LOSCHMIDT LABORATORIES

12. Molecular Biotechnology in Medicine II

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Outline

- Protein therapeutics
- Recombinant proteins
  - Monoclonal antibodies
- Gene therapy
  - Antigen and antisense oligonucleotides
  - Ribozymes / deoxyribozymes
  - Chimeraplasts
  - Triplex Forming Oligonucleotides
  - Human Artificial Chromosomes
- Clinical Trials

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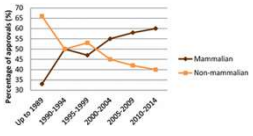
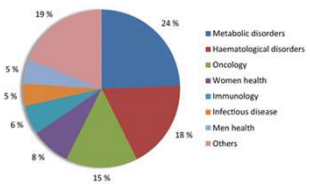
Recombinant proteins

- Interferons
- Human Growth Hormone
- Enzymes
  - DNase I
  - Alginate Lyase
  - Phenylalanine Ammonia Lyase
  - α_1-Antitrypsin
  - Glycosidases
- Alginate Lyase
- Antibodies
- Etc.





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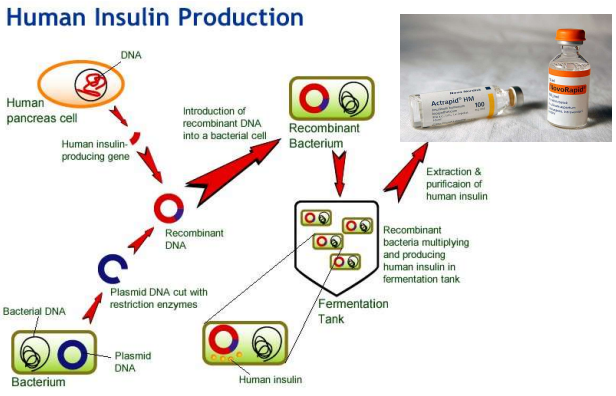
Recombinant proteins - Insulin



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U.S. FDA-approved protein therapeutics (2016)

40 11/30/2015	elotuzumab [Empliciti; Bristol Myers Squibb]	mAb [humanized anti-CD319(SLAMF7)]	oncology [cancer (multiple myeloma)]
41 12/8/2015	sebelipase alfa [Kanuma; Alexion Pharmaceuticals]	enzyme [lysosomal acid lipase]	cardiology/vascular diseases/genetic disease [lysosomal acid lipase deficiency]
42 3/18/2016	obiltoxaximab [Anthim; Elusys Therapeutics]	mAb [mouse/human chimeric anti-*Bacillus anthracis*]	infections and infectious disease [infectious disease (inhalational anthrax)]
43 3/22/2016	ixekizumab [Taltz; Eli Lilly and Company]	mAb [humanized anti-IL-17a]	dermatology/immunology [autoimmunity (plaque psoriasis)]
44 3/23/2016	reslizumab [Cinqair; Teva Respiratory]	mAb [humanized anti-IL-5]	pulmonary/respiratory disease [asthma]
45 4/5/2016	infliximab-dyyb [Inflectra; Celltrion]	mAb [mouse/human chimeric anti-TNFα]	musculoskeletal/rheumatology [inflammatory (Crohn's disease/ulcerative colitis/rheumatoid arthritis/ankylosing spondylitis/psoriatic arthritis/plaque psoriasis)]

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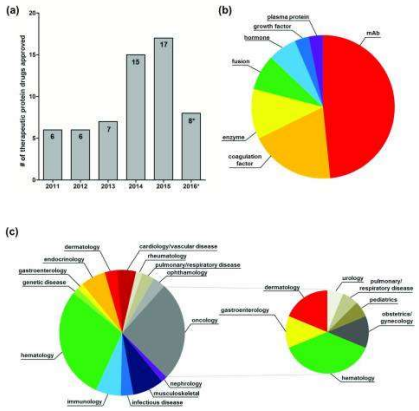
U.S. FDA-approved protein therapeutics (2011-2016*)



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THPdb

A database of FDA-approved therapeutic peptides and proteins

HOME · SEARCH · BROWSE · TOOLS · INFORMATION · DOWNLOADS · DEVELOPERS · HELP

Basic Search in THPdb

This page is designed to facilitate user to search THPdb database using query. This search module allows user to perform search on any or all fields of THPdb. It also allows to DISPLAY all fields or user selected fields. For more information see HELP page.

Query Submission Form

Please paste/insert/type your query to be searched:

Select Fields to be SEARCHED:

Name [Leptin]
Chemical Formula [CO_2]
Category [Hormone]
Component [Tri Life]
Functional Classification [IC]
Indication [cystic fibrosis]
Sequence [PPVQVLLR]
Isoelectric Point [4.98]
Patent number [US7654321]
Physical Appearance [Lyophilized]
Brand [Advel]
Brand [Intravenous]

Please select field you wish to DISPLAY: (Max seven field he can selected once)

Therapeutic peptide Name
Chemical Formula
Half life
Pharmacokinetics
Absorption
Patent Number
Target
Prescribed for
Route of administration
Sequence
Isoelectric point
2-D Structure
Mechanism
Volume of Distribution
Date of Issue
Brand
Chemical name
Recommended Dosage
Functional Classification
Hydrophobicity
Description
Toxicity
Clearance
Date of Expiry
Company
Formulation
Contraindication
Molecular Weight
Melting Point
Inclusion
Metabolism
Category
Drug Interaction
Brand Description
Physical Appearance
Side effect
Useful link
Published ID

Clear or Reset | Search

https://webs.iiitd.edu.in/raghava/thpdb/index.html

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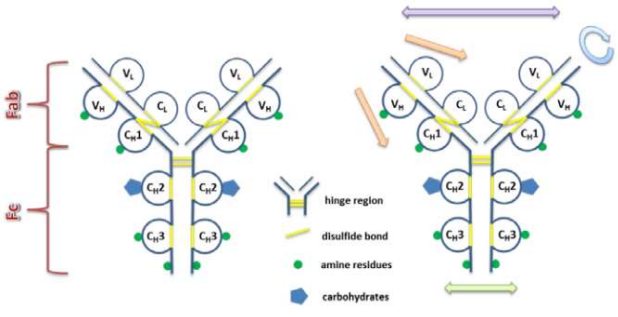
Antibodies – Basic Knowledge



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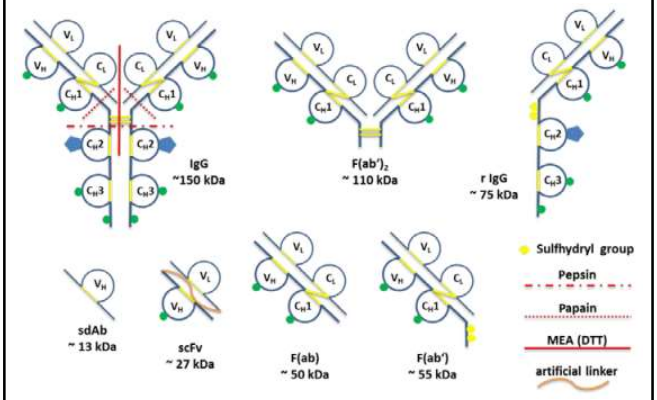
Antibodies – Basic Knowledge



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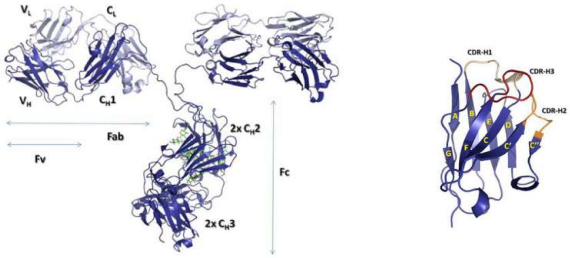
Antibodies – Basic Knowledge



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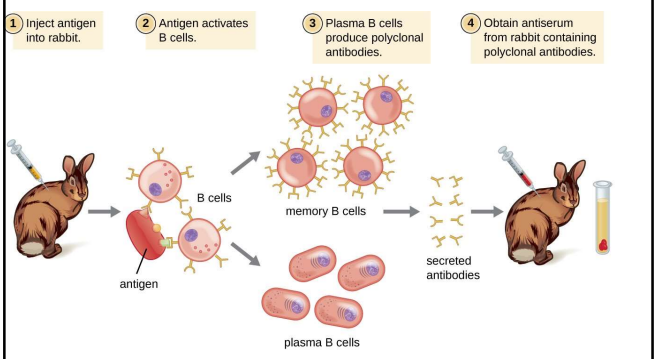
Polyclonal Antibody Production



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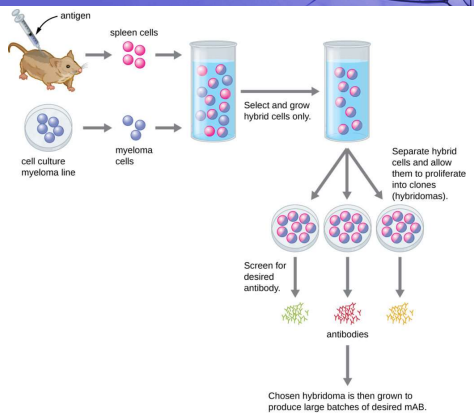
Monoclonal Antibody Production



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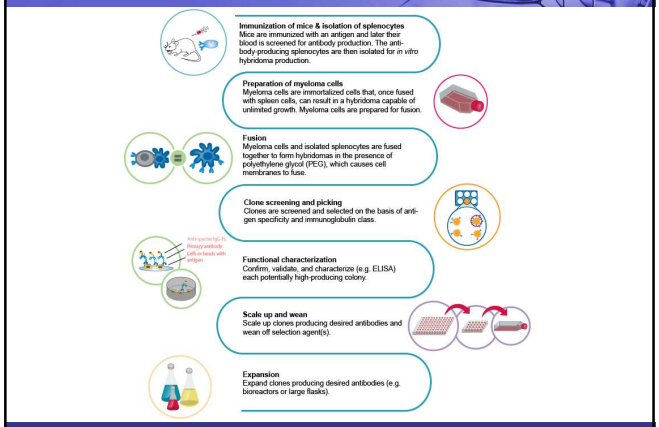
Monoclonal Antibody Production



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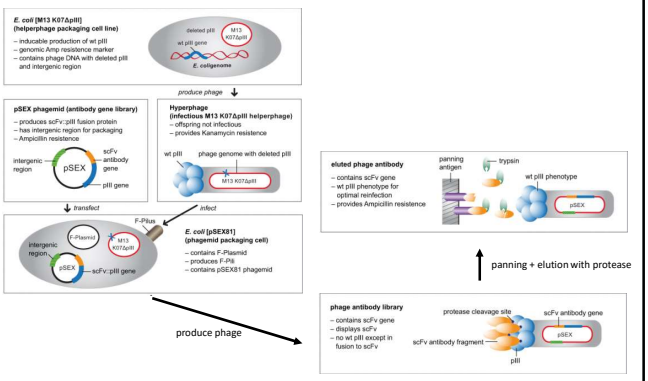
Phage Display Technology



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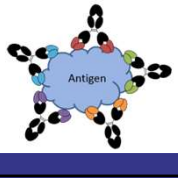
Monoclonal vs. Polyclonal Antibodies

Monoclonal Antibodies	Polyclonal Antibodies
Expensive production	Inexpensive production
Long production time	Rapid production
Large quantities of specific antibodies	Large quantities of nonspecific antibodies
Recognize a single epitope on an antigen	Recognize multiple epitopes on an antigen
Production is continuous and uniform once the hybridoma is made	Different batches vary in composition

Monoclonal antibody

Polyclonal antibody



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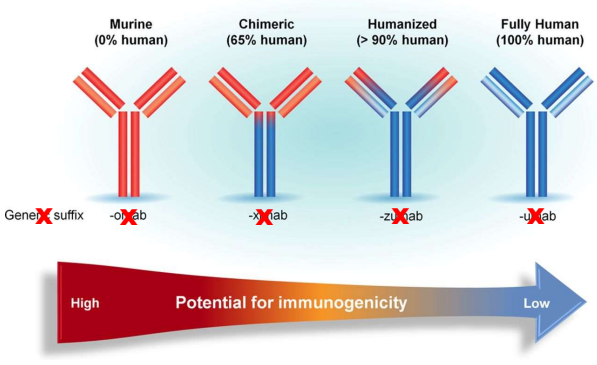

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Monoclonal antibody (mAb) nomenclature scheme

- **WHO - Geneva, 26 May 2017**

Table 1: Previous mAb nomenclature scheme.

Prefix:	Substem A: target class	Substem B: the species	Stem:
random	-b(a)- bacterial -ami(-)- serum amyloid protein (SAP) amyloidosis *(pre-substem)* -c(i)- cardiovascular -f(u)- fungal -gro(-)- skeletal muscle mass related growth factors and receptors *(pre-substem)* -k(i)- interleukin -l(i)- immunomodulating -n(e)- neural -o(s)- bone -tox(a)- toxin -t(u)- tumour -v(i)- viral	-a- rat -axo- rat-mouse *(pre-substem)* -e- hamster -i- primate -o- mouse -u- human -vet- veterinary use *(pre-substem)* -xi- chimeric -xizu- chimeric-humanized -zu- humanized	-mab

Table 2: New mAb nomenclature scheme.

Prefix:	Substem A*: target class	Stem:
random	-ba- bacterial -ami- serum amyloid protein (SAP) amyloidosis *(pre-substem)* -ci- cardiovascular -fung- fungal -gros- skeletal muscle mass related growth factors and receptors *(pre-substem)* -ki- interleukin -li- immunomodulating -ne- neural -os- bone -toxa- toxin -tu- tumour -vet- veterinary use *(pre-stem)* -vi- viral	-mab

* The substem A is currently under revision.

International Nonproprietary Name, INN

World Health Organization

doi: 10.3233/HAB-180347

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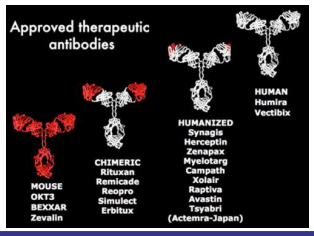
Therapeutic mAb

- **Function**
  - activate, repress, or alter endogenous immune responses to specific cells or molecules
- **Treatment of**
  - cancer, inflammatory and autoimmune disease, and many other types of disease



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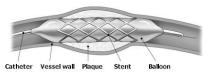
Chimeric and Humanized Therapeutic mAb

- **1st chimeric mAb**
  - Abciximab, for percutaneous coronary intervention
  - platelet aggregation inhibitor
  - since 1994
- **1st humanized mAb**
  - Daclizumab, to prevent rejection in organ transplantation
  - binds to CD25, the alpha subunit of the IL-2 receptor of T-cells
  - since 1997



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Fully Human Therapeutic mAb

- **phage-display platforms**
- **transgenic mouse platforms**
- **1st human mAb**
  - Adalimumab (Humira)
  - rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis, Crohn's disease, ulcerative colitis, chronic psoriasis, hidradenitis suppurativa, and juvenile idiopathic arthritis.
  - binds to TNFα receptors
  - since 2005

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SARS-CoV-2 „treatment“

- Bamlanivimab (plus Etesevimab), Casirivimab Plus Imdevimab
- Sotrovimab
- Granted by FDA and EMA
- Emergency Use Authorization (EUA)

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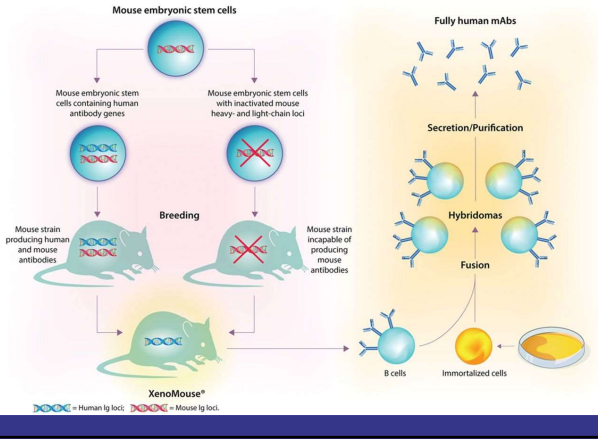
XenoMouse Hybridoma Technology



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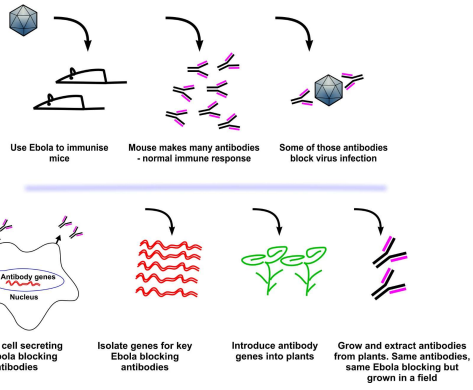
Therapeutic antibody grown in plants



Ref. 10.1073/pnas.1213709109 and 10.1126/scitranslmed.3006608

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Break 5 min

25

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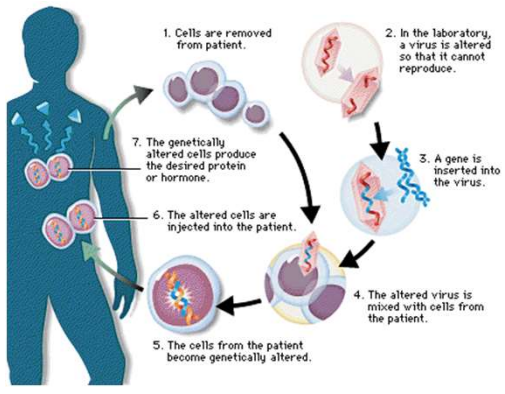
Gene Therapy



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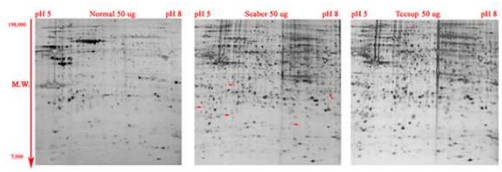
Nucleic Acids as Therapeutics

- Human disorders result in the overexpression of a normal protein
- Treatment approach
  - Lowering of transcription or translation
- Antigen oligonucleotide – binds to the gene and block the transcription
- Antisense oligonucleotide – base pairs with a specific mRNA



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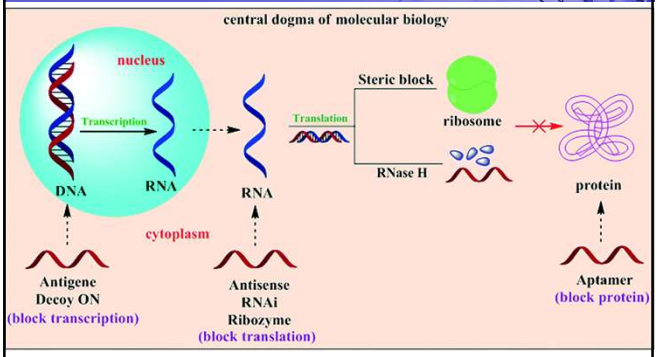
Antisense DNA



- Susceptibility to degradation by intracellular nucleases!

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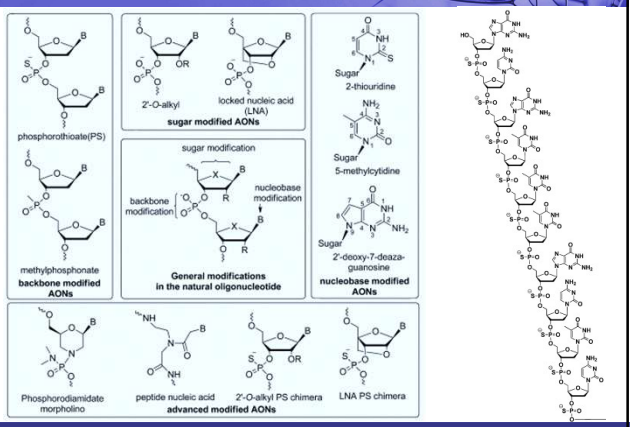
Synthetic Antisense Oligonucleotides



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Ribozymes

- Catalytic RNAs that cut things, make things, and do odd and useful jobs
- RNA metalloenzymes ~40 to 50 nucleotides in length
- can be engineered to specifically cleave any mRNA sequence
- separate catalytic and substrate-binding domains

The Nobel Prize in Chemistry 1989

Sidney Altman
Prize share: 1/2

Thomas R. Cech
Prize share: 1/2

"for their discovery of catalytic properties of RNA"

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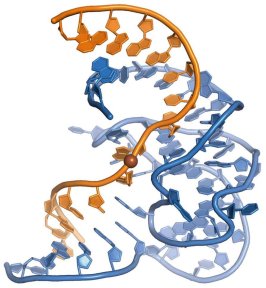
Deoxyribozymes

- No naturally occurring
- Artificially synthesized
- 1,000-fold more stable against hydrolytic destruction than protein
- 100,000-fold more stable than RNA

Structure of deoxyribozyme 9DB1, where we can see the synthetic strand of DNA (in blue) once it has catalysed the ligation of two RNA strands (in orange), joined at the point which is represented by a sphere.



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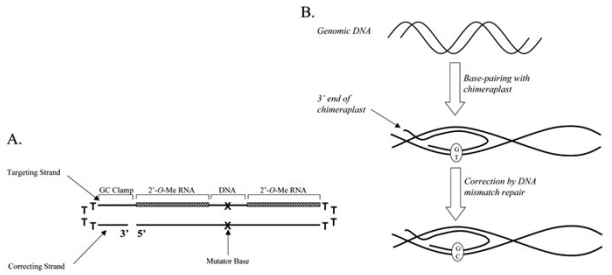
Chimeric RNA-DNA molecules - chimeraplasts

- site-specific point mutations within that sequence



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Gene Replacement Therapy vs. Corrective Gene Therapy

Gene Replacement Therapy (Gene Augmentation)	Corrective Gene Therapy
Random insertion of healthy counterpart of defective gene somewhere in genome so that its product could be available.	Directing insertion of healthy gene at specific site to displace defective gene is required.
Suitable for recessive disorders and for single gene mutations.	Possible for dominant disorders.
No recombinant event required and non specific insertion will work so long as appropriate regulatory controls are provided for expression.	Insertion at specific site would require some form of induced recombinational event.
Approach is not useful for dominant nature disorders or where errant(defective) gene gives destructive or interfering substance.	This approach would be ideal where errant gene produces destructive or interfering substance.
This approach is feasible today and has effect similar to transplantation approach only thing it bring done still at root level of the defect.	Extensive study is still required to direct gene at correct position in the genome.

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Aptamers

- sequences of NAs that are capable of recognizing and binding to a specific target
  - including metal ions, small molecules, peptides and proteins
  - high affinity and specificity
- Systematic Evolution of Ligands by EXponential enrichment (SELEX)
- DNA (are more stable) or RNA

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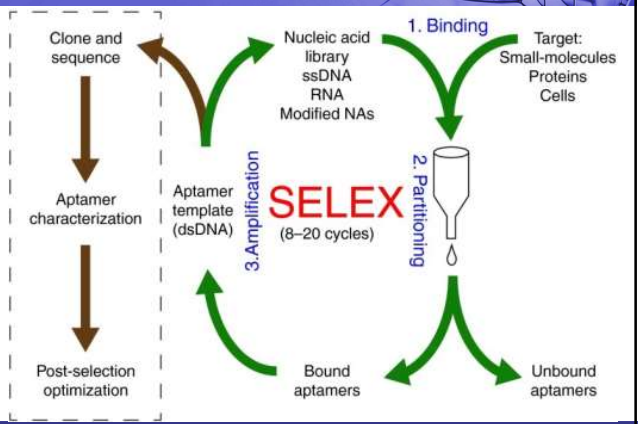
SELEX protocol



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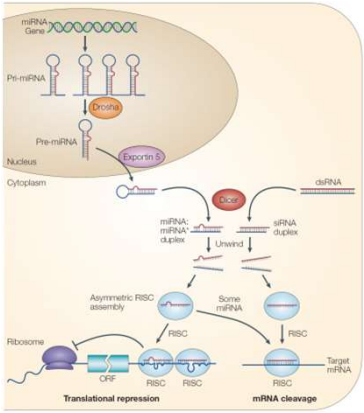
siRNA (miRNA) silencing pathway



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Triplex Forming Oligonucleotides

- Formation of triple helices discovered in 1957 (Felsenfeld *et al.*)
- Morgan (1968) demonstrated ability of a bound RNA third strand to inhibit transcription
- Sequence-specific tools for gene targeting (purine-rich strand)
  - Established binding code
- TFOs bind to a major groove of duplex DNA
  - High specificity and affinity ☻
  - Stabilized by divalent cations
- Homing devices for genetic manipulation *in vivo*
- Potential toll for gene knock out in mammalian cells
- Includes natural and modified DNA, PNAs, polyamides
- Typically 20-30 nt in length

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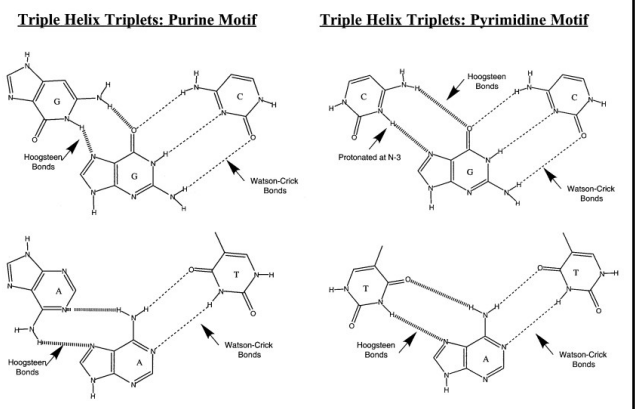
Binding code for TFOs



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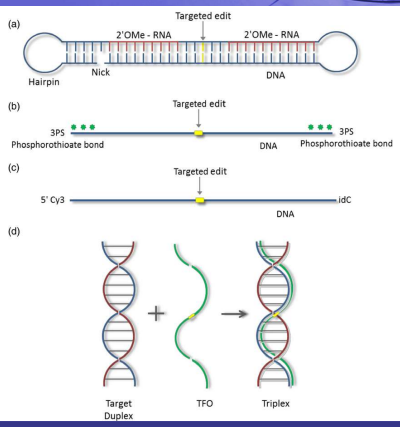
Triplex Forming Oligonucleotides



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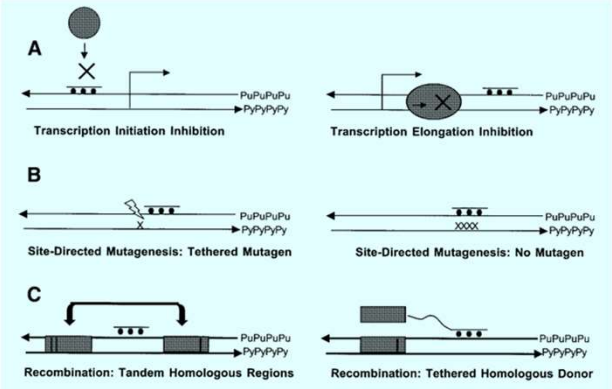
TFOs – gene alteration



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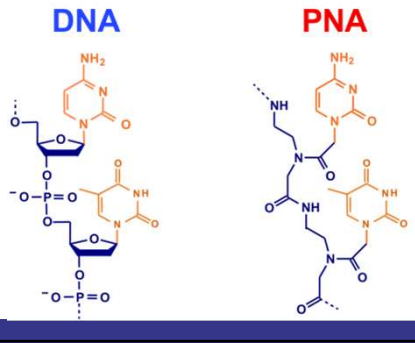
Peptide Nucleic Acids

- Invented in 1991
- Chemically stable and resistant to hydrolytic cleavage



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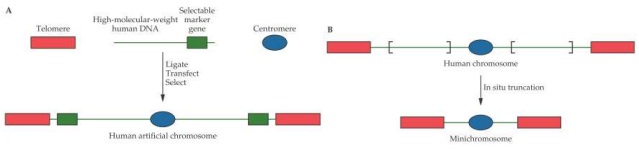
Human Artificial Chromosomes (HAC)

- Two ways for creation
  - Ligation of individual chromosome components (telomers, centromere, origins of replicon)
  - Paring down an existing human chromosome by deleting material to form "minichromosome"



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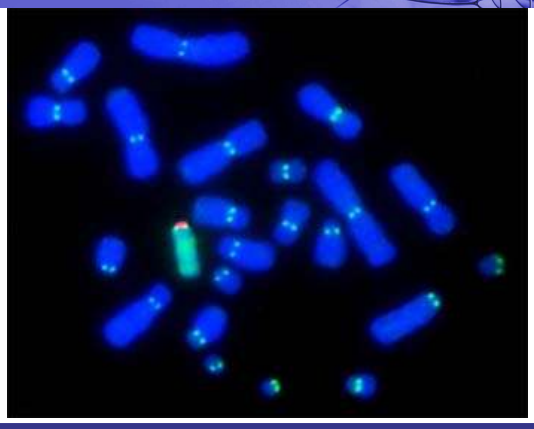

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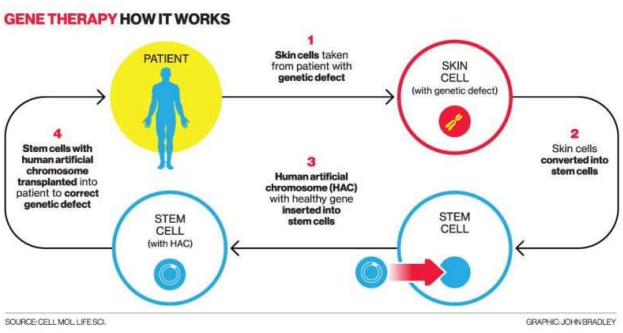
Gene Therapy using HAC



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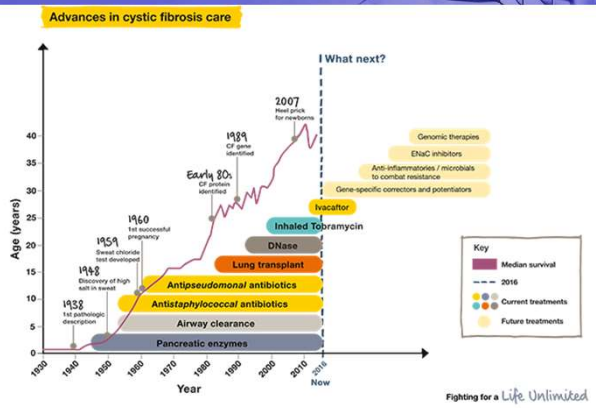
Cystic fibrosis treatment/ hopefully near future



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Clinical Trials

- Phase I
  - first-in-man trials
  - Usually, small group 20-80
  - Screening for safety and dosage
- Phase II
  - Larger group (200-300)
  - Determine efficacy, usually against placebo
- Phase III
  - Large group (1000-3000)
  - Confirmation of safety and efficacy (compare to commonly used treatments)

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Reading

- Vogenberg FR, Isaacson Barash C, Pursel M. Personalized Medicine: Part 1: Evolution and Development into Theranostics. Pharmacy and Therapeutics. 2010;35(10):560-576.

Personalized Medicine

Part 1: Evolution and Development into Theranostics

F. Randy Vogenberg, PhD, RPh; Carol Isaacson Barash, PhD; and Michael Pursel, RPh, MBA

This article is the first in a three-part series on the topic of medicine that is geared toward the individual patient. Part 2 will explore key ethical, legal, and regulatory issues facing the future of personalized medicine, and Part 3 will cover the anticipated challenges in implementing pharmacogenomics and genetic testing into routine clinical practice.

Key words: personalized medicine, pharmacogenomics, pharmacogenetics, pharmacodiagnostics, theranostics, personal genomics, human genome, gene testing

INTRODUCTION

Personalized medicine (PM) has the potential to tailor therapy with the best response and highest safety margin to ensure better patient care. By enabling each patient to receive earlier diagnoses, risk assessments, and optimal treatments, PM holds promise for improving health care while also lowering costs.

HISTORY AND LANDSCAPE

Over the past six decades, much evidence has emerged indicating that a substantial portion of variability in drug response is genetically determined, with age, nutrition, health status, environmental exposure, epigenetic factors, and concurrent therapy playing important contributory roles. To achieve individual drug therapy with a reasonably predictive outcome, one must further account for different patterns of drug response among geographically and ethnically distinct populations.

These observations of highly variable drug response, which began in the early 1950s, led to the birth of a new scientific discipline arising from the confluence of genetics, biochemistry, and pharmacology known as pharmacogenetics. Advances in molecular medicine have spawned the newer field of pharmacogenomics, which seeks to understand all of the molecular underpinnings of drug response. Commercialization of this research application is now known as personalized medicine (PM). Demonstrated success is emerging for several conditions and treatments, but whether PM will achieve

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