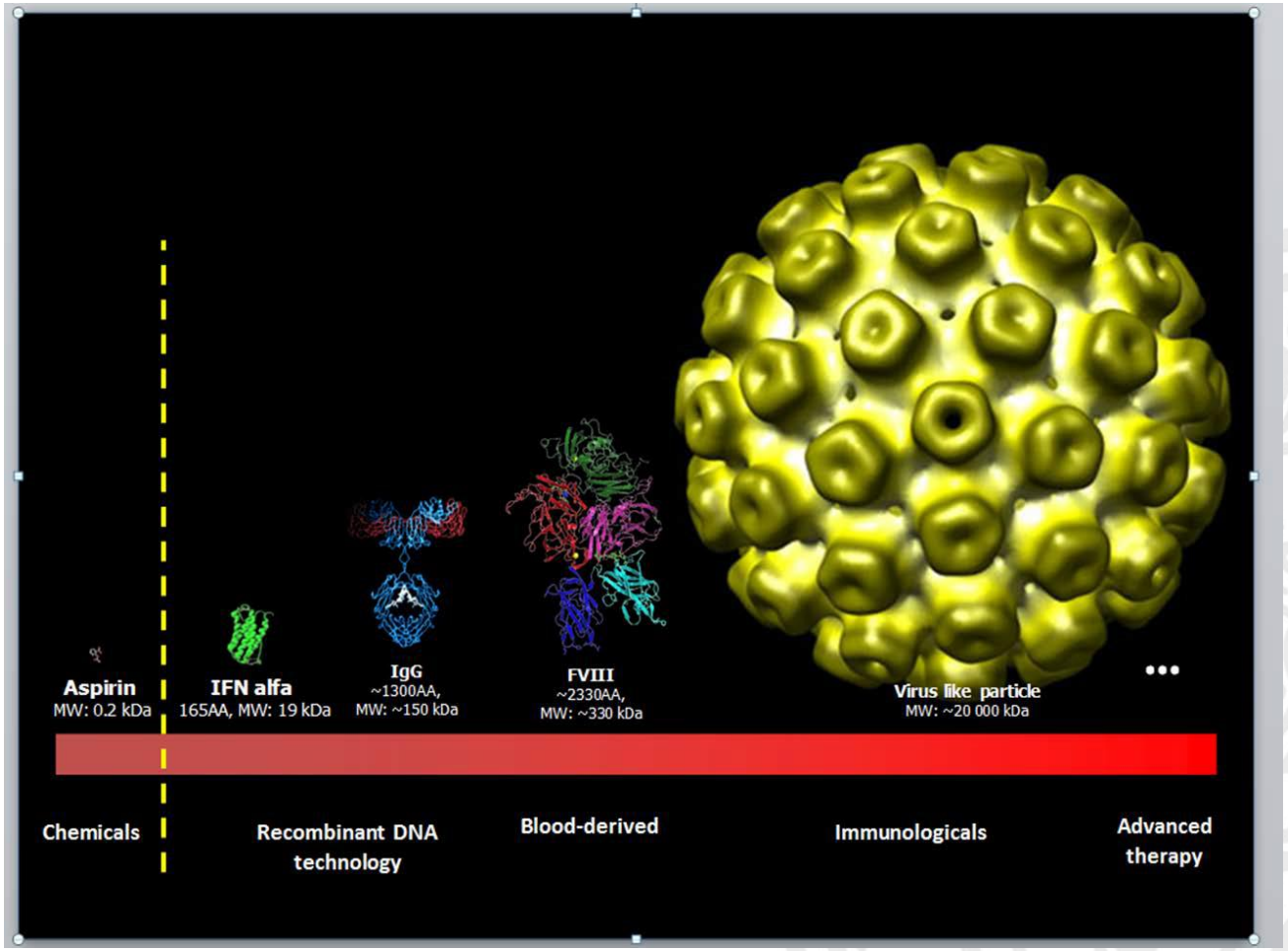
Molecular sizes



Difference peptides and proteins

Both are made of chains of amino acids (AA)
Size and structure distinguish them

Peptides 2 to 50 AA (approximately)

oligopeptide (2 to 20 AA): di- tripeptide (2 and 3 AA) etc
polypeptide, a long (>20 AA) continuous unbranched peptide
can adopt complex conformations

examples substance P (11 AA), calcitonin (32 AA), growth hormone–releasing hormone (GHRH, 44 AA)

Proteins > 50 AA (approximately)

formed from one or more polypeptides joined together → essentially very large peptides
often bound to coenzymes and cofactors, another protein, macromolecule

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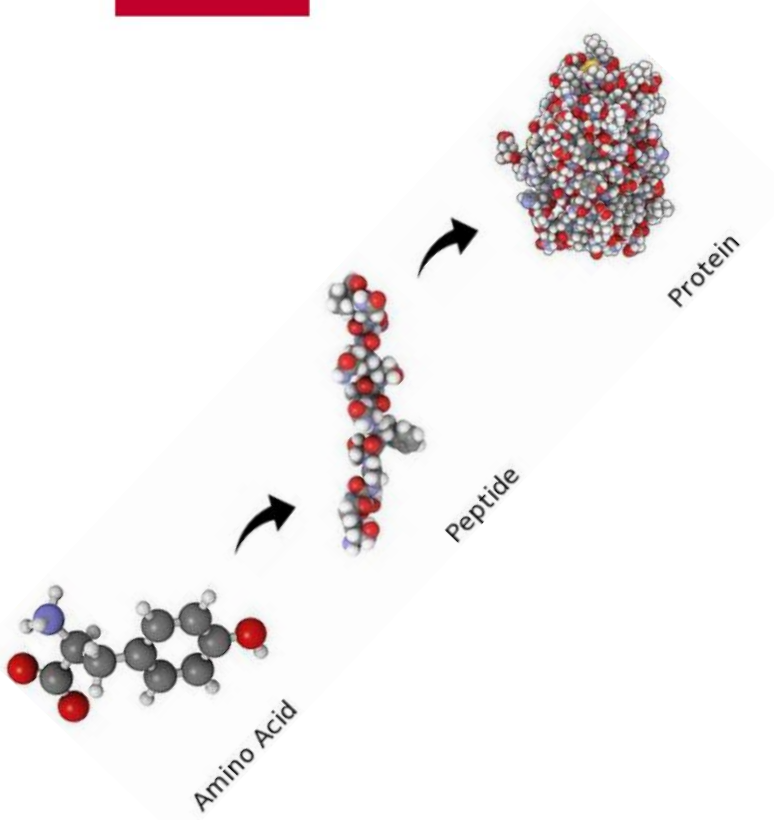

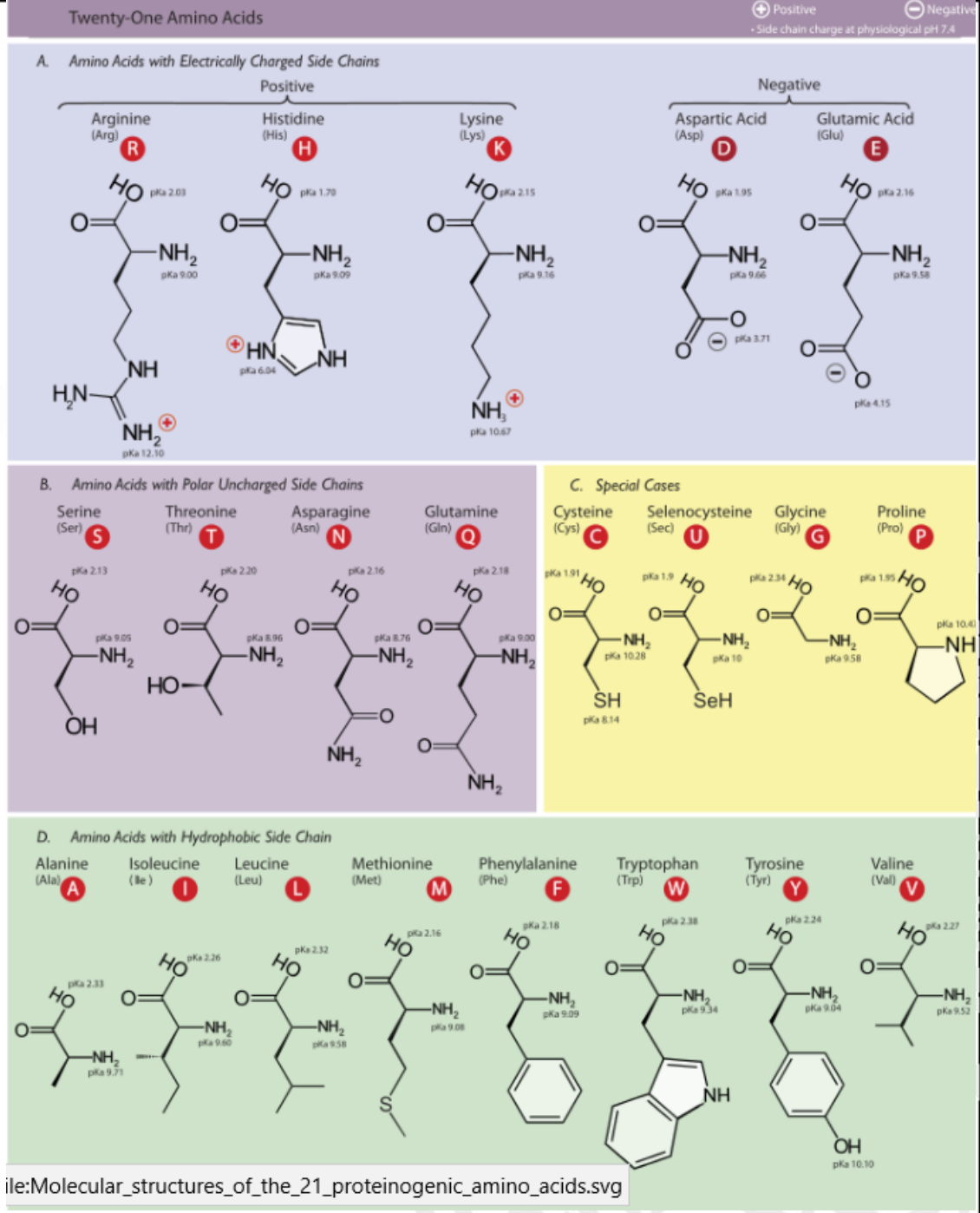

https://en.wikipedia.org/wiki/Proteinogenic_amino_acid ile:Molecular_structures_of_the_21_proteinogenic_amino_acids.svg

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Why use proteins as therapeutics?

- Highly specific and regulates complex functions that cannot be mimicked by chemicals
- Possible to engineer them due to the large binding interface
- Less problem of off-target effects due to their specificity
- Recombinant technology offerts today greater opportunities for novel function/activity
- Often well tolerated as it is a protein that is normally similar to a protein in the body that immune system then will not react to
- Can provide replacement when a person lack a protein
- May have faster approval time lines

However
Today protein therapeutics are used to target extracellular proteins, and there is a limitation in that

Recombinant DNA technology (landmark paper)

Proc. Nat. Acad. Sci. USA
Vol. 69, No. 10, pp. 2904–2909, October 1972

Biochemical Method for Inserting New Genetic Information into DNA of Simian Virus 40: Circular SV40 DNA Molecules Containing Lambda Phage Genes and the Galactose Operon of *Escherichia coli*

(molecular hybrids/DNA joining/viral transformation/genetic transfer)

DAVID A. JACKSON*, ROBERT H. SYMONS†, AND PAUL BERG

Department of Biochemistry, Stanford University Medical Center, Stanford, California 94305

Contributed by Paul Berg, July 31, 1972

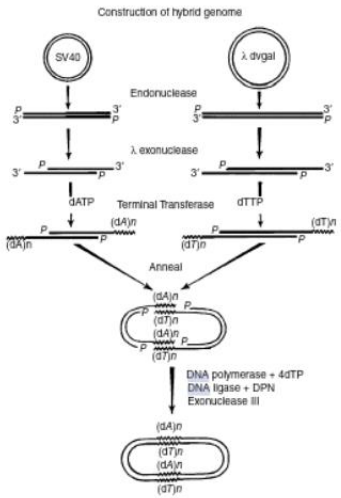


Figure 1.1 Paul Berg's construction of hybrid genome. (From his speech: The Nobel Prize in Chemistry 1980. Nobelprize.org. Nobel Media AB 2014. Web. 12 Jan 2017. http://www.nobelprize.org/nobel_prizes/chemistry/laureates/1980/ [24].)

Our goal is to develop a method by which new, functionally defined segments of genetic information can be introduced into mammalian cells. It is known that the DNA of the transforming virus SV40 can enter into a stable, heritable, and presumably covalent association with the genomes of various mammalian cells (1, 2). Since purified SV40 DNA can also transform cells (although with reduced efficiency), it seemed possible that SV40 DNA molecules, into which a segment of functionally defined, nonviral DNA had been covalently integrated, could serve as vectors to transport and stabilize these nonviral DNA sequences in the cell genome. Accordingly, we have developed biochemical techniques that are generally applicable for joining covalently any two DNA molecules.‡ Using these techniques, we have constructed circular dimers of SV40 DNA; moreover, a DNA segment containing λ phage genes and the galactose operon of *Escherichia coli* has been covalently integrated into the circular SV40 DNA molecule. Such hybrid DNA molecules and others like them can be tested for their capacity to transduce foreign DNA sequences into mammalian cells, and can be used to determine whether these new nonviral genes can be expressed in a novel environment.

The biotechnology business

The first biotechnology patent was granted in 1980 to Boyen and Cohen

In 1976 Herbert Boyer founded Genentech as the first company to work on biotechnology, this was founded together with Robert A Swanson

Since then, more than 2000 biotechnology companies have been founded

The history of insulin

1921 – purification of pancreatic insulin by Frederick Banting, Charles Best, James Collip and John LcLeod (University of Toronto)

First industry product by bovine or porcine origin (Novo Nordisk key player)

First protein to be sequenced (1955) earning Fred Sanger the nobel prize in 1958

First protein to be assembled by total peptide synthesis (1964)

X-ray crystallography achieved in 1969

First insulin injection pen (1985)

First DNA recombinant protein therapeutic on the market in 1982 (Humulin)
-kick started the biotechnology era

Recombinant human insulin

It is not clear if the recombinant human insulin gave an advantage over the purified animal derived product

The major advance from the success of producing recombinant insulin came from the later opportunity to prepare insulin analogues with improved PK and PD properties

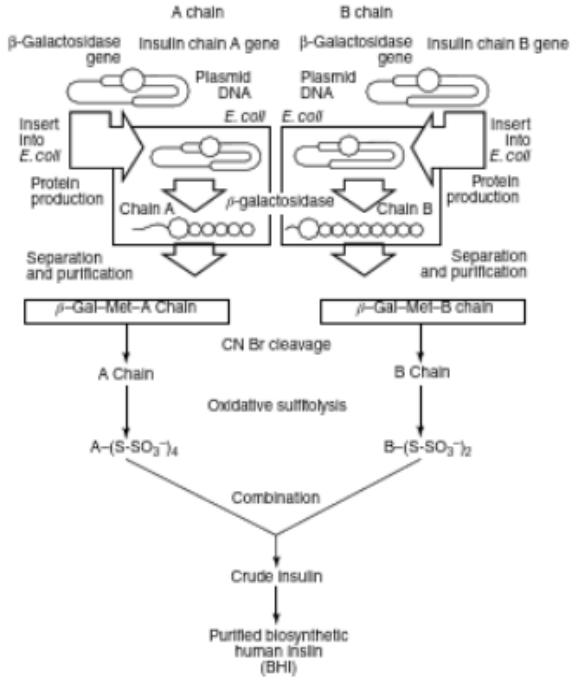


Figure 1.3 Production of insulin from separate genes encoding the A and B chains of human insulin. (From Ref. [37], used with permission. © E.P. Kroeff.)

Recombinant insulin

Novo made a semisynthetic insulin version using a 2-step enzymatic process to exchange 1 amino-acid in porcine insulin to prepare human insulin (not even a necessary step for biological function)

Novo and Nordisk merged in 1989 and after this they also got yeast DNA recombinant insulin on the market 1991

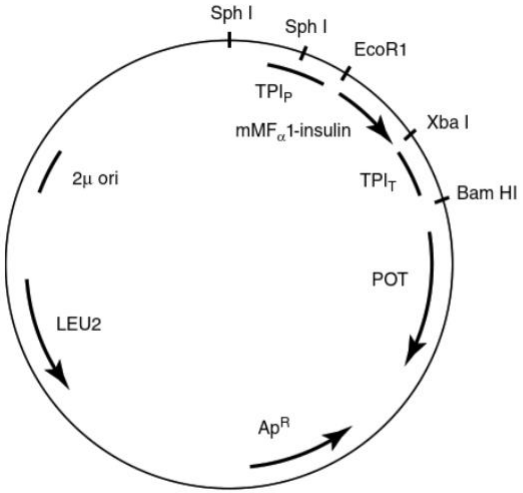


Figure 1.5 Yeast expression plasmid for dibasic insulin precursors. (From Ref. [52], used with permission © PNAS 1986.)

Growth hormones

Up to 1985 growth hormone deficiency was treated with hormones extracted from the pituitary glands of human cadavers (which lead to prion-disease manifests many years later)

Growth hormone is 191 amino acids, so longer than the 51 aa insulin

The first recombinant growth hormone approved 1985 by FDA (initially named Protropin and later Nutropin). Research funded by AB Kabi (later fused with Uppsala-based Pharmacia).

The first cytokine to be produced, interferon

Interferon is a signal substance that protects cells from viral infections

It was discovered in 1957

Complex class of proteins encoded by over 20 genes

Charles Weissmann (University of Zurich) and Peter Lenguyel (Yale) decided to work together to clone mouse interferon

Weismann initaited, together with VC, Biogen. An European version of Genentech

1980 reports on the cloning and expression of human IFN came

FDA approvals came 1986 for treatment of hairy cell leukemia

Antibody therapeutics development

Landmark paper by Köhler and Milstein in 1975, letter to *Nature* on how to make antibody producing cells of a specific species

In 1986 the first therapeutic monoclonal antibody was FDA approved, that was muromonab-CD3 (OKT3) – landmark approval to treat clinical transplantation and graft rejection

Later chimeric and humanized antibodies came that tackled the immunogenicity problem with the murine monoclonal antibodies

The first full-length chimeric antibody approved was anti-CD20 rituximab (1997). Also the first oncology product.

The first humanized antibody therapeutic was anti-interleukin 2 receptor (daclizumab)

Antibody therapeutics development

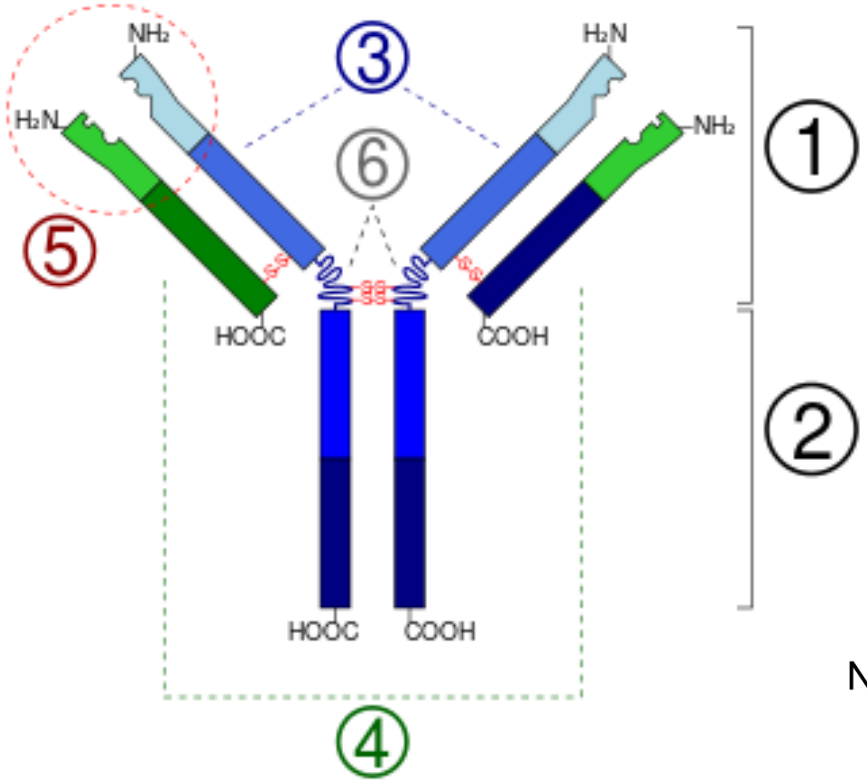
2002 was the first approval for an anti-TNF antibody (adalimumab)

The ADC (antibody drug conjugates) came during 2000 with gemtuzumab ozogamicin (anti-CD33 IgG4 conjugated to calicheamicin). Later withdrawn due to lack of efficacy

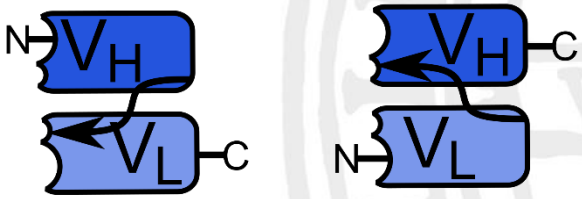
Also the radiolabeled ibritumomab got approval in 2002, which provides a chelation site for yttrium-90 (damage cells through beta-emission)

First bispecific antibody, catumaxomab was approved as a milestone in EU 2008. However, bispecifics are still hampered by production problems

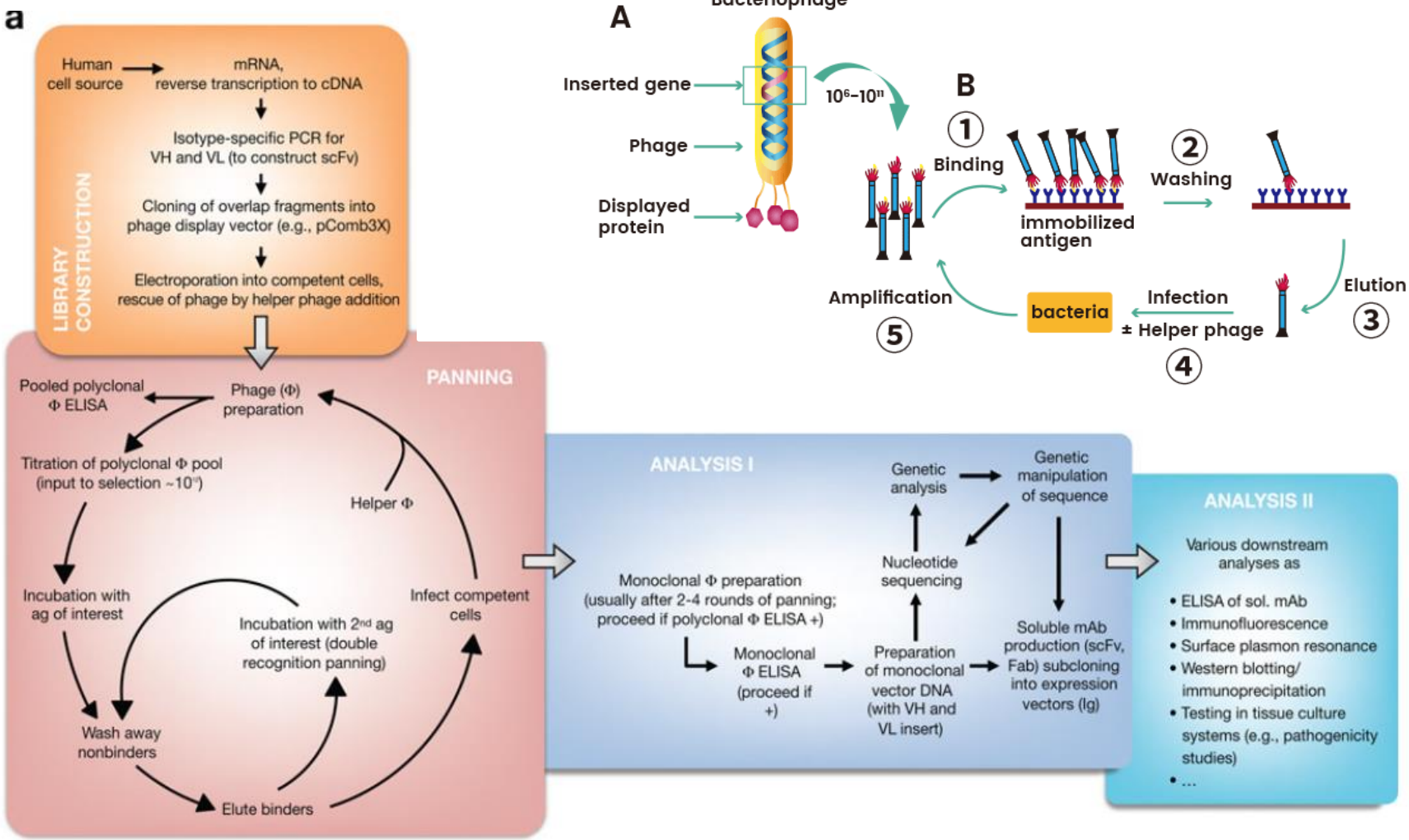
The bulding blocks of an IgG antibody



1.Fab region
2.Fc region
3.Heavy chain (blue) with one variable (VH) domain followed by a constant domain (CH1), a hinge region, and two more constant (CH2 and CH3) domains
4.Light chain (green) with one variable (VL) and one constant (CL) domain
5.Antigen binding site (paratope)
6.Hinge regions



Antibody phage display processes boosted the antibody therapeutics



https://cn.sinobiological.com/resource/antibody-technical/phage-display-antibody

Nomenclature of antibodies

Prefix must distinguish an antibody from other products.
Infix specifies the target of the antibody, such as a tumor or bacterial target, and the origin from the specifies the sequence was derived, so antibodies that were derived from a mouse would contain the substem *-o-*.
The suffix *–mab* is a common stem for all monoclonal antibodies."

Sequence of Stems and Infixes

The key elements of a monoclonal antibody name appear in the following order:

1. Prefix
2. Infix representing the target and origin
3. Stem used as a suffix (-mab or –pab)

Naming scheme	*prefix +*	*Target*	*Origin*	*suffix*
abciximab	ab	ci	xi	mab
rituximab	ri	tu	xi	mab

https://www.bioatla.com/appendix/antibody-nomenclature/
https://www.ama-assn.org/about/united-states-adopted-names/monoclonal-antibodies

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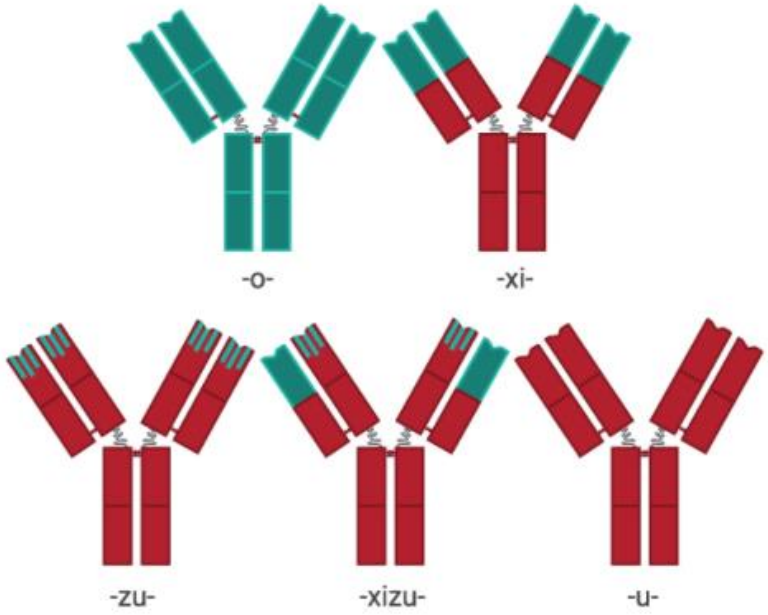
Source substems

Source substems: mouse (top left), chimeric (top right), humanized (bottom left), chimeric/humanized (bottom middle), and human (bottom right) monoclonal antibodies. Human parts are shown in red, non-human parts in blue.



Old and new naming system

NEW CONVENTION

•Olaratumab is an antineoplastic. Its name is composed of the components olara-t-u-mab. This shows that the drug is a human monoclonal antibody acting against tumors.

•The name of benralizumab, a drug designed for the treatment of asthma, has the components benra-li-zu-mab, marking it as a humanized antibody acting on the immune system.

OLD CONVENTION

•Adalimumab is a drug targeting TNF alpha. Its name can be broken down into ada-lim-u-mab. Therefore, the drug is a human monoclonal antibody targeting the immune system. If adalimumab had been named after 2009, it would have been adalumab.[6]

•Abciximab is a commonly used medication to prevent platelets from clumping together. Broken down into ab-ci-xi-mab, its name shows the drug to be a chimeric monoclonal antibody used on the cardiovascular system. This and the following two names would look the same if the new convention were applied.[12]

•The name of the breast cancer medication trastuzumab can be analyzed as tras-tu-zu-mab. Therefore, the drug is a humanized monoclonal antibody used against a tumor.[13]

•Alacizumab pegol is a PEGylated humanized antibody targeting the circulatory system.[8]

•Technetium (99mTc) pintumomab and technetium (99mTc) nofetumomab merpentan are radiolabeled antibodies, merpentan being a chelator that links the antibody nofetumomab to the radioisotope technetium-99m.[14]

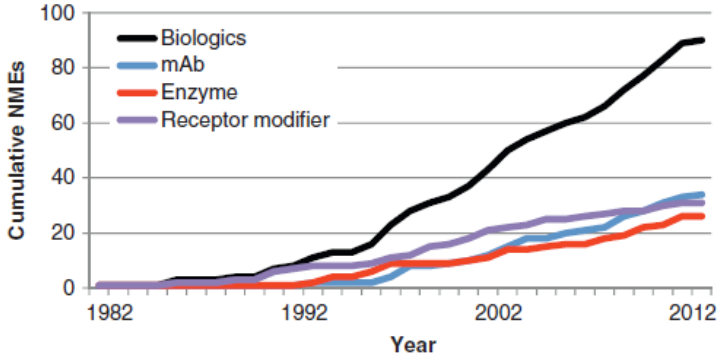
•Rozrolimupab is a polyclonal antibody. Broken down into rozro-lim-u-pab, its name shows the drug to be a human polyclonal antibody acting on the immune system. The suffix -pab shows it is a polyclonal antibody.

First 5 biologics approved in major categories

TABLE 2

First five biologics approved in major categories.

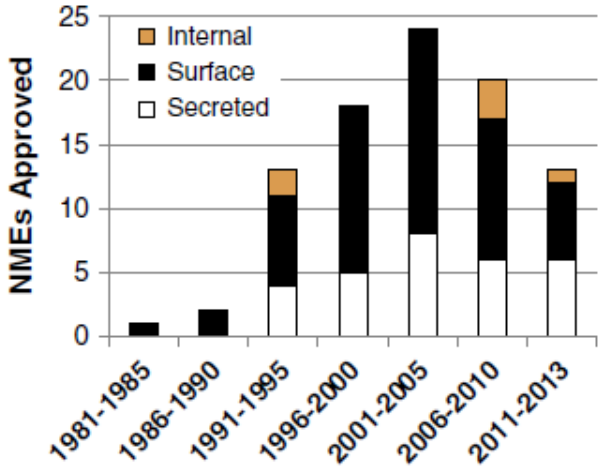
Monoclonal antibody	Receptor modulator	Enzyme modulator
Muromonab CD3 (Ortho, 1982)	rhInsulin (Eli Lilly, 1982)	Dornase alfa Genentech (1993)
Abciximab (Centocor, 1993)	Interferon alpha-2a (Roche, 1986)	Pegaspargase (Enzon, 1994)
Rituximab IDEC (1997)	Epoetin alfa (Amgen, 1989)	Imiglucerase (Genzyme, 1994)
Basiliximab (Novartis, 1998)	Filgrastim (Amgen, 1991)	Alteplase (Genentech, 1996)
Palivizumab (MedImmune, 1998)	Sagramostim (Immunex, 1991)	Reteplase (Boehringer-Mannheim, 1996)



Drug Discovery Today Volume 20, Number 4
April 2015

Biologics can be broadly distinguished based on their target types

- soluble cytokines acting on lymphoid cells
- soluble growth factors impacting non-lymphoid cells
- nonsoluble modulators of cell signaling and adhesion
- proteases
- other mechanisms



Drug Discovery Today Volume 20, Number 4
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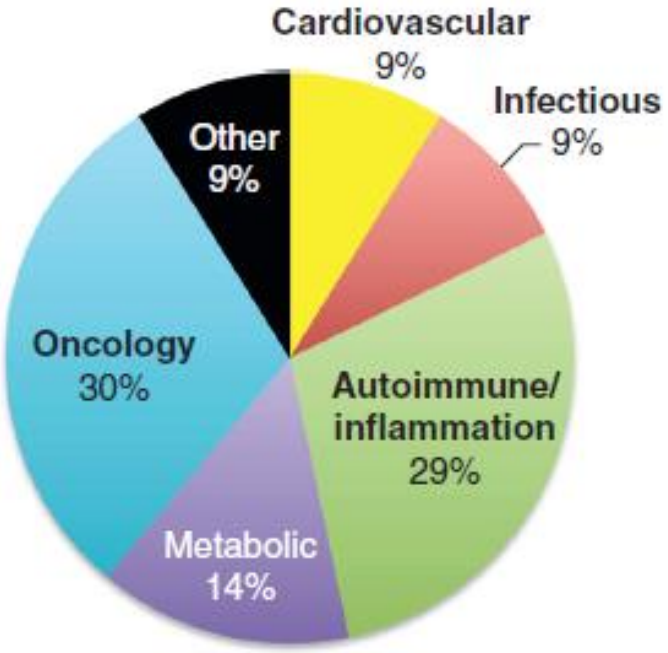
The indications for which biologics gained their initial approval



Drug Discovery Today Volume 20, Number 4
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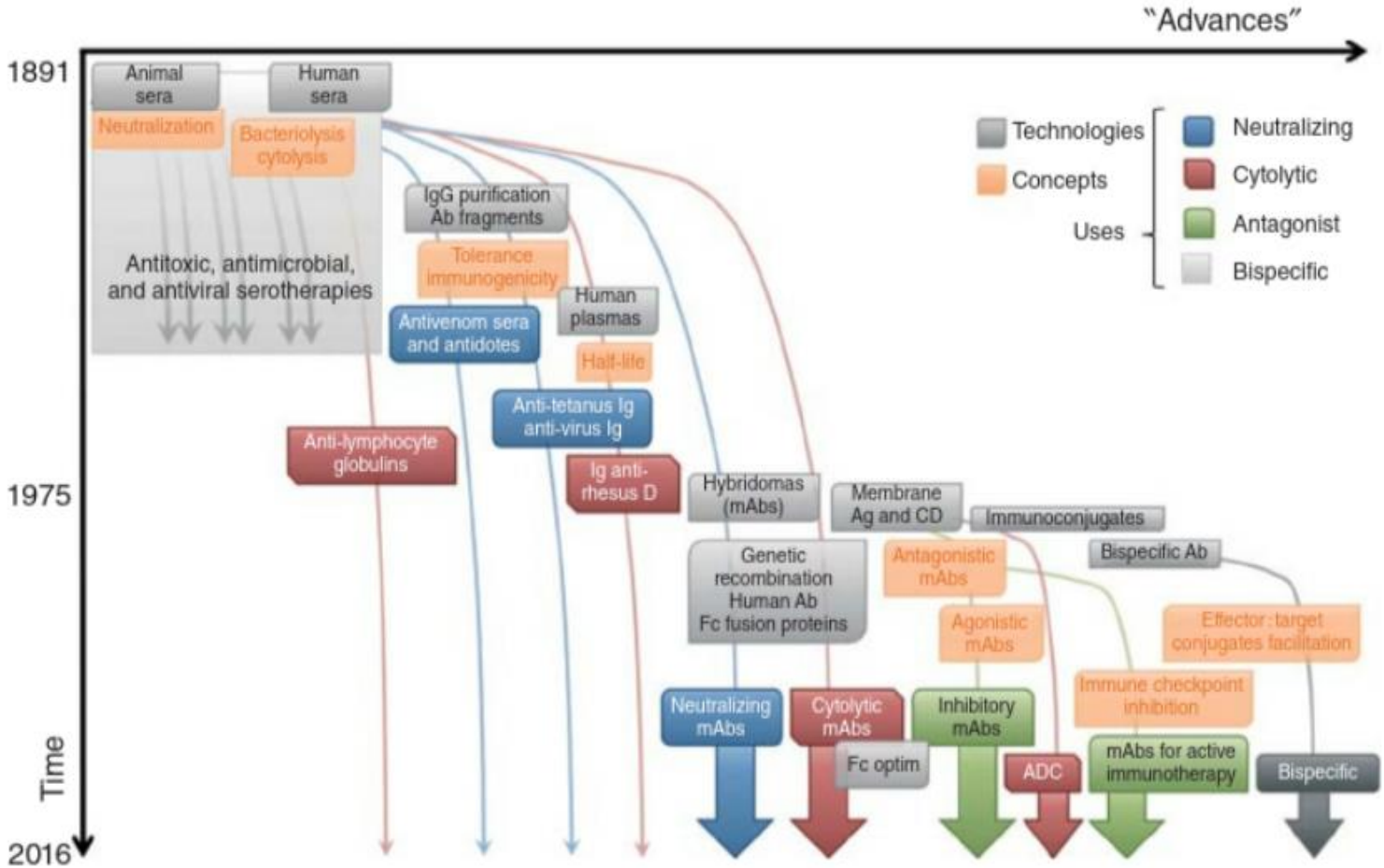
The evolution of Antibody therapeutics



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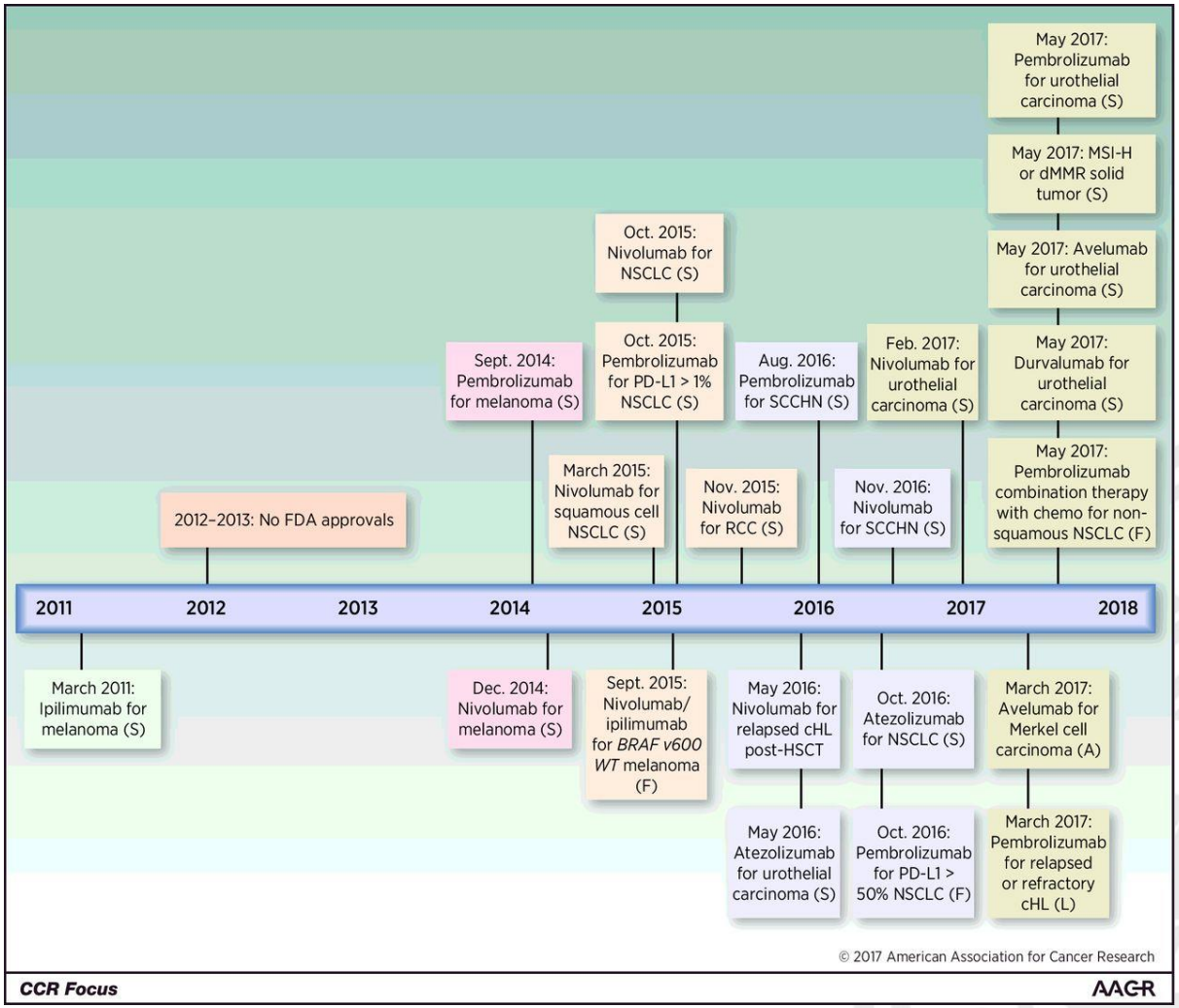
FDA approval timeline of immune checkpoint inhibitors in advanced/metastatic malignancies (https://www.fda.gov/drugs, retrieved May 31, 2017).



Christina S. Baik et al. Clin Cancer Res 2017;23:4992-5002

Clinical Cancer Research

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91% increase in the number of new agents in only 2 years in the IO field

IO= Immuno-onology

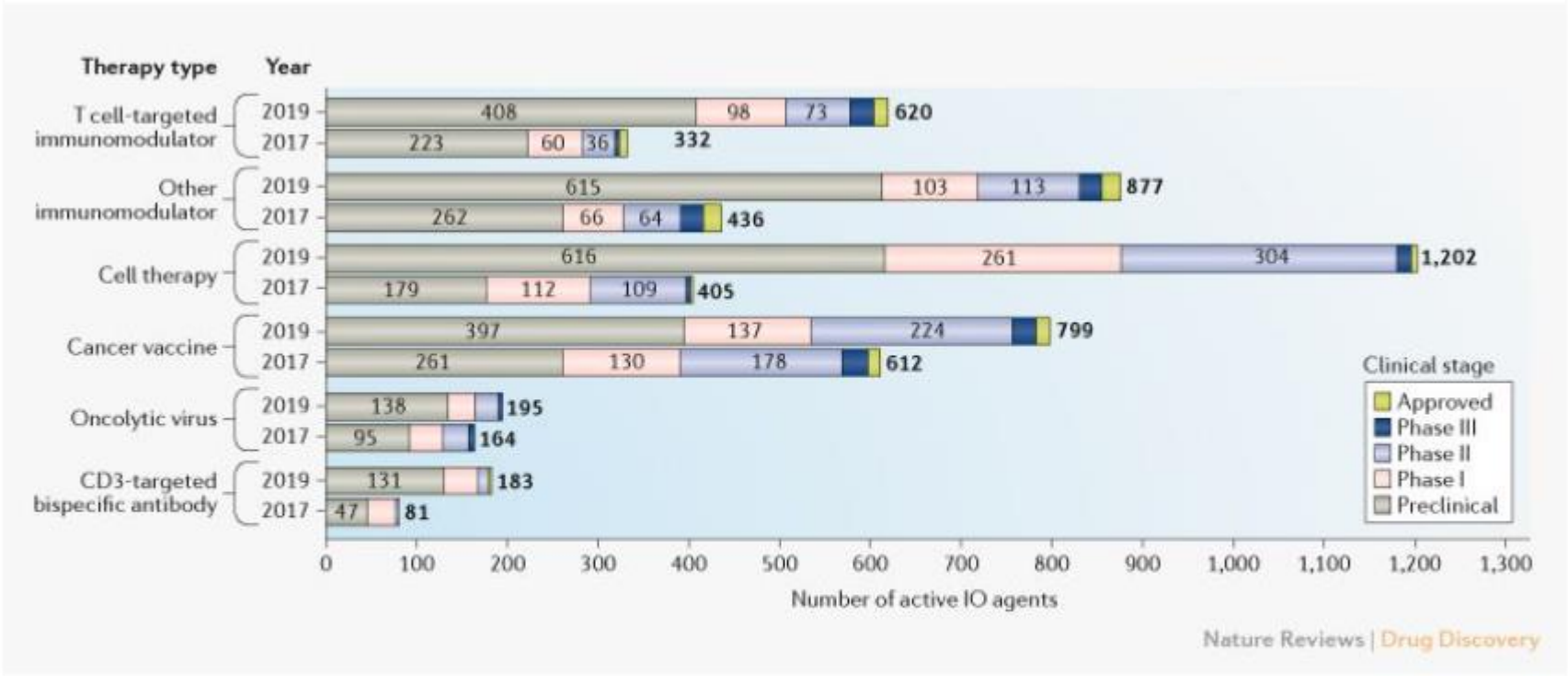
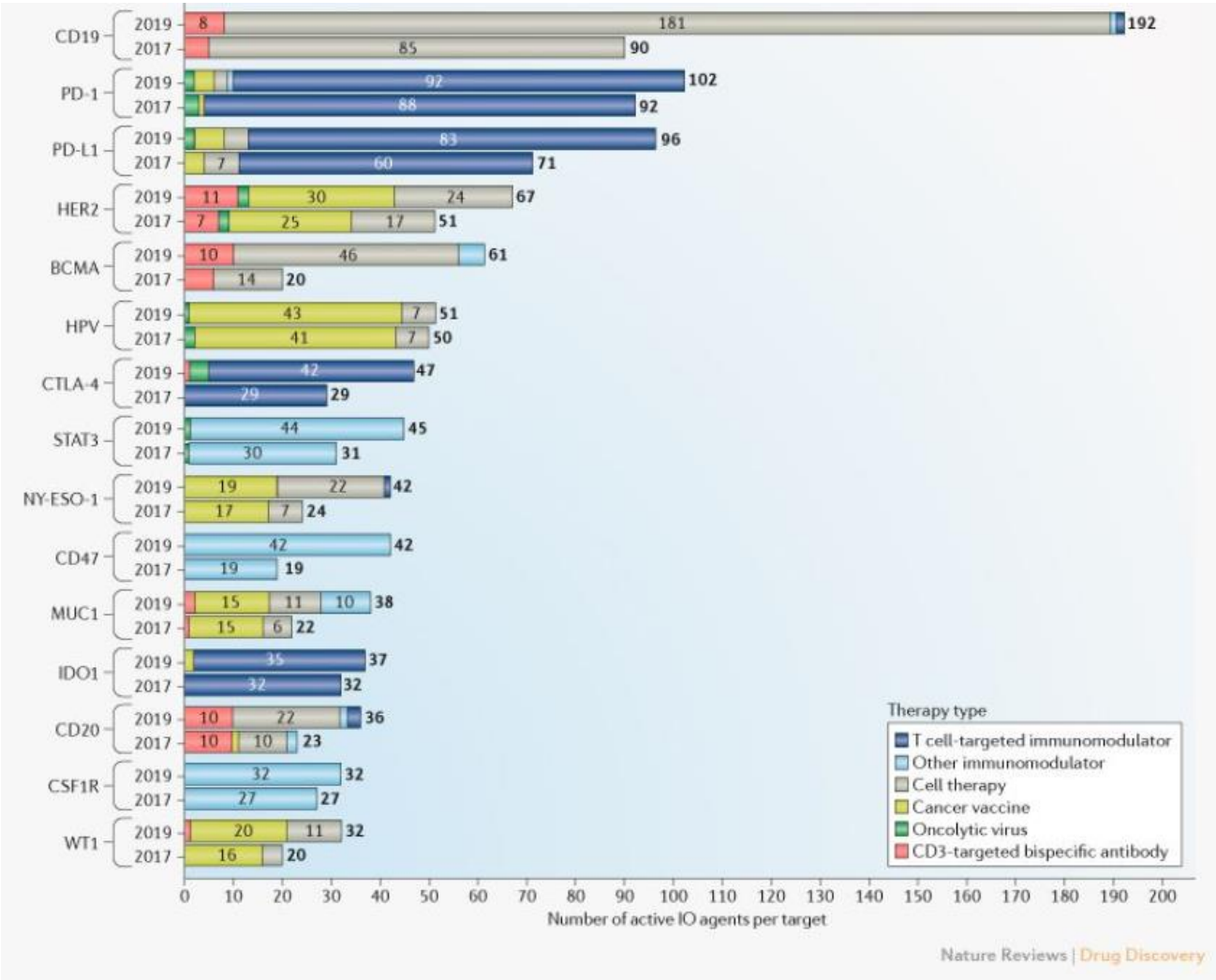


Fig. 1 | **Overview of all 3,876 active IO agents in the current global drug development pipeline.** In the past 2 years, 1,846 new agents have been added to the immuno-oncology (IO) pipeline, an increase of 91%.

https://www.nature.com/articles/d41573-019-00167-9

The top 15 targets within the IO field



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-allergens

Advanced therapy medicinal products (ATMPs)

"Human cell– or tissue-based products are highly heterogeneous and regulatory authorities will always apply their rulings on a case-by-case basis. Nevertheless, at present, most of the cell- and tissue-based products are considered biological medicinal products in those countries with more developed regulatory structures. "

Cytotherapy Volume 20, Issue 11, November 2018, Pages 1401-1413

Classes:

1. gene therapy medicinal products (GTMPs),
2. somatic cell therapy medicinal products (sCTMPs),
3. tissue-engineered products (TEPs)
4. combined products (tissue or cell associated to a device)

Criterias for the different products

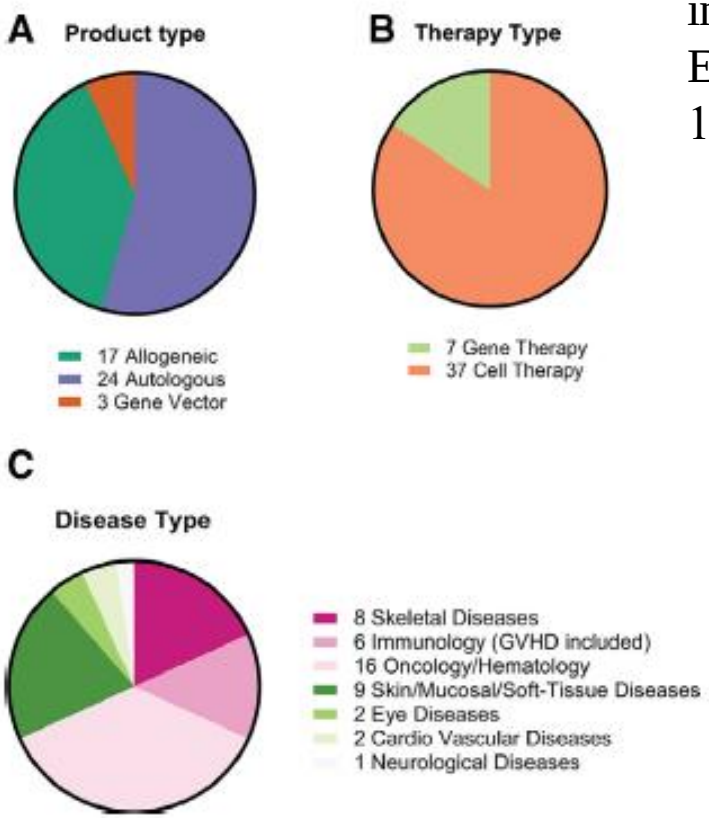
GTMPs should fulfil the three following criteria:

- Have biological origin.
- Contain recombinant nucleic acid(s).
- The therapeutic, prophylactic, or diagnostic effect should relate directly to the recombinant nucleic acid sequence it contains or to the product of genetic expression of this sequence.

sCTMPs and TEPs both contain or consist of engineered cells or tissues. To be considered engineered, cells or tissues should fulfil at least one of the following criteria:

- Substantial manipulation: biological characteristics, physiological functions, or structural properties relevant for the intended regeneration, repair, or replacement are achieved during their manipulation.
- Non-homologous use: the cells and tissues are not intended to be used for the same essential function (s) in the recipient and the donor.

ATMP



"The legal and regulatory framework for ATMPs in the European Union (EU) was established by the EU Commission in 2007 (Regulation EC No. 1394/2007) and first applied in December 2008"

J Mark Access Health Policy. 2016; 4: 10.3402/jmahp.v4.31036.

Cytotherapy Volume 20, Issue 11, November 2018, Pages 1401-1413

Examples of ATMP with MA in EU

Gene therapy:

- alipogene tiparvovec for lipoprotein deficiency
- tisagenlecleucel for B cell acute lymphoblastic leukemia (ALL) and diffuse large B-cell lymphoma (DLBCL)

Cell therapy:

- Sipuleucel-T for metastatic castrate-resistant prostate cancer

TEP:

- Autologous cartilage cells
- Autologous human corneal epithelial cells

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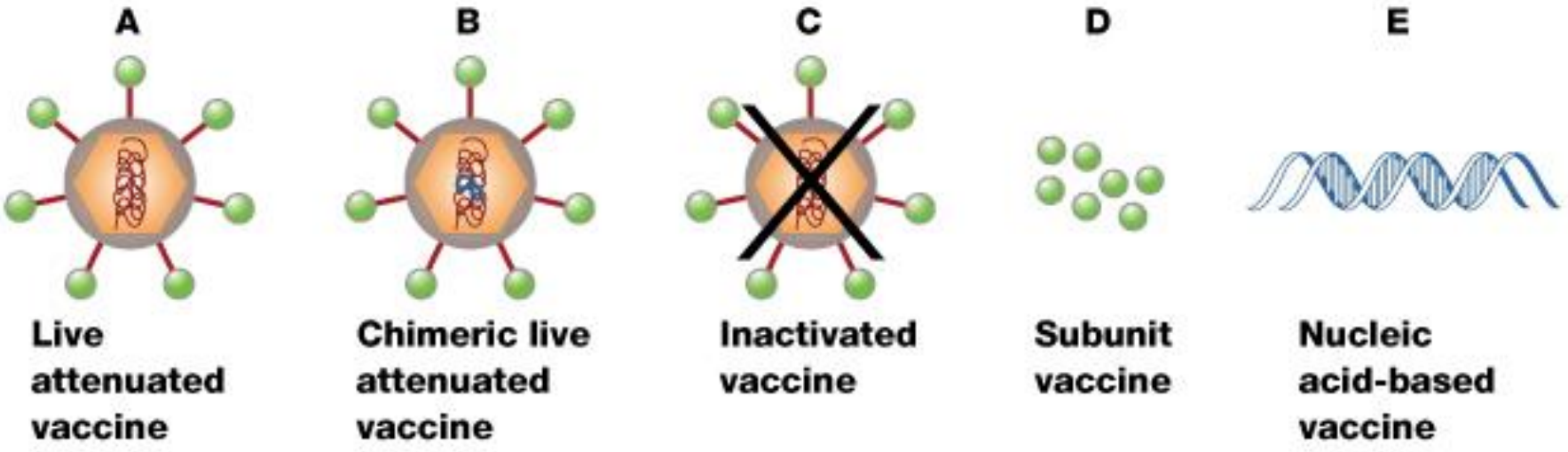
Vaccine components

Vaccines can be built up in different ways



https://www.nature.com/scitable/topicpage/future-dengue-fever-treatments-22404960

Viral vaccines

Viral Vaccines[*†]

Virus	Vaccine Components	Who Should Receive Vaccinations
Polio, inactivated	Trivalent (Salk vaccine)	Children
Attenuated polio	Live (oral polio vaccine, Sabin vaccine)	Children in epidemic areas
Measles	Attenuated	Children
Mumps	Attenuated	Children
Rubella	Attenuated	Children
Varicella-zoster	Attenuated Larger dose	Children Adults (>60) years
Rotavirus	Human-bovine hybrids	Infants
	Attenuated	
Human papillomavirus	VLP	Girls and boys ages 9-26 yr
Influenza	Inactivated	Children, adults, especially medical personnel and the elderly
	Attenuated (nasal spray)	Ages 2-50 yr
Hepatitis B	Subunit (VLP)	Newborns, health care workers, high-risk groups (e.g., sexually promiscuous, intravenous drug users)
Hepatitis A	Inactivated	Children, child care workers, travelers to endemic areas, Native Americans, and Alaskans
Adenovirus	Attenuated	Military personnel
Yellow fever	Attenuated	Travelers at risk to exposure, military personnel
Rabies	Inactivated	Anyone exposed to virus Preexposure: veterinarians, animal handlers
Smallpox	Live vaccinia virus	People seeking protection from bioterrorism, military

Anti-serum

Human or animal serum containing antibodies specific for one or more antigens and that is used to passively protect against a diasease

Examples:
Anti-Toxin
Anti-venom

First use around mid-1890 and initially horses were used to extract antibodies from

Behring received the Nobel prize 1901 for his work on diphtheria and antiserum

Summary

Protein therapeutics have revolutionized health-care as a new drug class, when insulin as the first protein therapeutics came, it truly saved lifes

Recombinant DNA technology have made an huge impact to spur protein drug development and accelerated clinical drug approvals in this drug class

Antibodies most often target extracellular soluble or membrane bound targets

Other formats of biologics are also in development or in clinical use, among these for example CAR T cells as well as bispecific antibodies