R E G I S T E R N O W !

1,000,000,000 *CHF investment*

7,000,874 *hours of work*

6,587 *experiments*

423 *researchers*

1 *medicine*

Health Hackathon 4.12 & 11.12.2025

THE MAKING OF AN INNOVATIVE MEDICINE

Introductory workshops on translational biomedical research, drug discovery and development

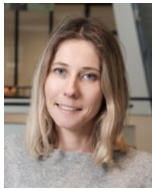
Judge Prof Dr med Olaia Naveiras

BIO-698 resumes Thursday September 11. 2025
4:15 PM @ CM013

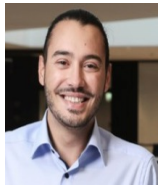
With Timothee Ferrari MD PhD cand

EPFL

Sciences de la Vie -SV



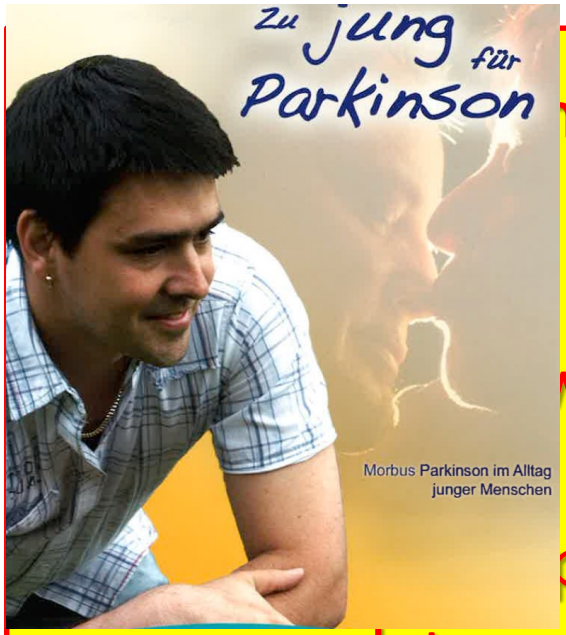
With Justine Epiney MD PhD cand



Mehdi AliGadiri MD PhD cand

Prof Roger G. Clerc

Each of You Knows a Relative, a Friend, a Neighbor Who Suffered from a Disease



nt-side of life, a more onerous citizenship.

born holds a dual citizenship, in the
vell and in the kingdom of the sick

prefer to use only the goo
sooner or later each of us is obliged, at lea
identify ourselves as citizen of the other p

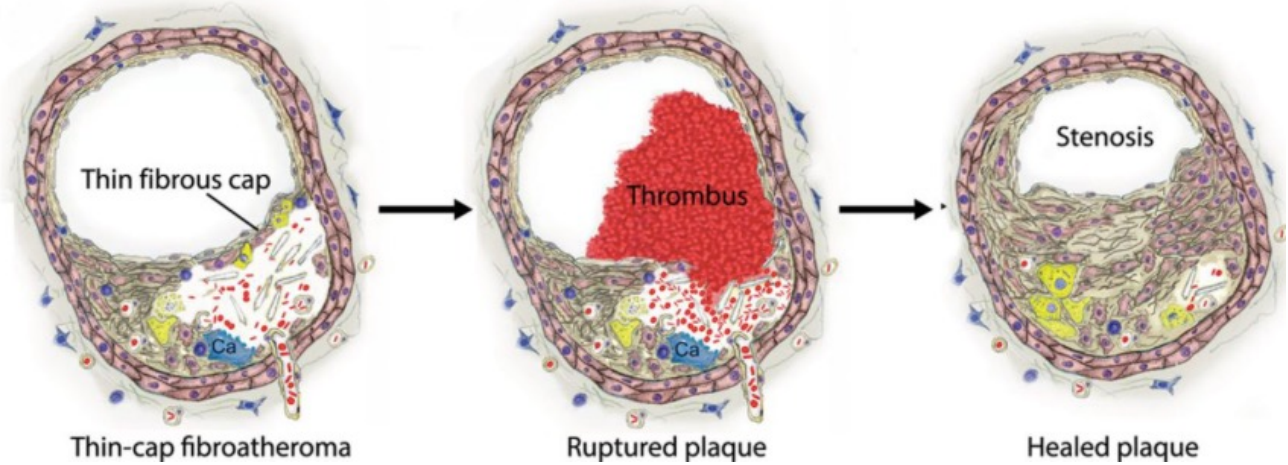
DO WHAT PATIENTS NEED NEXT !

Susan Sontag

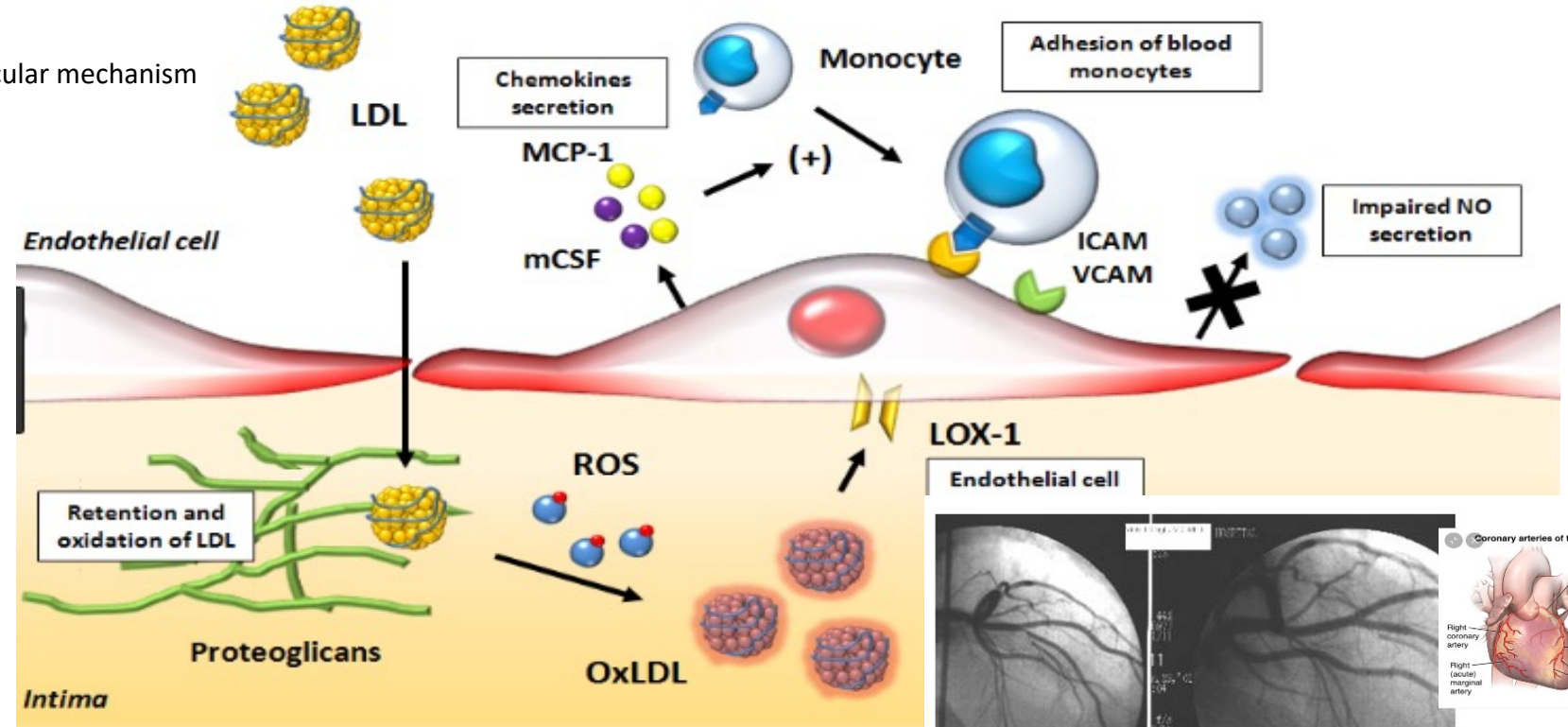
CVDs are the number 1 cause of death globally: more people die annually from CVDs than from any other cause.

Stroke and acute heart failure : the unstable atheroma plaque

at histopathological level



at molecular mechanism



The Making Of An Innovative Medicine – class schedule

Thursday's @ 4-6 PM except 04.12/11.12.25 @2-6 PM

Session 1: **Scope of the course _ general organization _ case study**
11.09.25 *Embracing a career at the heart of biomedical research !?*
CM013

Session 2: **Historical perspective: the modern pharmacy**
18.09.25 *Advent of modern medicines - placebo controlled drug development*
CM013

Session 3: **Introduction to translational research: crossing the bridge**
25.09.25 *A chasm has opened wide between biomedical research and patients in need*
CM013

Sessions 4-5: **Therapeutic target identification I & II**
02-09.10.25 *"me too" vs a wealth of innovative targets _ small MW cpds vs biologicals*
CM013 *Early front loading of biomarker identification for cohort stratification*

Session 6: **Structure based drug design _medicinal chemistry_low/high throughtput**
16.10.25 **screening assays_ multiple parallel optimization_ML-powered screens**
CM013 *Setting up screening assays, the robotics, the million cpds librairies*

Session 7: **Therapeutic modalities biologicals–peptides : today's - tomorrow's**
30.10.25 **pharmacy NBEs**
CM013 *Challengies (cost of goods - healthcare payers) and opportunities*

WORKSHOP LISTING - THE MAKING OF AN INNOVATIVE MEDICINE BIO 698-HS2025 in CM013			
! NON EXHAUSTIVE LISTING - SUGGESTIONS WELCOME !			
sessions		**workshops**	**speaker/s**
S02 (18-09-25)			
historical medicines		penicilin: impact, whose invention ?	
hopping on giant shoulders		prozac at the core of psychiatry	
		vaccine discovery:smallpoxJennerTodaymRNAvaccine	Eugenio
		artemisinin and malaria	
		insulin-Banting Best et al. beagle dog	
		slide51-X-ray image DNA--Rosalind Franklin	
		cyclosporin from soil sample to life saver	
S03 (25-09-25)			
translational research		expanding scope of translational therapies	
from bench to bedside and back		chronotherapy,circadian clock,sex,longevity	Solomon
		CAR-T, TCR-T cell therapies in "cold" tumors	
		Y chrom loss in immune cells drives cancer	
S04 (02-10-25)			
therapeutic target identification		th. target identification using a phenocopy screen	Justine
S05 (09-10-25)		nocosomial inf/MRSA/phage antibacterials	
therapeutic target identification		Crispr/Cas9 gene editing huntington disease	
		AI in drug discovery / ML-powered medicine	Lou
		AIDS - Lenacapavir : end of plague ?	
S06 (16-10-25)			
structure based drug design		macrocycles and non druggable targets	Benedikt
		chemoproteomics - NMEs	
		AIDS HIV from deadly virus to chronic disease	
S07 (30-10-25)			
therapeutic modalities - NBEs		rare diseases repurposing medicines	Jana
		biologics on the rise-MABs medicines & more	Eleni
		RNA therapeutics, antisense medicines	
		Wnt pathway - PROTACs vs molecular GLUEs	
S08 (06-11-25)			
PHC personalized healthcare		BRCA1/2 preventive surgery/tumor board	
Human genomics		4P medicine-GWAS-**P**ersonalized **H**ealth **C**are	Frederico
		disease enabling biomarkers/micro RNAs	
		AZ-biomarker BD-tau yet still no curative drug	
		centenarian host isoallo-LCA bile acid bacteria	

Workshops
The Making Of An Innovative Medicine
(today's class)

Prof O Naveiras

Prof J Shukry

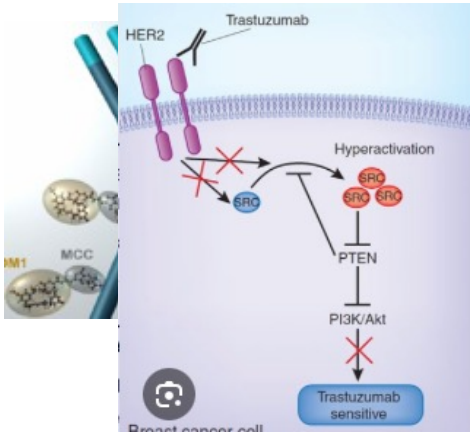
World Health Organization

Session 7 : biologicals – biotherapeutics : older and emerging therapeutic modalities

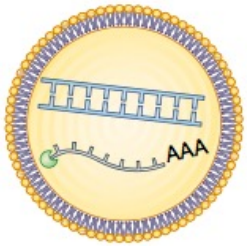
Health Topics

Home / Health topics / Biologicals

MABs



Peptides



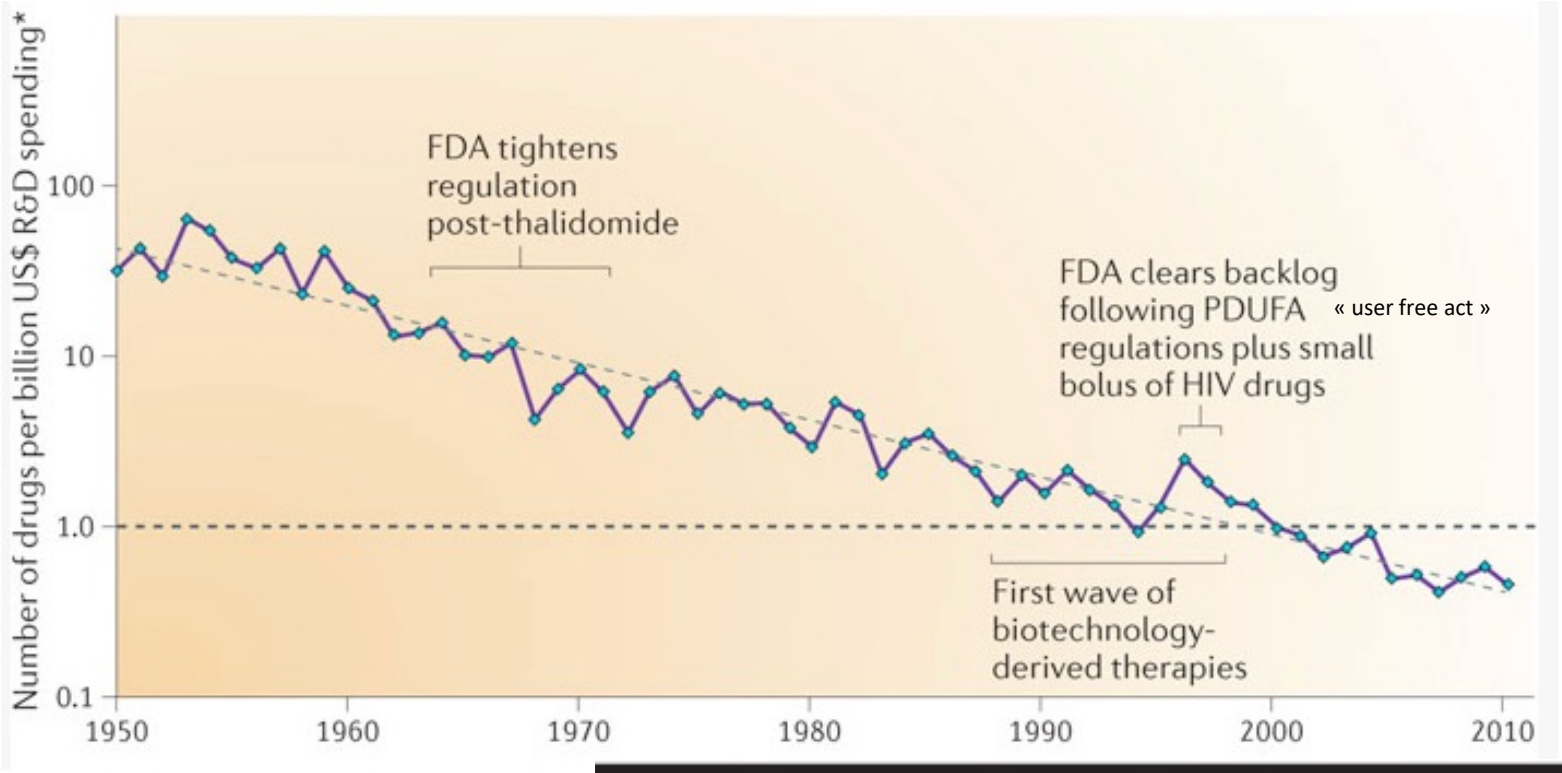
ASOs
(antisense oligonucleotides)
RNAs
(miRs, siRNAs etc)

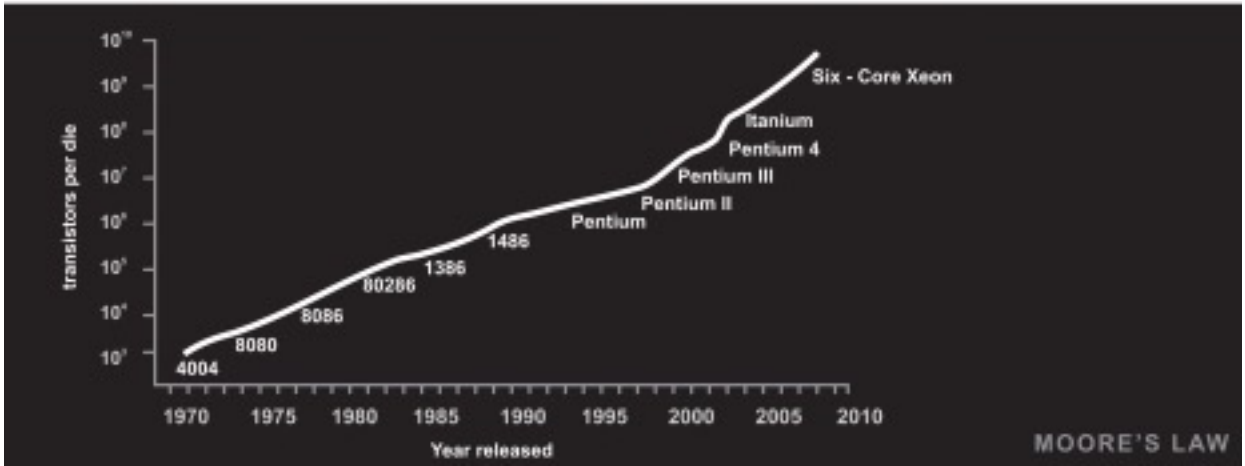
- Therapeutic peptides, eg. insulin, incretins, hormones
- Monoclonal antibodies mono, bi, tri-specific, armed
- Novel therapeutic modalities, DNA, RNA, Macrolides, non biologicals complex drug (NBCDs)

- Costs of goods NME's vs NBEs – healthcare payers ! ££$$

EROOM'S LAW
MOORE'S LAW

The biggest risk is NOT to get a medicine !
productivity declines – why ?

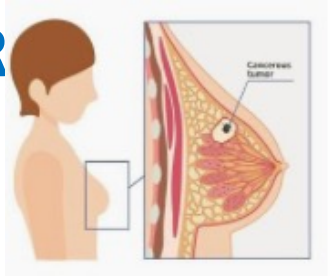




Disease biology_Molecular Biology_Drug discovery

- **DISEASE BIOLOGY STARTS WITH THE UNDERSTANDING OF THE UNDERLYING MOLECULAR MECHANISMS AT THE CAUSE OF THE DISEASE**

- **DISEASE BIOLOGY IS COMPLEX: SEVERAL THOUSANDS OF ELEMENTS ARRANGE IN A VERY INTRICATE MANNER**

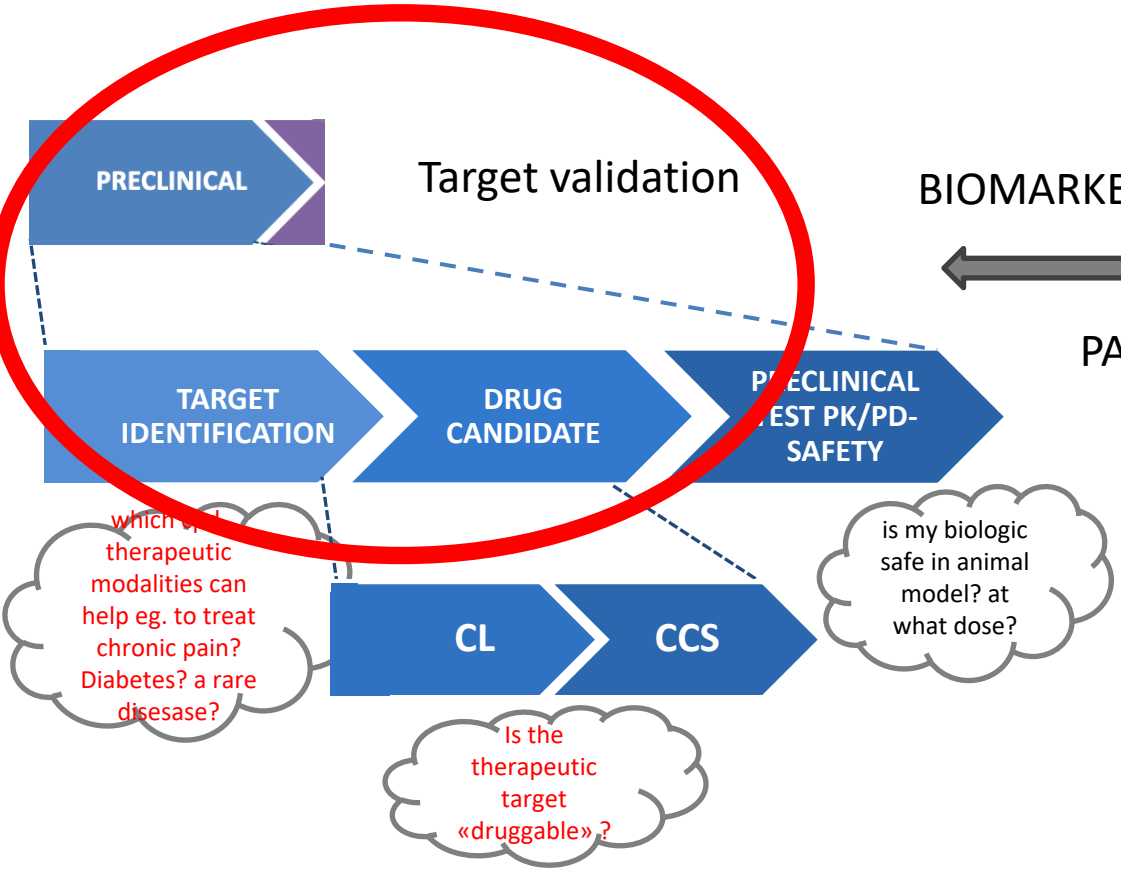


patients first !

- **ONCE THE BIOLOGICAL TARGET TO BE ADDRESSED HAS BEEN IDENTIFIED, MOLECULAR BIOLOGISTS START TO DESIGN A BIOLOGICAL WITH THE RIGHT CHARACTERISTICS TO BECOME A MEDICINE**

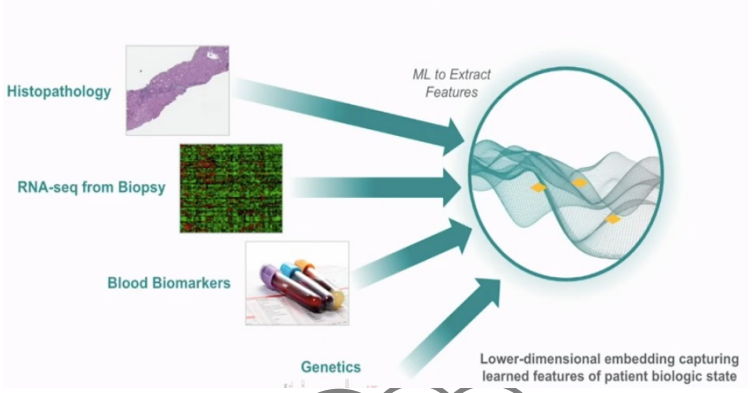
Moellering RE et al. 2002 Nature 462:182-188

Therapeutic modality selection

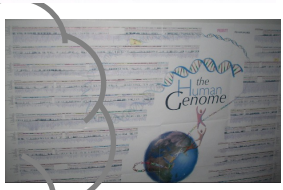


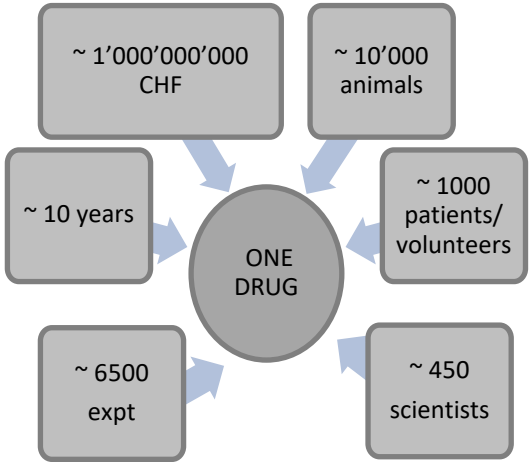
BIOMARKER

PATIENT

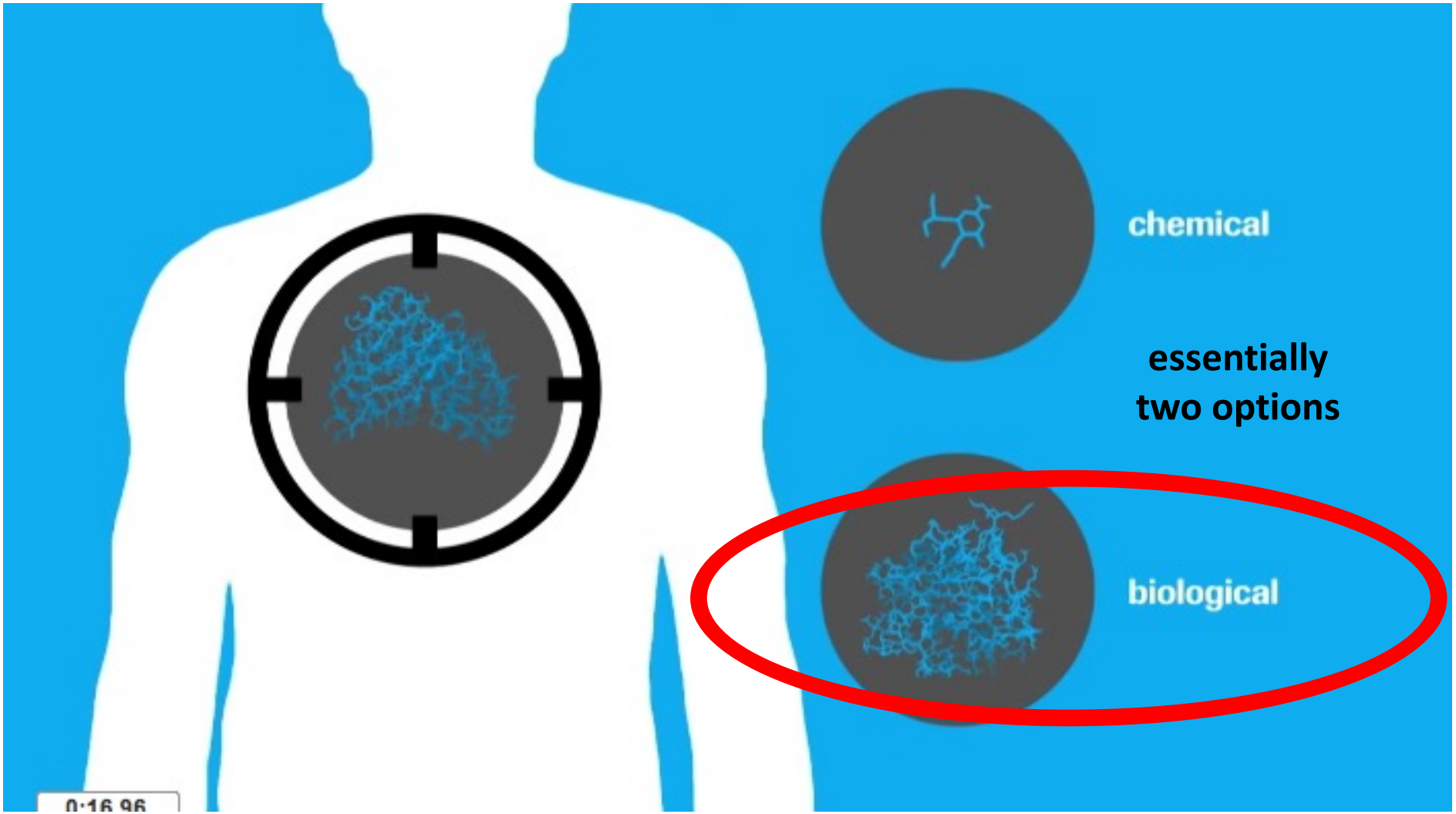


Do we have compelling genetic evidence underlying the symptoms/disease phenotype?

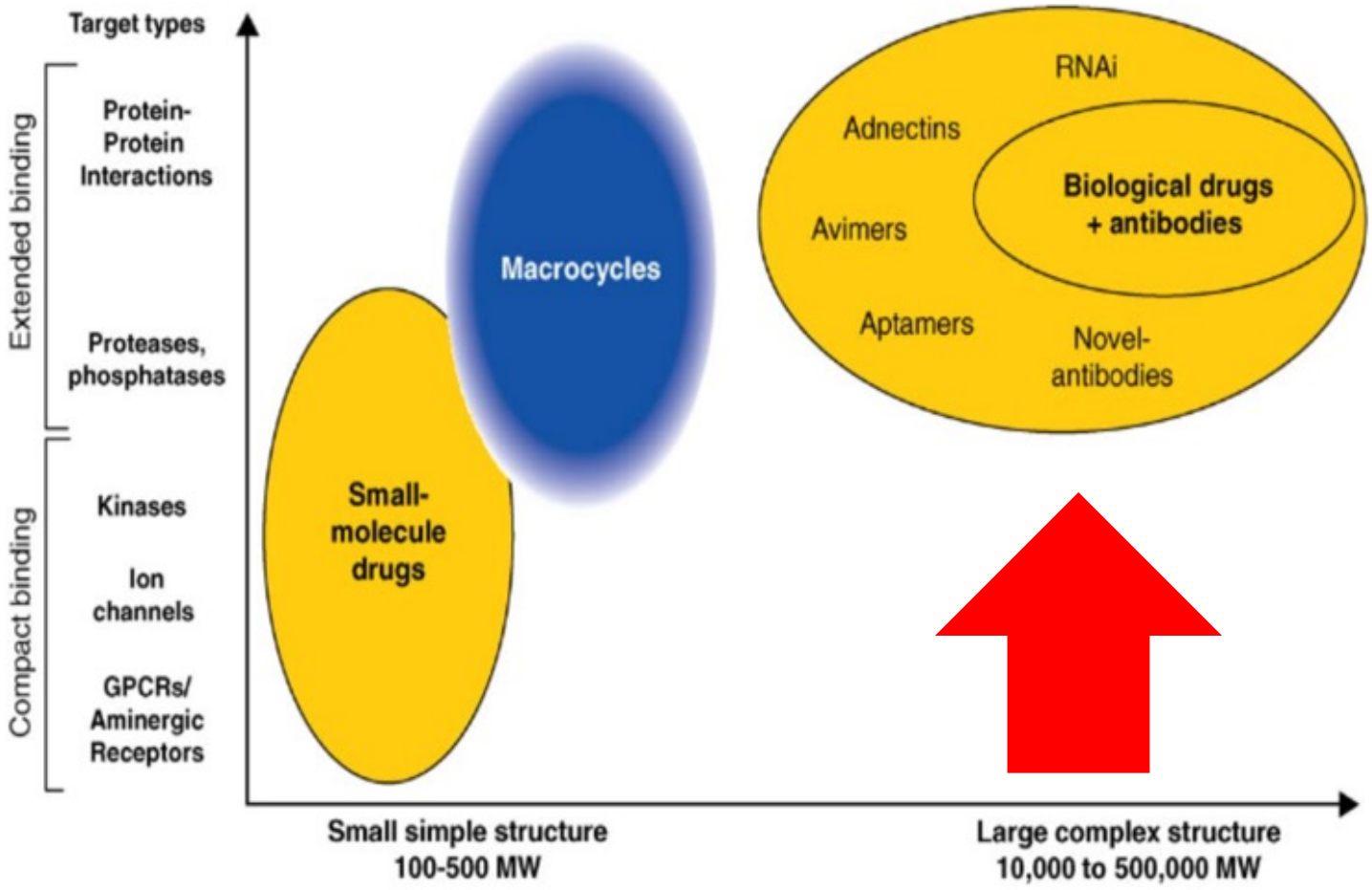




Adressing a novel therapeutic target : small chemical cpds versus large biological moieties : what‘s best ?



Large complex macromolecules for non small MW druggable targets



BIOLOGICALS - NBEs VERSUS CHEMICALS - NMEs

When a small M_R moiety (CHEMICALS) approach is not an option) eg large flat protein: protein interaction surfaces, then another therapeutic modality (BIOLOGICALS) eg. peptides, monoclonal antibodies or RNA, DNA is considered

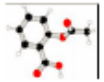
When eg. a druggable therapeutic target is known for its absence of a solvent accessible surface of an hydrophobic "pocket" peptide groove (enzyme, ion channel, receptor etc) invagination lined up with hydrophobic amino acid side chains

then candidates for other therapeutic modalities, such as biologicals, RNA and DNA moeities shall be considered

Only 3000 out of >25000 proteins encoded in the human genome possess a hydrophobic "pocket"!

MABs biotherapeutics and safety

Small molecules



- chemical synthesis
- single molecule species
- well defined structure, stable, half life hours
- MW < 500-1000 Da
- standard models for safety testing (2 species: rodent and non-rodent)
- non-immunogenic
- Typically given oral
- distribution extra- and intracellular
- metabolised
- efficacy and toxicity from parent and metabolite(s)

Antibodies

- biotechnology derived molecules
- Therapeutic mAb = mixture of molecule variants
- complex structure, heat sensitive, long half life
- 150'000 Da for IgGs
- high target selectivity & species specificity (for safety testing often only monkey cross-reacts)
- may be immunogenic
- Parenteral administration (i.v., s.c.)
- distribution extracellular in blood (low V_{ss})
- catabolised (proteolytic degradation)
- effects secondary to MoA and exaggerated pharmacology

Aspirin half life 1h ; mAB several weeks !

Biotherapeutics in drug research and clinical application

- WHAT ARE BIOTHERAPEUTICS ? Peptides, antibodies, DNA, etc
- PRODUCTS DERIVED FROM CHARACTERIZED CELLS THROUGH THE USE OF VARIOUS EXPRESSION SYSTEMS
- INCLUDING BACTERIA, YEAST, INSECT, PLANT, MAMMALIAN CELLS ETC
- THERAPEUTIC MONOCLONAL ANTIBODIES ARE THE LARGEST SUBGROUP OF BIOTHERAPEUTICS IN CLINICAL USE
- 2800 LISTED CLINICAL TRIALS CURRENTLY REFER TO MONOCLONAL ANTIBODIES

Immunoglobin as biotherapeutics on the rise

BIOLOGICALS are increasingly being developed as INNOVATIVE MEDICINE

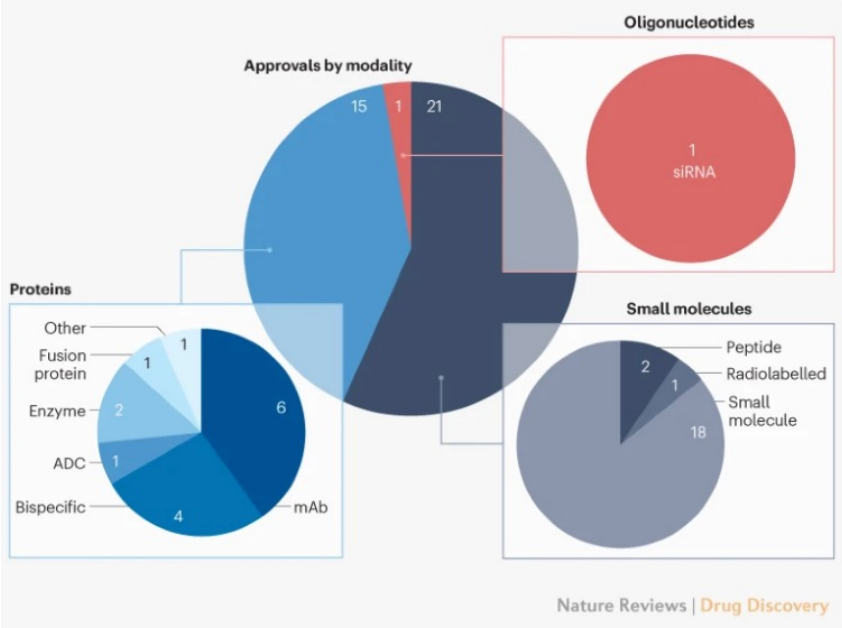


Fig. 3 | CDER approvals by modality. Small molecules, including peptides of up to 40 amino acids in length, and oligonucleotides are approved as new molecular entities (NMEs). Protein-based candidates

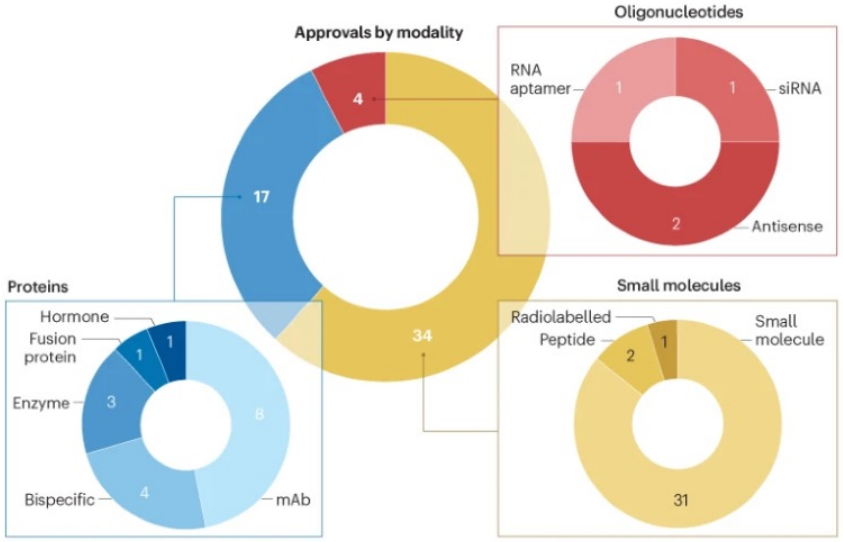


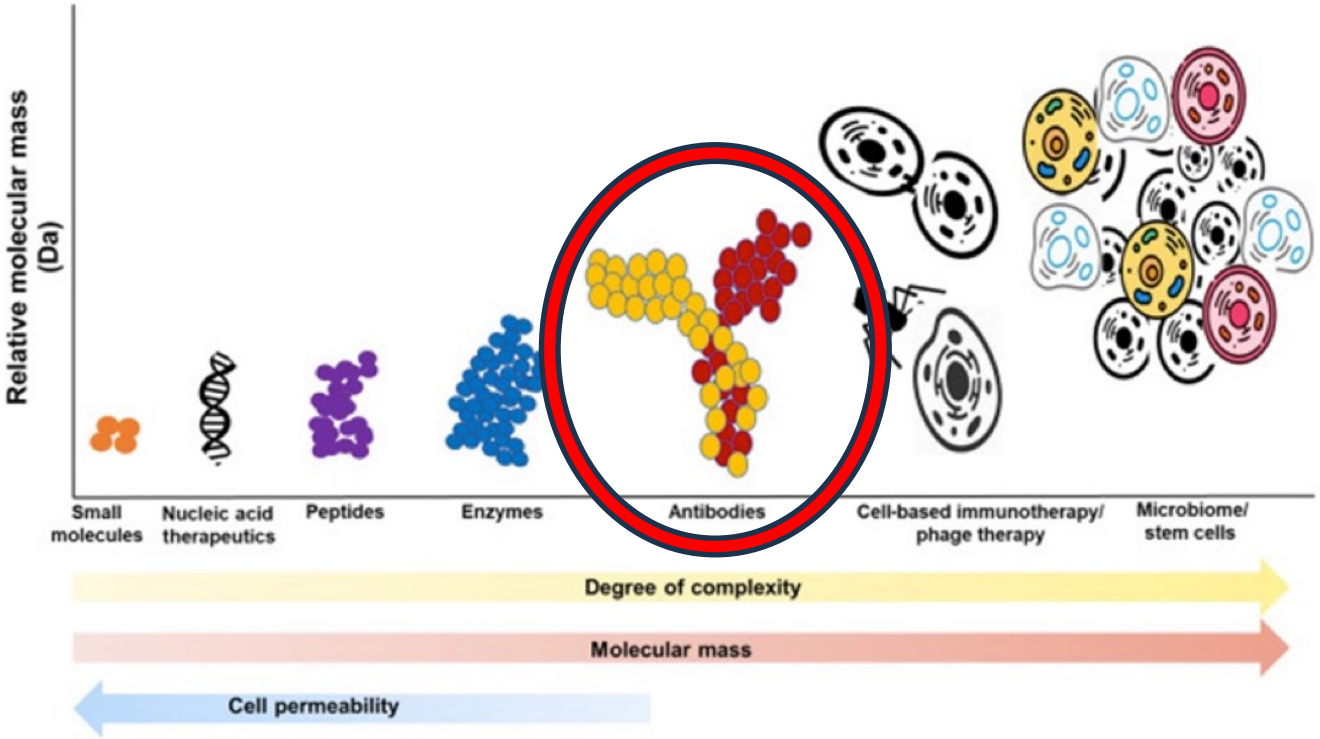
Fig. 3 | CDER approvals by modality. Small molecules, including peptides of up to 40 amino acids in length, and oligonucleotides are approved as new molecular entities (NMEs). Protein-based candidates are approved through biologics license applications (BLAs). mAb, monoclonal antibody; siRNA, small

2022 FDA approvals

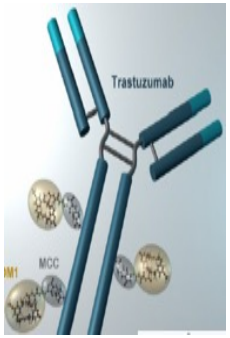
2023 FDA approvals

Nature Reviews Drug Discovery **22**, 83-88 (2023)

Degree of complexity of target modalites comes with challenges !

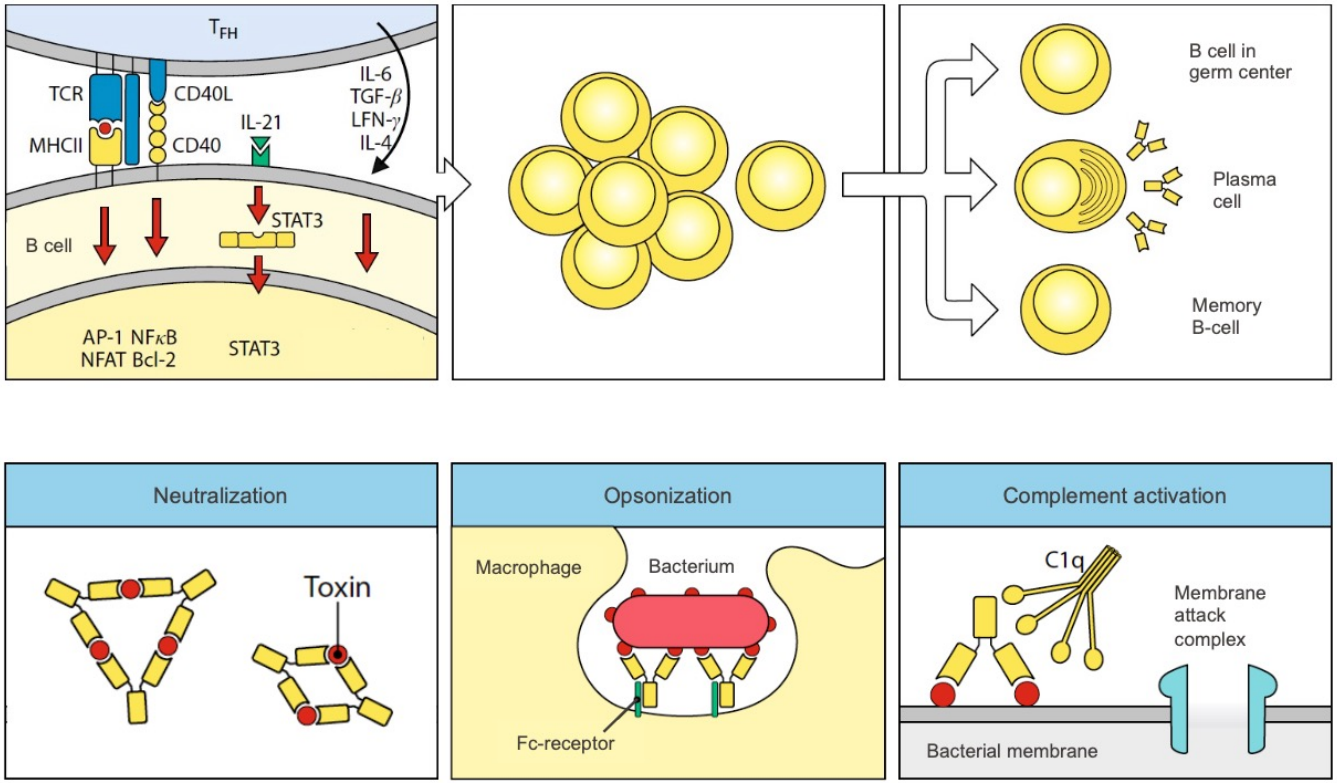


MABs



Features	Monoclonal Antibodies (mAbs)	Small Molecules
Size	**150 kilodaltons** (kDa)	**500 daltons**
Production	Produced using recombinant DNA technology in **living cells**, resulting in complex manufacturing processes.	**Chemically synthesized**, allowing for precise structural control.
Administration	Administered parenterally, usually via **intravenous** (IV) or **subcutaneous** (SC) injection	Can be taken **orally**, as they are typically absorbed through passive diffusion in the gastrointestinal tract.
Target specificity	**Highly specific** to a single epitope on their target antigen, minimizing off-target effects.	**May interact with multiple targets**, potentially leading to off-target effects
Mechanism of action	Bind to **extracellular targets**, such as cell surface receptors or soluble antigens, to modulate immune responses or block disease pathways	Can target both **intracellular and extracellular sites**, influencing various cellular processes.
Pharmacokinetics	**Exhibit long half-lives** (approximately 11–30 days in humans) and are cleared through linear and nonlinear processes. Low frequent dosing	Typically have **shorter half-lives**, necessitating more frequent dosing.

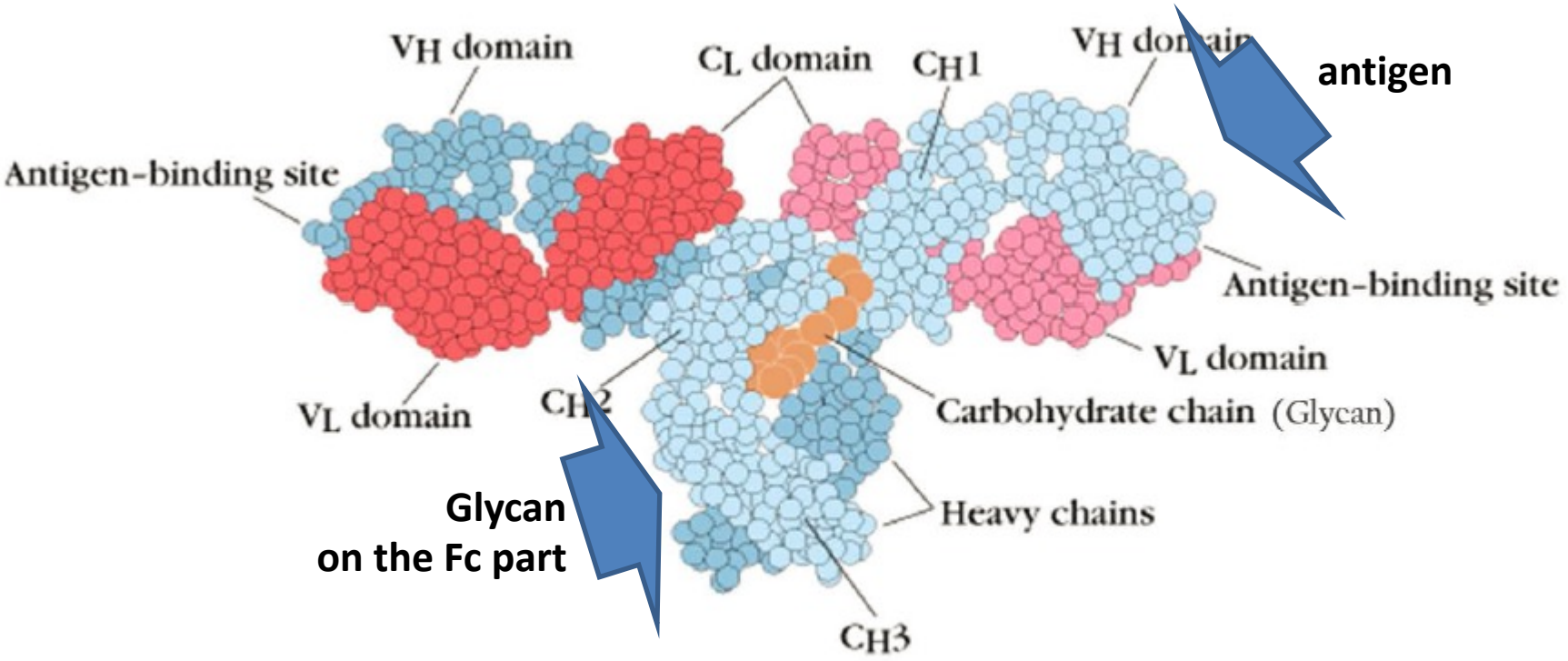
The adaptive humoral immune response



Murphy, K., & Weaver, C. (2018) Janeway Immunologie. 9th Edition (2018). Springer Berlin Heidelberg.

Monoclonal antibodies _ mABs

mABs _ monoclonal antibodies are a product from a fused B cell with a tumor cell (hybridoma)

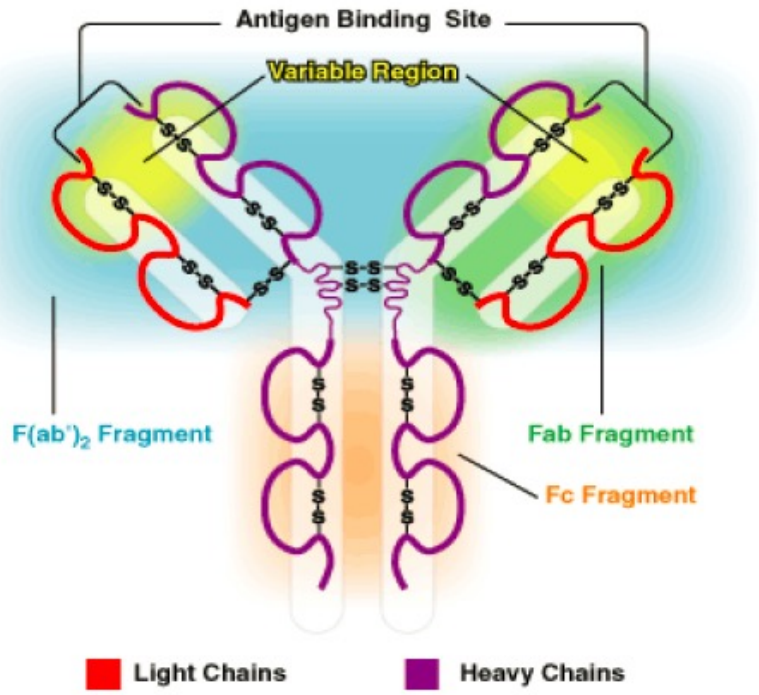


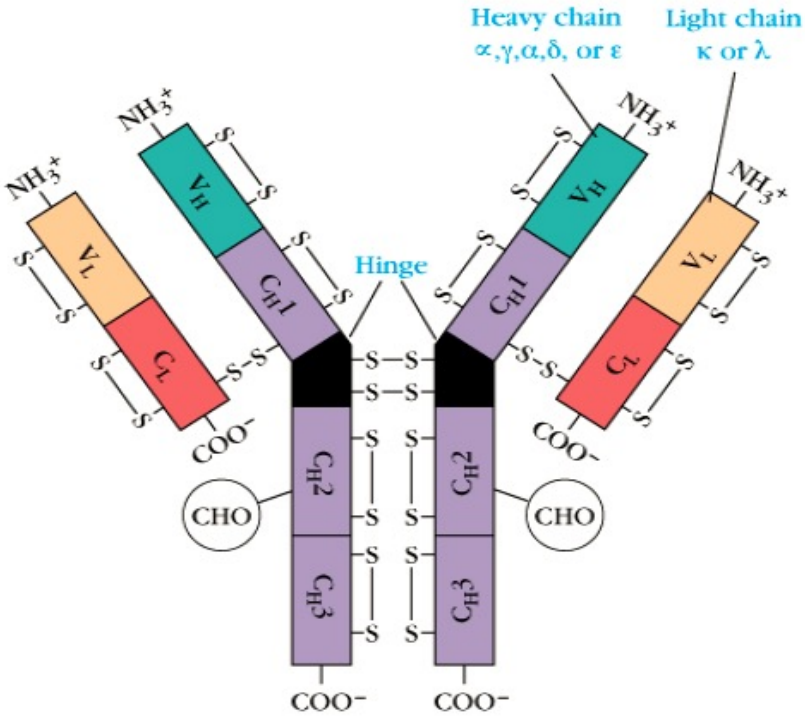
Immunglobulins are glycoproteins

Immunoglobin as biotherapeutics in various clinical setting

Immunglobulin G (IgG)

BIOLOGICALS are increasingly being developed as INNOVATIVE MEDICINE



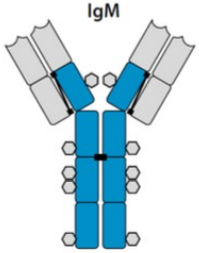


Immunglobulins consist of two heavy chains (μ, γ, α, δ, or ε, respectively), and two light chains (κ or λ)

S. Kronenberg 2017

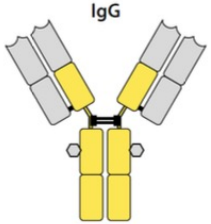
Several distinct classes of Immunoglobins
IgGs most prevalent biotherapeutics

IgM



- First soluble immunoglobulin during immune reaction
- Usually low affinity
- Pentamer
  → avidity
  → complement activation

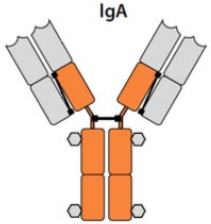
IgG



- Most prevalent human antibody
- Mediates immunity in extracellular space
- Requires class switch (IgM → IgG)

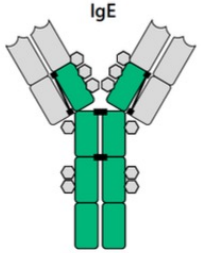
Therapeutic antibody format

IgA



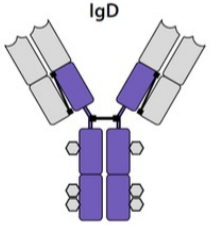
- Secreted antibody
- Mucosa-associated class (e.g. digestive tract)
- Can be monomer, dimer, or trimer

IgE



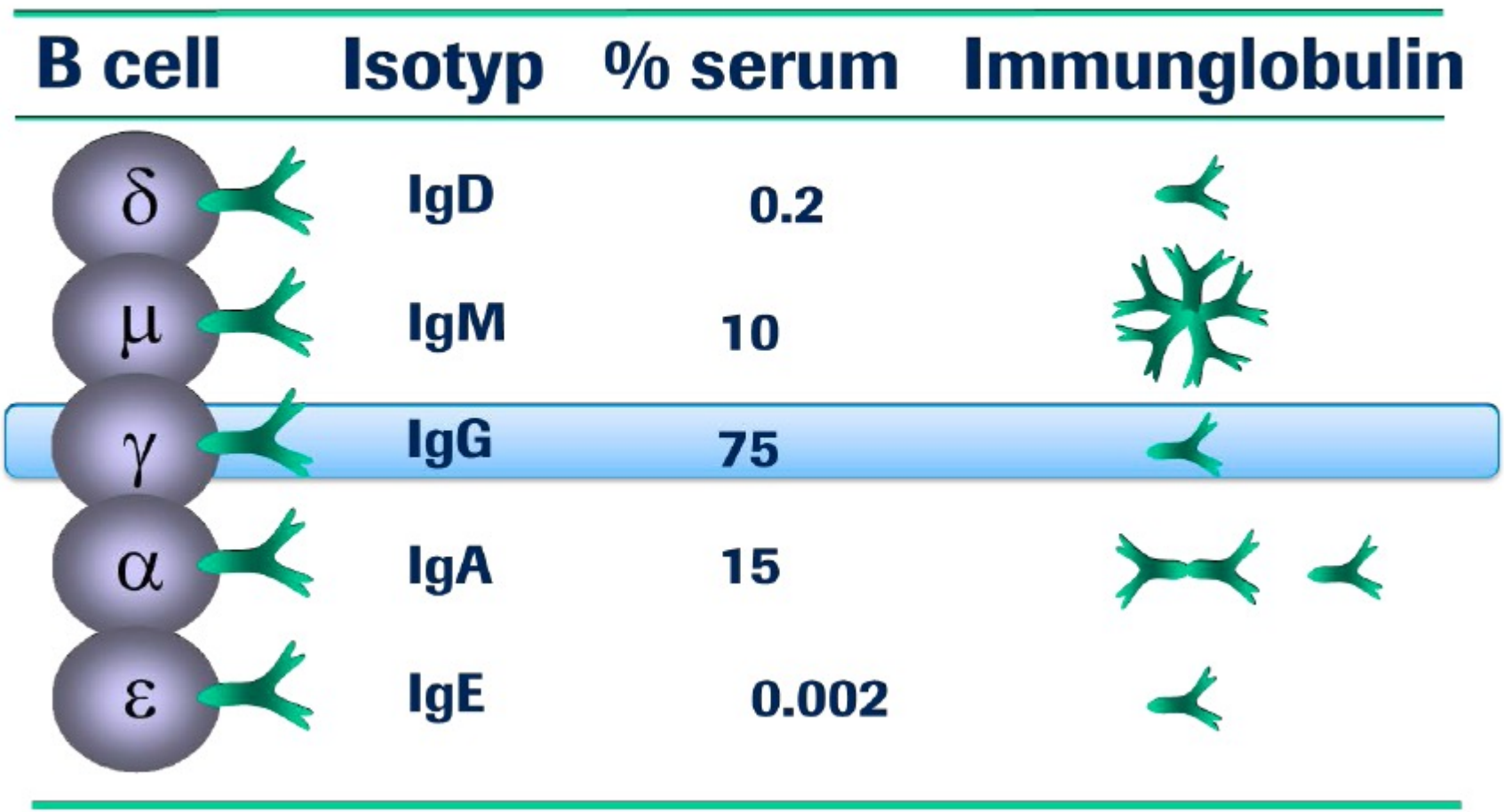
- Only low abundance
- Protection against parasites
- Associated with allergies

IgD

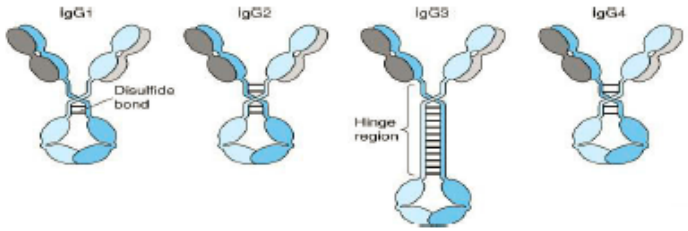


Funciton unclear

mABs in drug research



IgG1 is the most common in serum

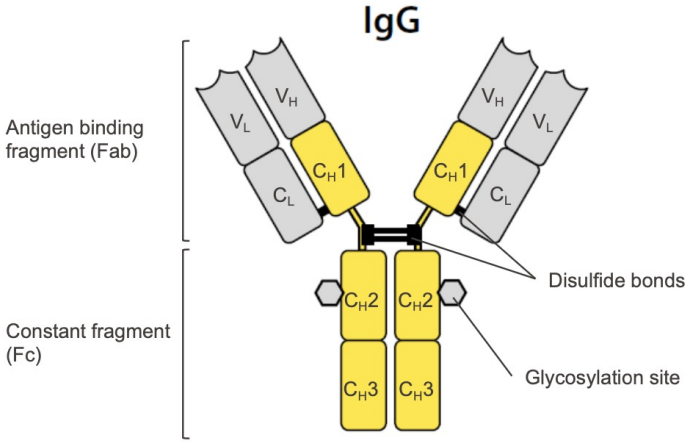


	IgG_1	IgG_2	IgG_3	IgG_4
Serum concentration (%)	60-65	20-25	5-10	<4
Serum half life (days)	21	20	7	21
Placental passage	++	±	++	++
Complement binding	+++	++	++++	±
FcγR binding	RI, RII, RIII	RII	RI, RII, RIII	
ADCC	+++	+	+++	±

antibody dependent cell cytotoxicity

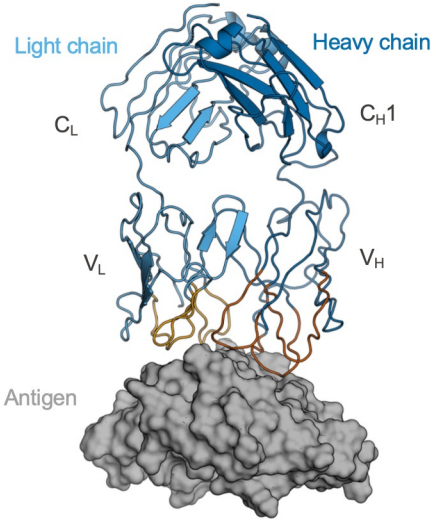
All 4 IgG subclasses are monomeric, but are different for their effector function which can be utilized for therapeutic mAbs

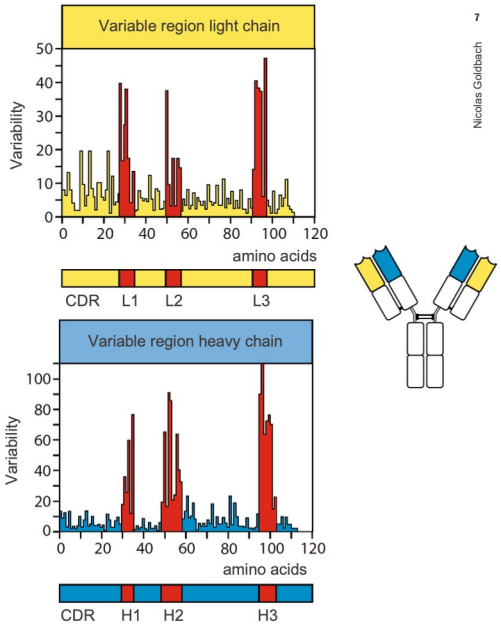
Several distinct classes of Immunoglobins
IgGs most prevalent biotherapeutics



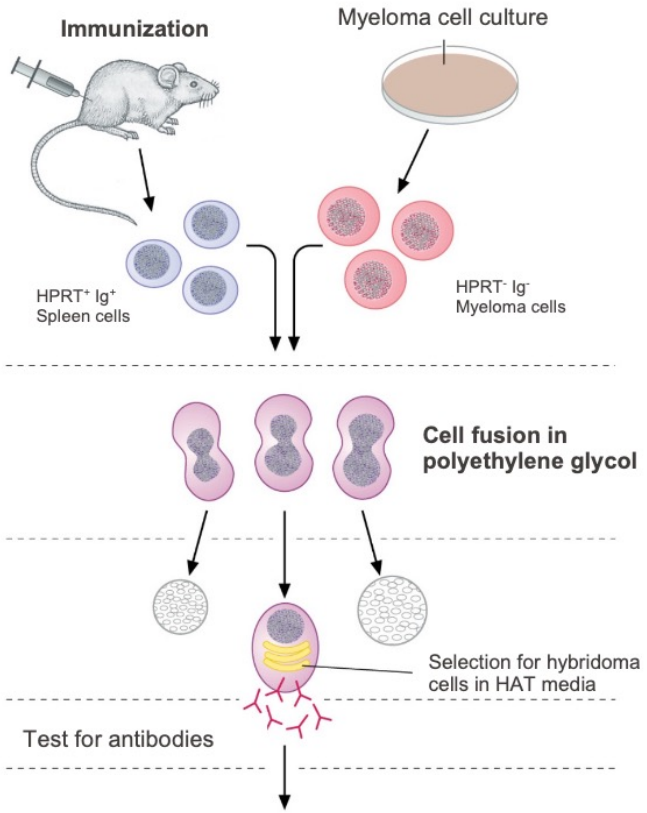
- IgG is a Y-shaped protein
- 2 light (L) and 2 heavy (H) chains
- Antibodies can be cleaved into antigen binding and constant fragments
- Constant (C) and variable (V) domains
- Stabilized by disulfide bnds
- Glycosylation on the Fc part

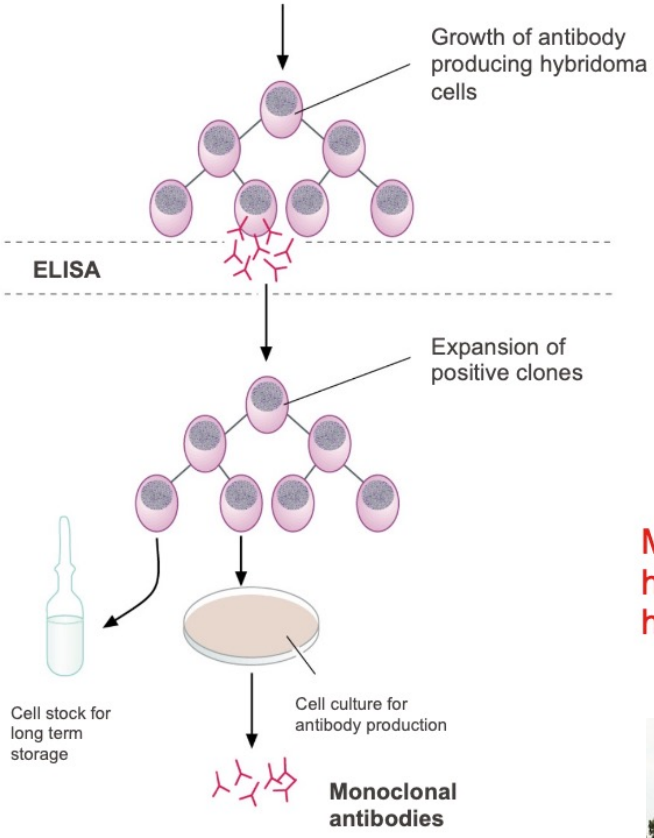
Fab: antigen binding





At the core of MABs : the Roche Basel Institute of Immunology : ”NL - hybridoma technology»





Murine antibodies are highly immunogenic to humans!

Köhler & Milstein, *Nature*, (1975), 495-497, 256(5517)

Basel Institute of Immunology

Basel, Switzerland

Connections Niels Jerne

Monoclonal antibodies _ mABs in new medicine development

Main therapeutic areas for mAbs & impact

- Cancer (~ 50%) (also CVD, AZ etc)
- Auto-immune diseases (~ 15%)
- > 2'800 clinical trials currently listed*

New biologics formats (antibody-based)

- Fc-engineered
- Antibody-drug conjugate
- Bispecifics /trispecific
- Trifunctionals (e.g. ADCC enhanced)

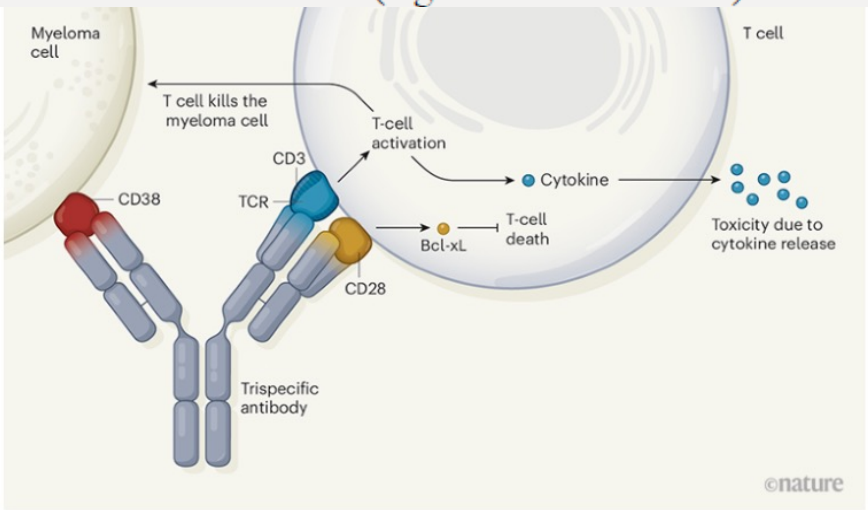
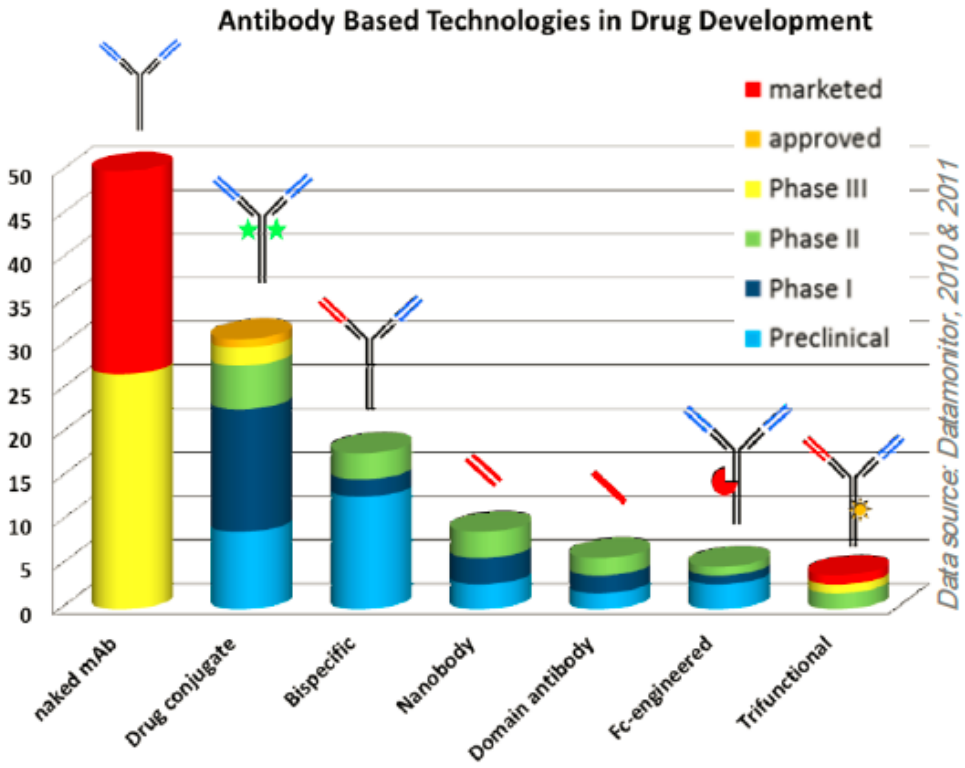


Figure 1 | An antibody that helps immune cells to target cancer cells. Wu *et al.*[1] report the

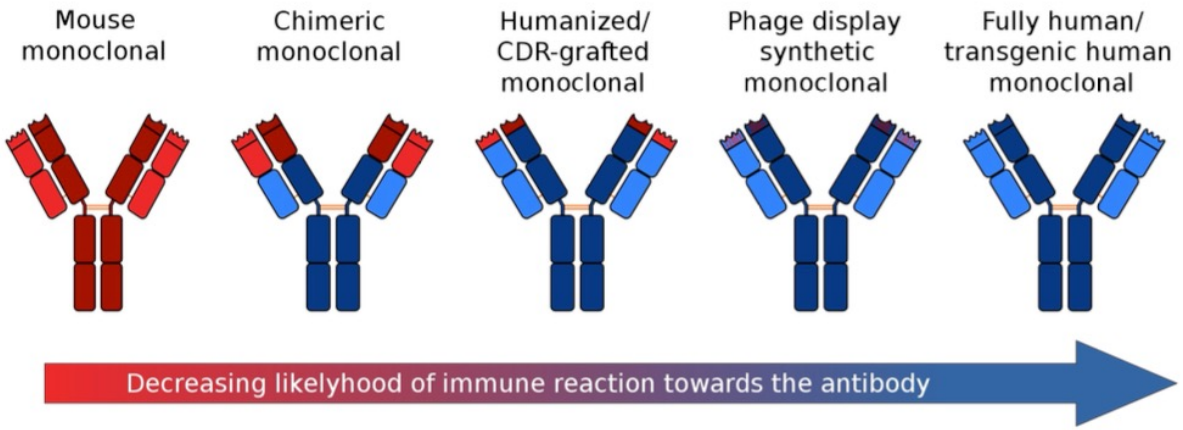


Wu et al (2020) Nature cancer 1:86-98

Tri-specific MABs enhance therapeutic efficacy through T cell receptor co-stimulation (blocking Bcl-x)

Humanization/fully human Immunoglobins have improved patient safety

(idiosyncratic immune reactions - anaphylactic shock)



- Chimeric: fusion of variable domains (e.g. from mouse antibody) with constant domains from human antibody
- Humanized: grafting (transferring) CDRs from mouse antibody to a human antibody
  - Selection of a human antibody closely matching the structure/sequence of the mouse antibody
  - Often results in reduced affinity (non-matching framework regions / vernier zones)
- Synthetic mAb: guided selection
- Humanization methods may be less important with the emergence of single B-cell sequencing for identification of fully human antibodies

Type d'Ac

Seules les IgG seront utilisées en thérapeutique.

- **Les anticorps murins (suffixe -omab)** sont des anticorps produits
utilisés comme agent thérapeutique chez l'homme. Aujourd'hui leur ut

- **Les anticorps chimériques (suffixe -ximab)** sont humains à 60%.
respectives (VH et VL) d'un anticorps murin.

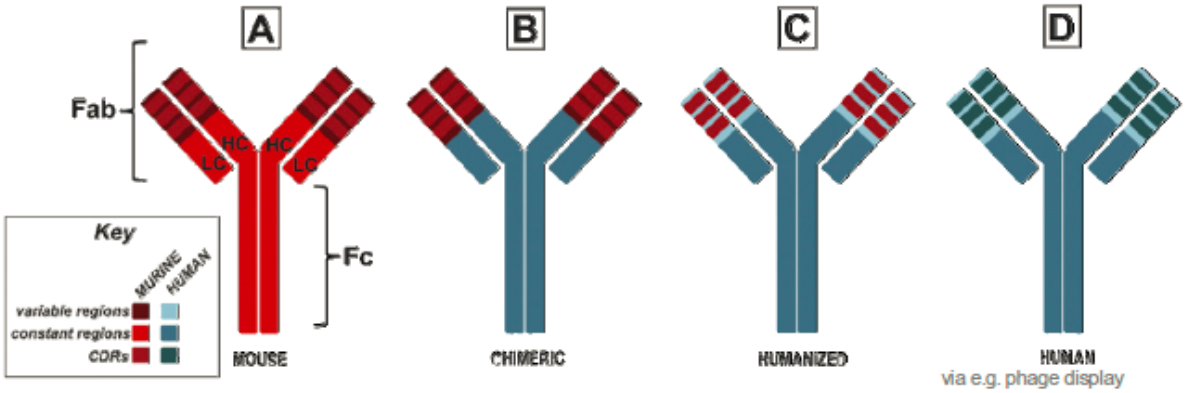
- **Les anticorps humanisés (suffixe -zumab)** sont humains à 90%.
humanisé est mieux toléré par l'organisme humain car ressemble plu

- **Les anticorps humains (suffixe -umab)**) sont humains à 100%. Ils
lorsque des anticorps chimériques et humanisés sont utilisés.

Le choix de l'**isotype** est important à considérer lorsque l'on veut utili
Il existe 4 isotypes d'IgG (IgG1 à IgG4). Les IgG1 sont les plus abon
d'IgG se différencient par le nombre de ponts S-S entre les chaînes l
disulfures intercaténaires.

mABs: murine, chimeric, humanized, human : towards safety

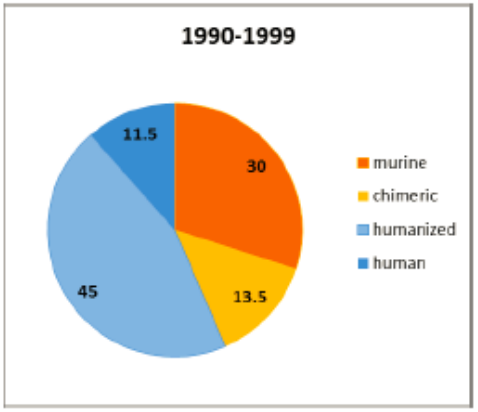
Classification of mAbs by level of humanisation

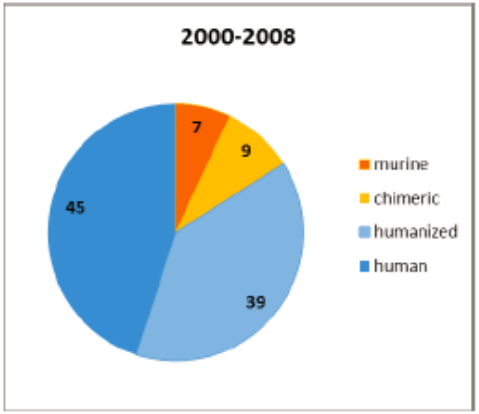


mAbs Nomenclature

Types of mAbs	Description	Nomenclature
Murine	Entirely murine amino acids	'o' = mouse e.g. murommonab
Chimeric	Human constant + murine variable regions	'xi' = chimeric e.g. rituximab
Humanized	Human except murine complementary determining regions (CDRs)	'zu' = humanized e.g. alemtuzumab
Human	Entirely human amino acids	'u' = humanized e.g. adalimumab

Types of Therapeutic mAbs in Pipeline (%)





Source: Nelson et al, Nature Rev, 2010

Biologicals - large scale production challengies

Pilot (1 liter fermenter)and large scale (1000 l scale) fermenters

Biotechnology production means tailor made downstream processing !

Biological generics (biosimilars) entered the market relentlessly, why ?

GMP large scale biological production

kg amounts = several 10^6 $$ costs !

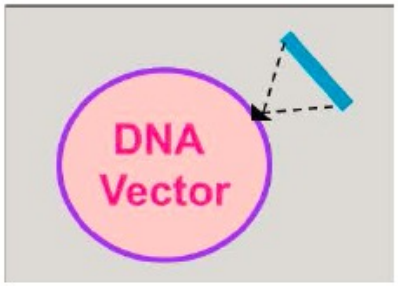
Cell lines (eg CHO) are reprogrammed with expression DNA vectors to ectopically express *bona fide* biologicals

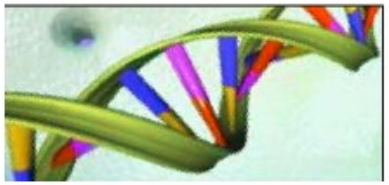
Custom designed posttranslational modifications (eg.glycoengineering) as well as humanized AA codons to improve T-cell recognition and reduce idiosyncratic reactions with patients (see Glycart Inc.- James Bailey)

Biologicals – challenges of large scale production

GMP PRODUCTION : A MULTISTEP PROCESS THAT REQUIRES STRICT CONTROLS

BIOTECHNOLOGY: KING IN THE KINGDOM OF UNCERTAINTY ?



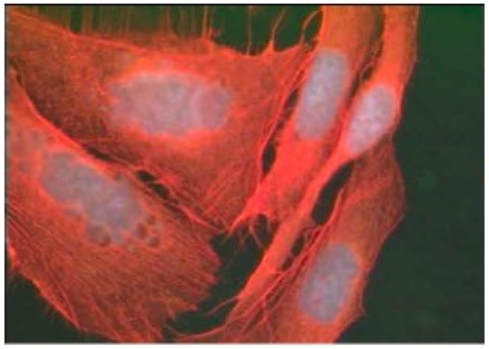


Cloning genes into
DNA Vector(s)

Downstreaming (purify)

Analytics

Transfer into Host Cell
for Expression

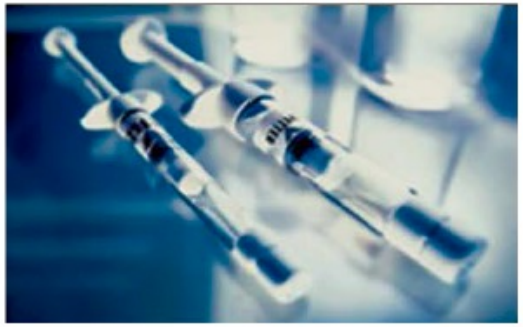


e.g., bacterial or mammalian cell

Large-Scale Fermentation

COST OF GOODS: who is going to support the financial burden of biologicals ?

Formulation

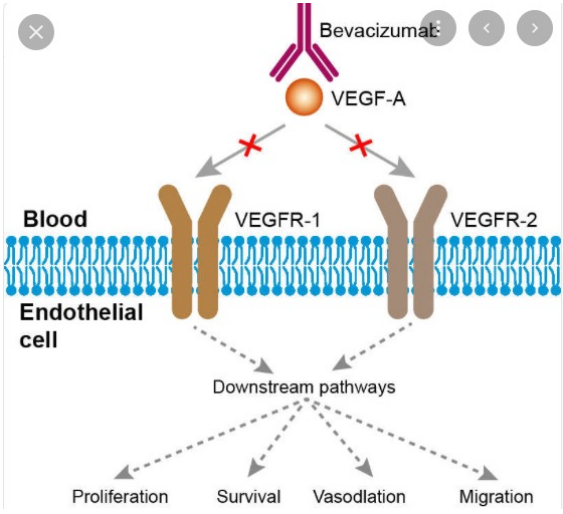


Biologicals - large scale production in Basel – Avastin production

100mg

GMP PRODUCTION OF AVASTIN (hVEGF MAB)

oncology
ophtalmology AMD
(controversy Lucentis)



Cancer patient life extension : average 4.7 months (phase 3 trials)
20’000.-US $ /y/patient
5mg/kg bi-monthly iv dose, 120mg/y

Biologicals - large scale production in Basel – Avastin production

100mg

GMP PRODUCTION OF AVASTIN (hVEGF MAB)

Controversy Lucentis vs Avastin off label

SANTÉ

Traitement de la DMLA : l'Autorité sanctionne 3 laboratoires pour des pratiques abusives

Publié le 09 septembre 2020 | Imprimer la page

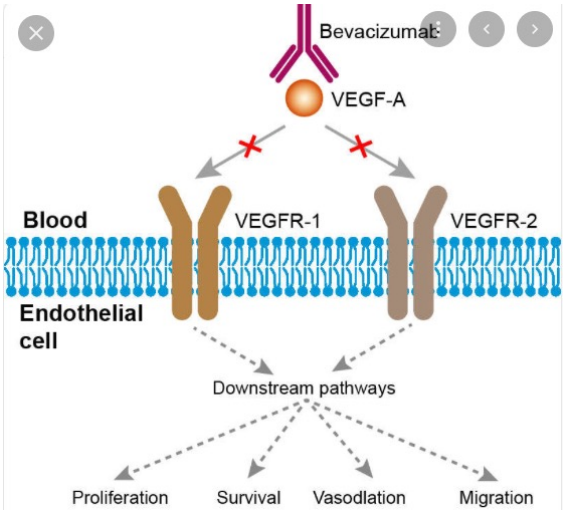
L'Autorité sanctionne les 3 laboratoires Novartis, Roche et Genentech à hauteur de 444 millions d'euros pour des pratiques abusives visant à préserver les ventes du médicament Lucentis pour le traitement de la DMLA au détriment d'Avastin (spécialité concurrente 30 fois moins chère).

L'essentiel

La dégénérescence maculaire liée à l'âge (DMLA), est la principale cause de malvoyance chez les sujets de plus de 50 ans dans les pays industrialisés. Elle entraîne une altération sévère de la vision centrale, qui se présente notamment sous la forme de taches sombres perçues par le patient au milieu de sa vision.

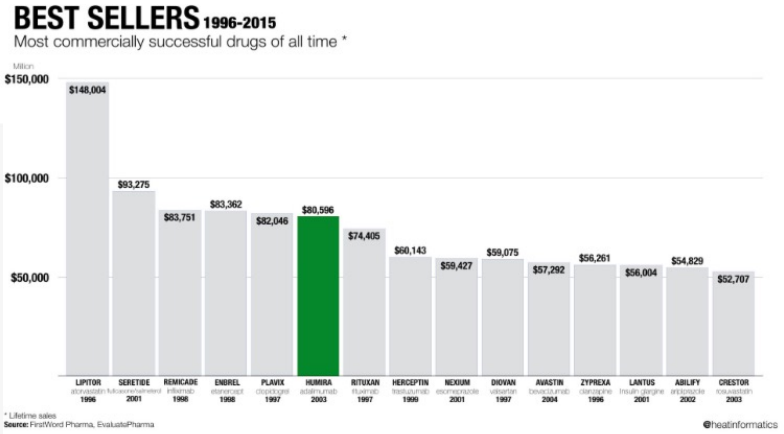
Le laboratoire Genentech a développé un médicament, le Lucentis, traitant la DMLA. Il

oncology
ophtalmology AMD
(controversy Lucentis)



More pioneer champions in Mabs biologicals

- Avastin : **entirely humanized anti VEGF** monoclonal
- Rituxan : **anti CD20** non Hodgkin's lymphoma
- Herceptin : **anti Her2** monoclonal
- and more…
- resistance : non responders !
- Patient costs : 5,000 US $ a month



Les AcM à usage thérapeutique

- Abatacept (ORENCIA®)
- Abciximab (REOPRO®)
- Adalimumab
- Aflibercept (ZALTRAP®, EYLEA®)
- Alemtuzumab (LEMTRADA®)
- Alirocumab (PRALUENT®)
- Anakinra (KINERET®)
- Atezolizumab (TECENTRIQ®)
- Avelumab (BAVENCIO®)
- Basiliximab (SIMULECT®)
- Belatacept (NULOJIX®)
- Belimumab (BENLYSTA®)
- Bevacizumab
- Beztoloxumab (ZINPLAVA®)
- Brentuximab vedotin (ADCETRIS®)
- Brodalumab (KYNTHEUM®)
- Burosumab (CRYSVITA®)
- Canakinumab (ILARIS®)
- Catumaxomab (REMOVAB®)
- Certolizumab pegol (CIMZIA®)
- Cetuximab (ERBITUX®)
- Daclizumab (ZINBRYTA®)
- Daratumumab (Darzalex®)
- Denosumab (PROLIA®, XGEVA®)
- Dinutuximab beta (QARZIBA®)
- Dupilumab (DUPIXENT®)
- Eculizumab (SOLIRIS®)
- Elotuzumab (Empliciti®)
- Erenumab (AIMOVIG®)
- Etanercept
- Idarucizumab (PRAXBIND®)
- Infliximab
- inotuzumab ozogamicin (BESPONSA®)
- Ipilimumab (YERVOY®)
- Ixekizumab (TALTZ®)
- Mepolizumab (NUCALA®)
- Natalizumab (TYSABRI®)
- Nivolumab (OPDIVO®
- Obinutuzumab (GAZYVARO®)
- Ofatumumab (ARZERRA®)
- Olaratumab (LARTRUVO®)
- Omalizumab (XOLAIR®)
- Palivizumab (SYNAGIS®)
- Panitumumab (VECTIBIX®)
- Pembrolizumab (KEYTRUDA®)
- Pertuzumab (PERJETA®)
- Ramucirumab (CYRAMZA®)
- Ranibizumab (LUCENTIS®)
- Reslizumab (CINQAERO®)
- Rituximab
- Sarilumab (KEVZARA®)
- Secukinumab (COSENTYX®)
- Siltuximab (SYLVANT®)
- Tildrakizumab (ILUMETRI®)
- Tocilizumab (ROACTEMRA®)
- Trastuzumab
- Trastuzumab emtansine (KADCYLA®)
- Trastuzumab (HERCEPTIN®)
- Ustekinumab (STELARA®)
- Vedolizumab (ENTYVIO®)

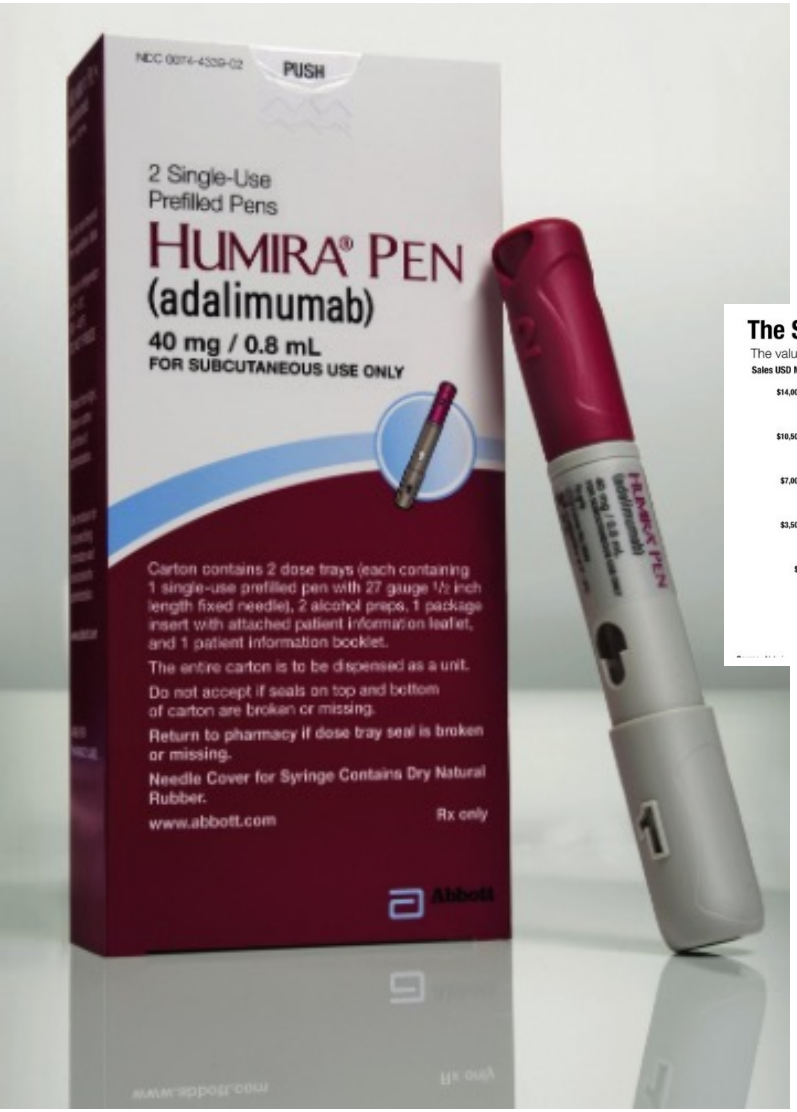
mABs in clinical practice oncology, neurology, inflammation (01. 2019)

Onkologie-Marktführer

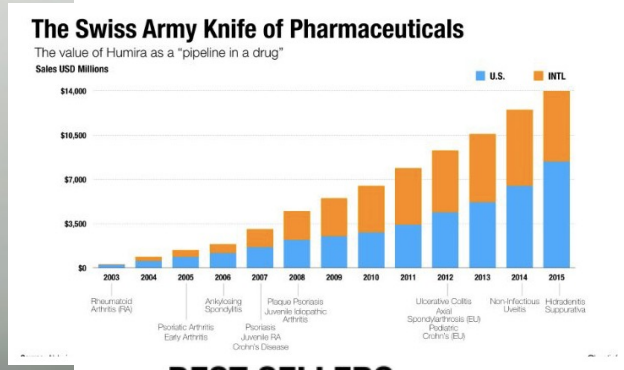
Umsätze 2017 mit Krebsmedikamenten in Milliarden US-Dollar		Veränderung zu 2016, in %
Roche	26,2	+3,7
Bristol-Myers + Celgene	8,4 + 10,8 = 19,2	+18,6
Novartis	12,3	–4,1
Johnson & Johnson	7,3	+25,0
Pfizer	6,1	+32,7
Merck & Co	4,6	+107,5
Astra-Zeneca	4,0	+19,1
Eli Lilly	3,5	–3,3
Abbvie	3,4	+28,2

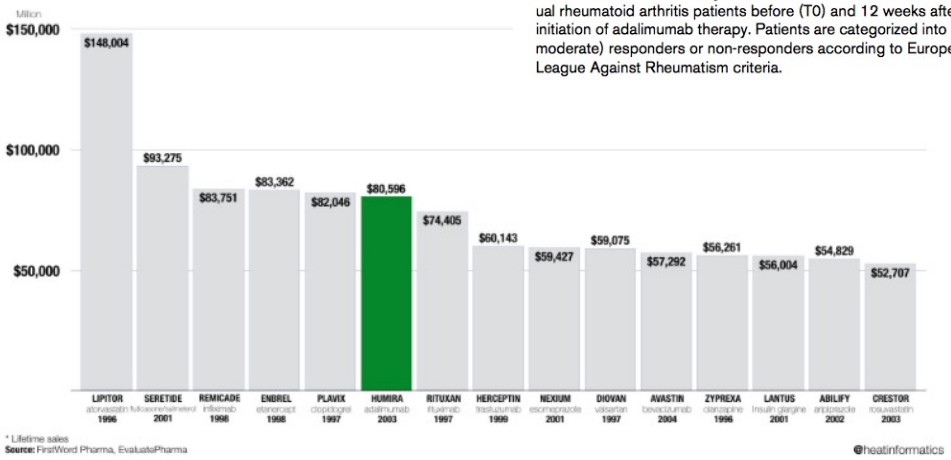
Grafik: ake / Quelle: «Handelsblatt»

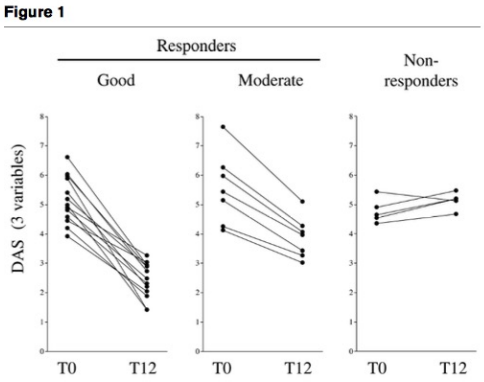
Anti TNF : rhumatoid arthris-Crohn bowel disease-ulcerative collitis



- Adalimumab : **entirely humanized** monoclonal antibody to TNFalpha cytokine
- Double response rate of methothrexate
- Resistance : non responders to adalimumab !
- Patient costs : 5,000 US $ a month

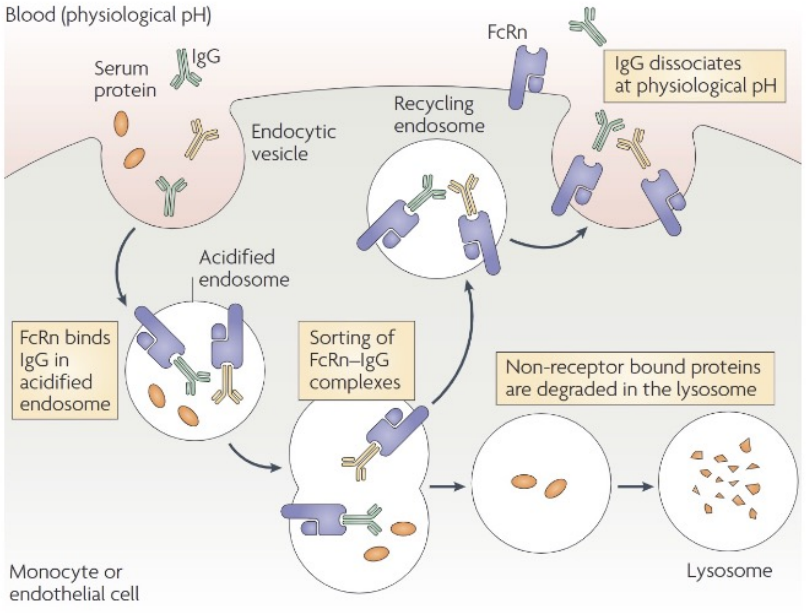


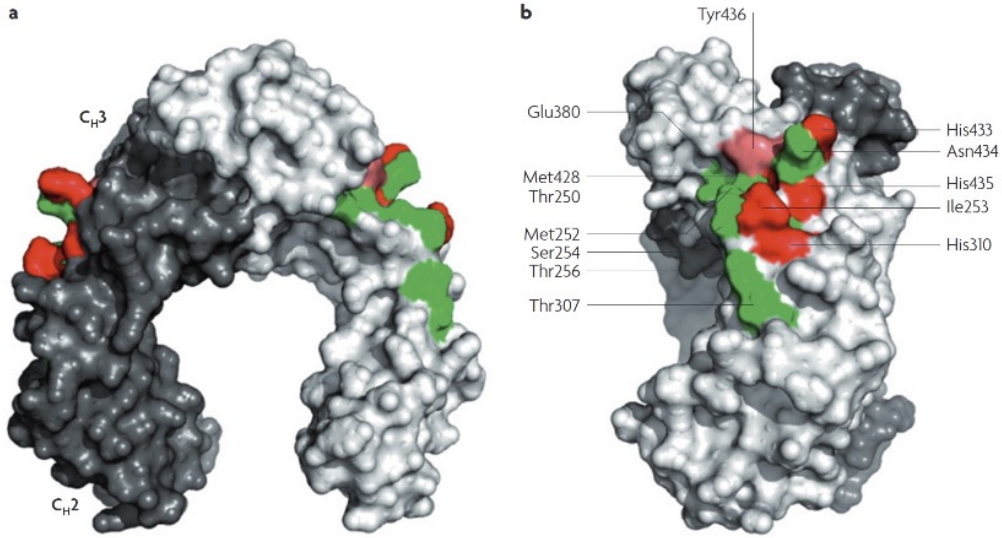




Evolution of disease activity score (DAS) (three variables) in 25 individual rheumatoid arthritis patients before (T0) and 12 weeks after (T12) initiation of adalimumab therapy. Patients are categorized into (good or moderate) responders or non-responders according to European League Against Rheumatism criteria.

The neonatal Fc receptor FcRn is unable to present AG but binds IgGs and albumin at low pH and regulates half lifes of both proteins





pH dependent binding of IgG Fc is mediated through histidine residues 310 and 435 located the C_H2 domain

→ Most (but not all) antibodies, which can bind to FcRn have an increased half-life in blood plasma

FcRn neonatal receptor belongs to a divergent family of MHC molecules

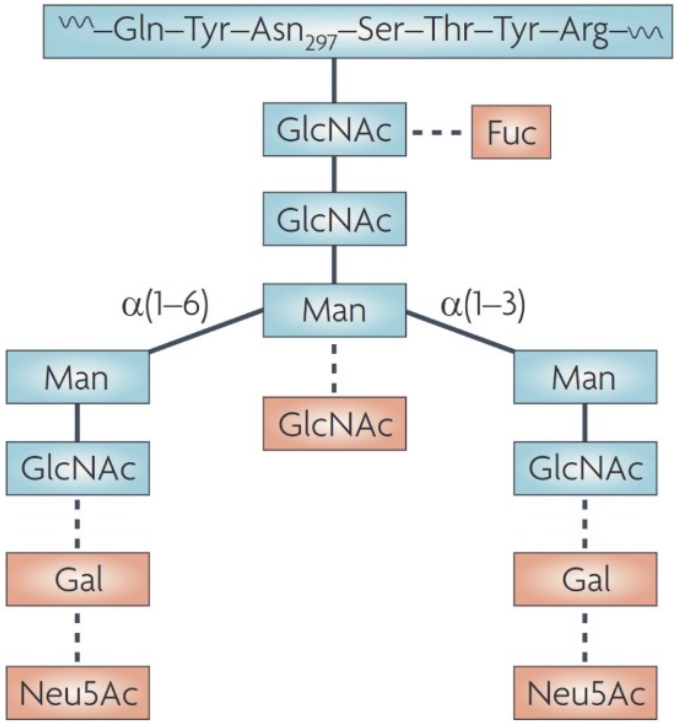
Nature Reviews Immunology, (2007), 715-725, 7(9)

Goal: mimic glycosylation of MABs to enhance efficacy and increase plasma exp

- IgG carries glycolylation at Asn297 in the Fc part
- Glycosylation is of complex diantennary type
- 20% of IgG glycosylated, about 128-512 glycoforms!
- Glycosylation is dependent on the enzyme repertoire of the producing cell

- terminal α-2,6-linked sialic acid
  → anti-inflammatory activity
- CHO, NS0, Sp2/0 cells: >90% fucosylated IgG
  → decreased ADCC

- Roche GlycoMAB: Cell line for recombinant expression of non-fucosylated antibodies



nAbs, (2012), 419-425, 4(4) & Nature Reviews Drug Discovery, (2009), 226-234, 8(3)

Glycosylation pattern may influence the biology and therapeutic index of mABs

Goal: mimic glycosylation of MABs to enhance efficacy and increase plasma exp

RESEARCH

GLYCART biotechnology

Engineered glycoforms of an antineuroblastoma IgG1 with optimized antibody-dependent cellular cytotoxic activity

Pablo Umaña, Joël Jean-Mairet, Radmila Moudry[1], Hanspeter Amstutz[1], and James E. Bailey*

*Institute of Biotechnology, ETH Zürich, CH-8093, Zürich, Switzerland. [1]ZLB Central Laboratory, I CH-3000 Bern 22, Switzerland. *Corresponding author (e-mail: bailey@bi*

Received 26 June 1998; accepted 1 December 1998

The glycosylation pattern of chCE7, an antineuroblastoma chimeric Ig hamster ovary cells with tetracycline-regulated expression of β(1,4)-*N*-ac (GnTIII), a glycosyltransferase catalyzing formation of bisected oligosacch ed in antibody-dependent cellular cytotoxicity (ADCC). Measurement of t duced at different tetracycline levels showed an optimal range of GnTIII e vitro ADCC activity, and this activity correlated with the level of consta complex oligosaccharides determined by matrix-assisted laser desorptio spectrometry. The new optimized variants of chCE7 exhibit substantial AD useful for treatment of neuroblastoma. The strategy presented here shou ADCC activity of other therapeutic IgGs.

Keywords: glycosylation, effector function, antitumor antibody,

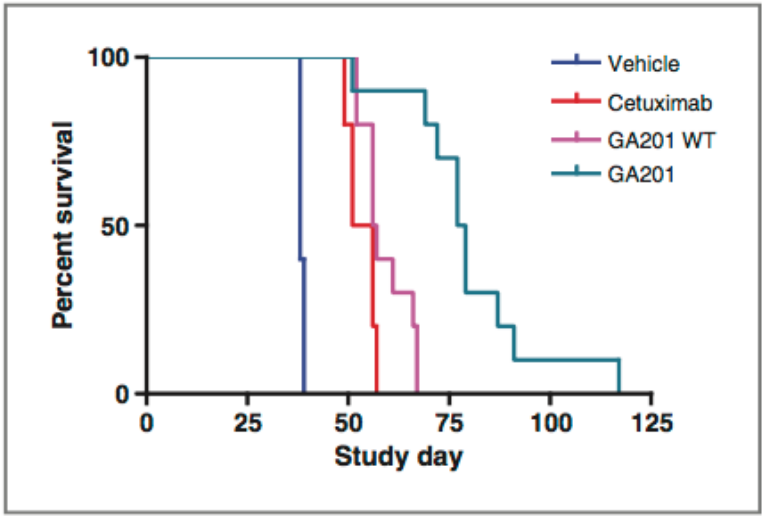
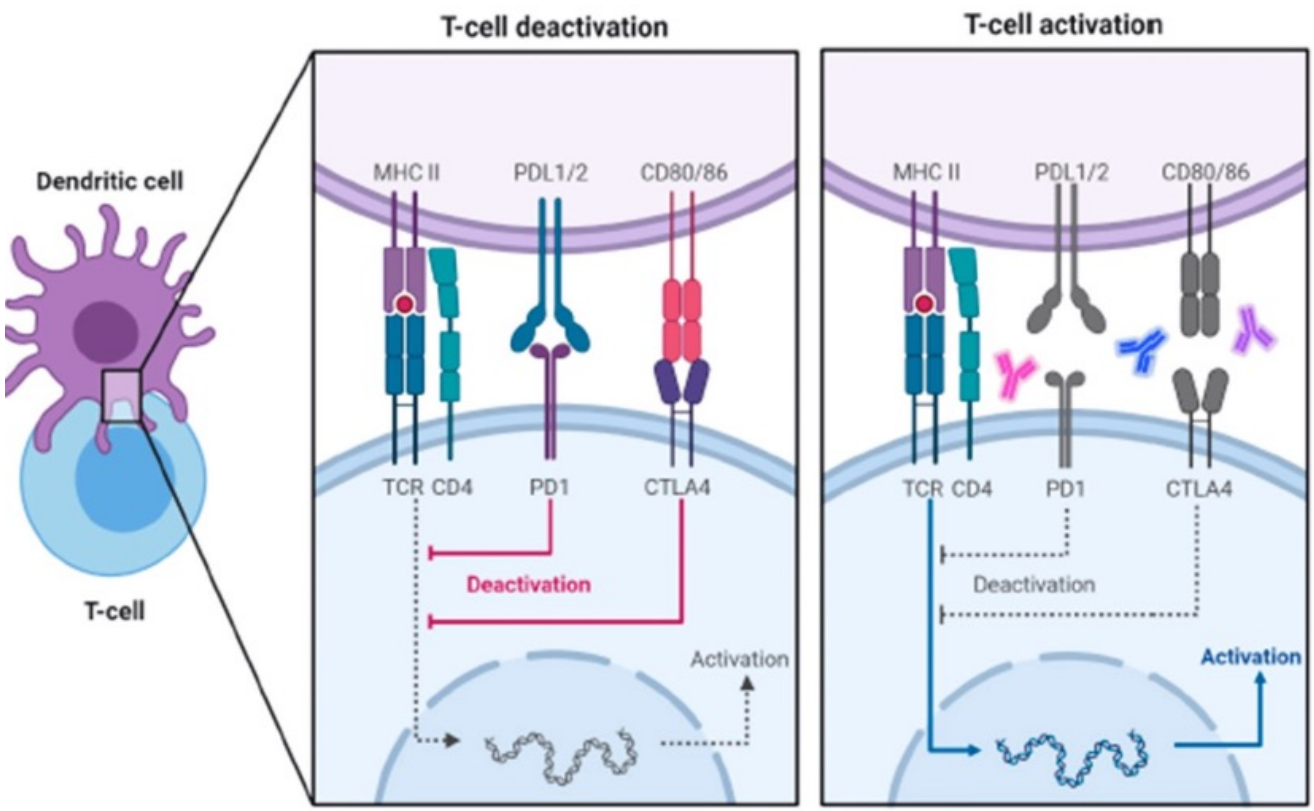


Figure 2. Superior efficacy of GA201 versus cetuximab and the wild type, nonglycoengineered version of GA201 in the A549 lung adenocarcinoma xenograft model in SCID/beige mice. All animals ($n = 10$ per

Glycosylation pattern may influence the biology and therapeutic index of mABs

Distinct immune check point blockers
or how to boost immunity against tumor cells

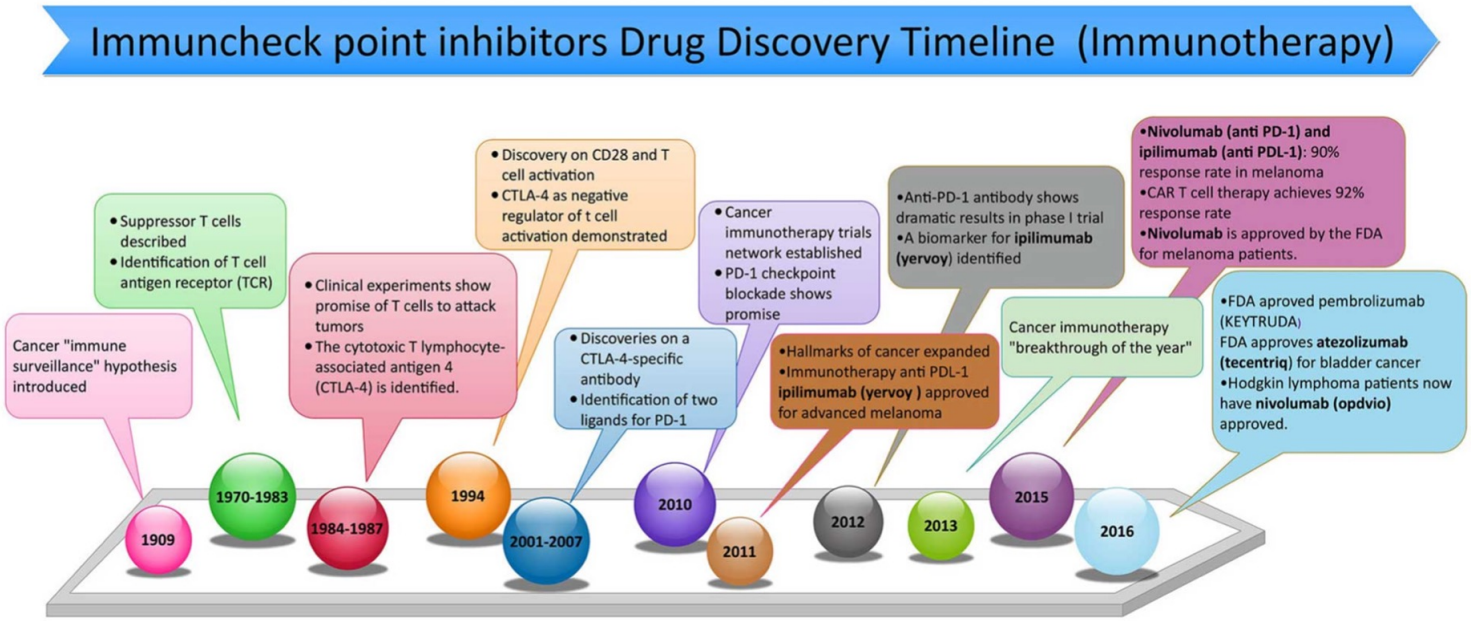


Anti-CTLA-4 Inhibitors:
Ipilimumab
Tremelimumab

Anti-PDL1 Inhibitors:
Durvalumab
Avelumab
Atezolizumab

Anti-PD1 Inhibitors:
Nivolumab
Pembrolizumab
Tislelizumab
Sintilimab
Camrelizumab
Toripalimab
Spartalizumab
Penpulimab

Immune checkpoint blockade : time line



Alsaab & al. frontiers in Pharmacology, 2017

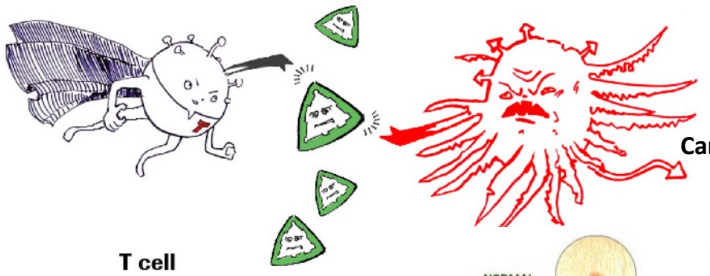
Important Dates for PD-1/PDL-1:

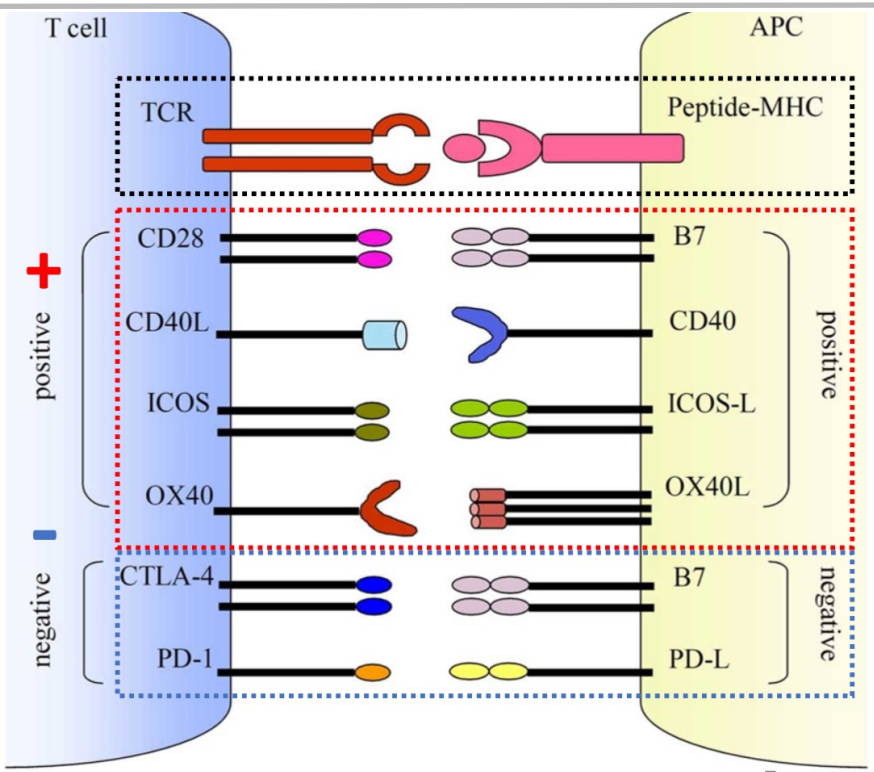
- 1992: Discovery of PD-1
- 1999: Discovery of B7-H1 (PDL-1)
- 2018 Honjo T. (PD1) Allison JP. (CTLA-4) Nobel Laureates in medicine and physiology

Beyond PD-1: next frontier immunotherapy T-cell exhaustion (eg resistant melanoma)

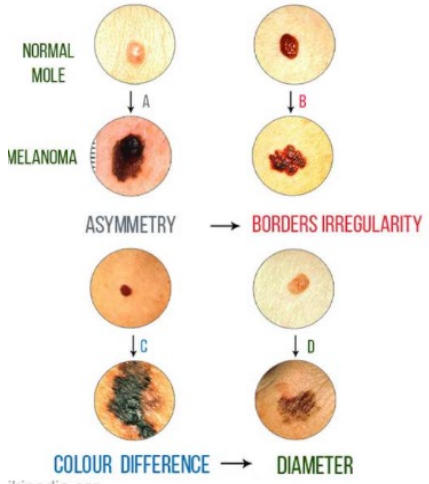
- Activation of T cell upon foreign antigen encounter **+ "Go"**

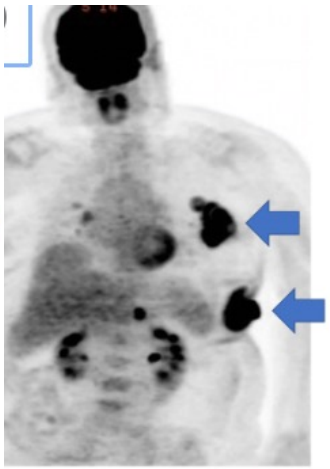
- Regulation and blocking of self-antigen recognizing T cells (that avoided self-tolerance check) **- "No Go"**





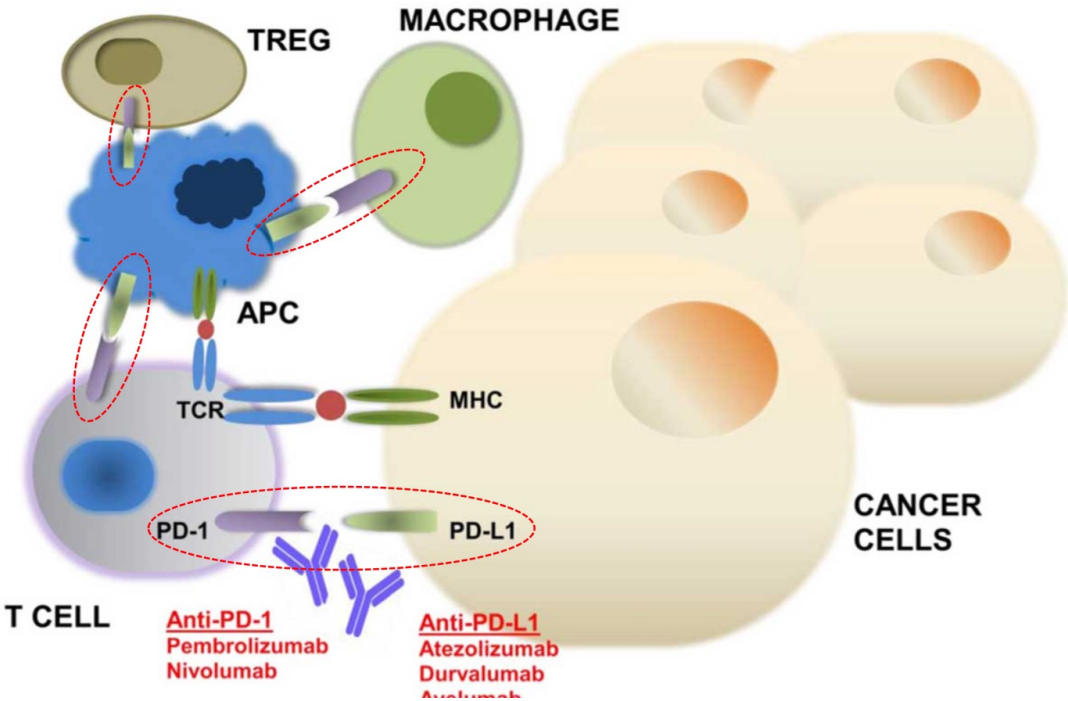
Huang & al. frontiers in Microbiology, 2011





PD-1/PDL-1 checkpoint blockade : a potent cancer treatment

- PD-1/PDL-1 Blockade could result in:
  - Cancer cell recognition & clearance
  - Prolonged immuno-activation
  - Induction of memory T cells
  - Neutralization of cancer-sustaining actors (M2 macrophages, stroma cells…)



- T-reg Nobel laureate 2025 Sakaguchi S, Brunkow M Ramsdell F.

Keytruda among the pioneers PD-1/PDL-1 checkpoint blockade

Approved PD-1/PD-L1 inhibitors

Name	Target	Approved
Nivolumab	PD-1	2014
Pembrolizumab	PD-1	2014
Atezolizumab	PD-L1	2016
Avelumab	PD-L1	2017
Durvalumab	PD-L1	2017
Cemiplimab	PD-1	2018
Tislelizumab	PD-1	2019
Dostarlimab	PD-1	2021
Retifanlimab	PD-1	2023
Toripalimab	PD-1	2023
Cosibelimab	PD-L1	2024

Case study: Keytruda / Pembrolizumab: 1st PD-1 Inhibitor approved for metastatic melanoma

- 5-year survival rate for metastatic melanoma = 16%
- Increase of 2% annually of cases in children between 0 and 19 years old
- Cost: $11,000 monthly / patient ---- 100'000 patients in 2018 US

$11,000-per-month

- **Keytruda**
  - First human PD-1 blocking antibody approved in the USA
  - Objective response rate (ORR): **19.4%** (~500 patients)
  - Most common side effects: fatigue and decreased appetite
- Medical conclusion (2015): Safe, effective for unresectable or metastatic melanoma

Of note SMW cpds are in 2022 in clinical trials as inhibitors targeting PD1/PDL1 ! (cost of good !)

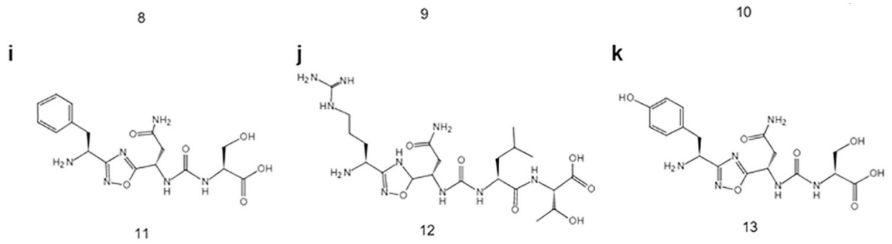
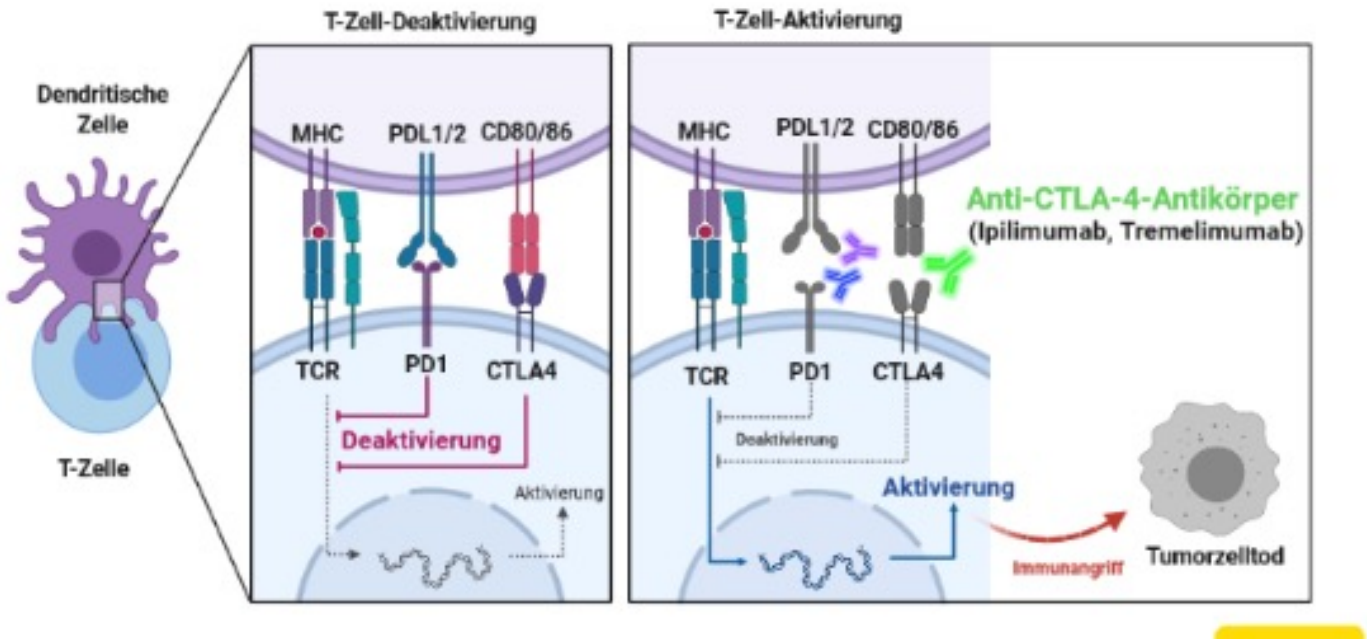


Fig. 3 Nonpeptide-based small molecule inhibitors targeting PD-1/PD-L1

Ipilumab, pembrolizumab : therapy for the treatment of metastatic melanoma

Approved PD-1/PD-L1 inhibitors

Name	Target	Approved
Nivolumab	PD-1	2014
Pembrolizumab	PD-1	2014
Atezolizumab	PD-L1	2016
Avelumab	PD-L1	2017
Durvalumab	PD-L1	2017
Cemiplimab	PD-1	2018
Tislelizumab	PD-1	2019
Dostarlimab	PD-1	2021
Retifanlimab	PD-1	2023
Toripalimab	PD-1	2023
Cosibelimab	PD-L1	2024



Of note SMW cpds are in 2022 in clinical trials as inhibitors targeting PD1/PDL1 ! (cost of good !)

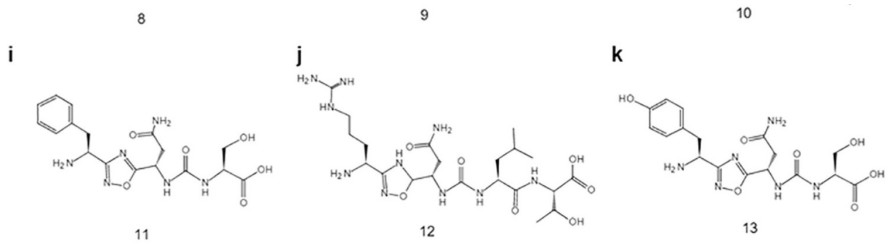


Fig. 3 Nonpeptide-based small molecule inhibitors targeting PD-1/PD-L1

pembrolizumab : therapy for the treatment of metastatic melanoma ; the J Carter success story -2015-2024-

PD-1 checkpoint blockade: **Hype vs Reality**

- *"I want what Jimmy Carter had"* syndrome
- **ORR 20% & cancer-dependent** VS **total remission & long-term cancer free**
- Works better in synergy with others (chemotherapy, cancer vaccine)

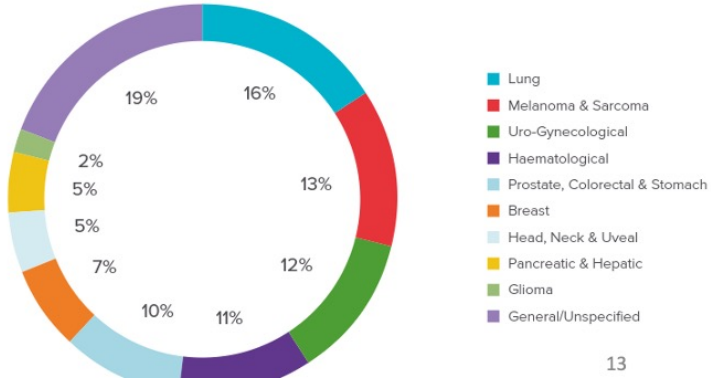
Major FDA Approvals of PD-1 / PD-L1 Inhibitors

Drug	Commercial name	Owner	Target	First approval date
Pembrolizumab	Keytruda	MSD	PD-1	September 2014
Nivolumab	Opdivo	BMS	PD-1	December 2014
Atezolizumab	Tecentriq	Roche	PD-L1	May 2016
Avelumab	Bevancio	EMD and Pfizer	PD-L1	March 2017
Durmalumab	Imfinzi	AstraZeneca	PD-L1	May 2017

Source: Drugs.com

LABIOTECH.eu

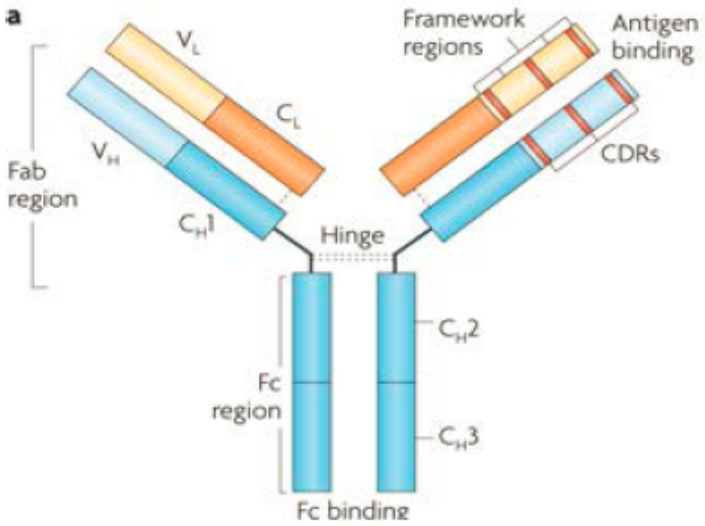
Anti-PD-1/PD-L1 MAb combination studies by (broad) indication (grouping)



13

pembrolizumab : the J. Carter success story in metastatic melanoma diagnosed in 2015, exitus in 2024, almost 10 years relapse free liver surgery, radiotherapy included **!**

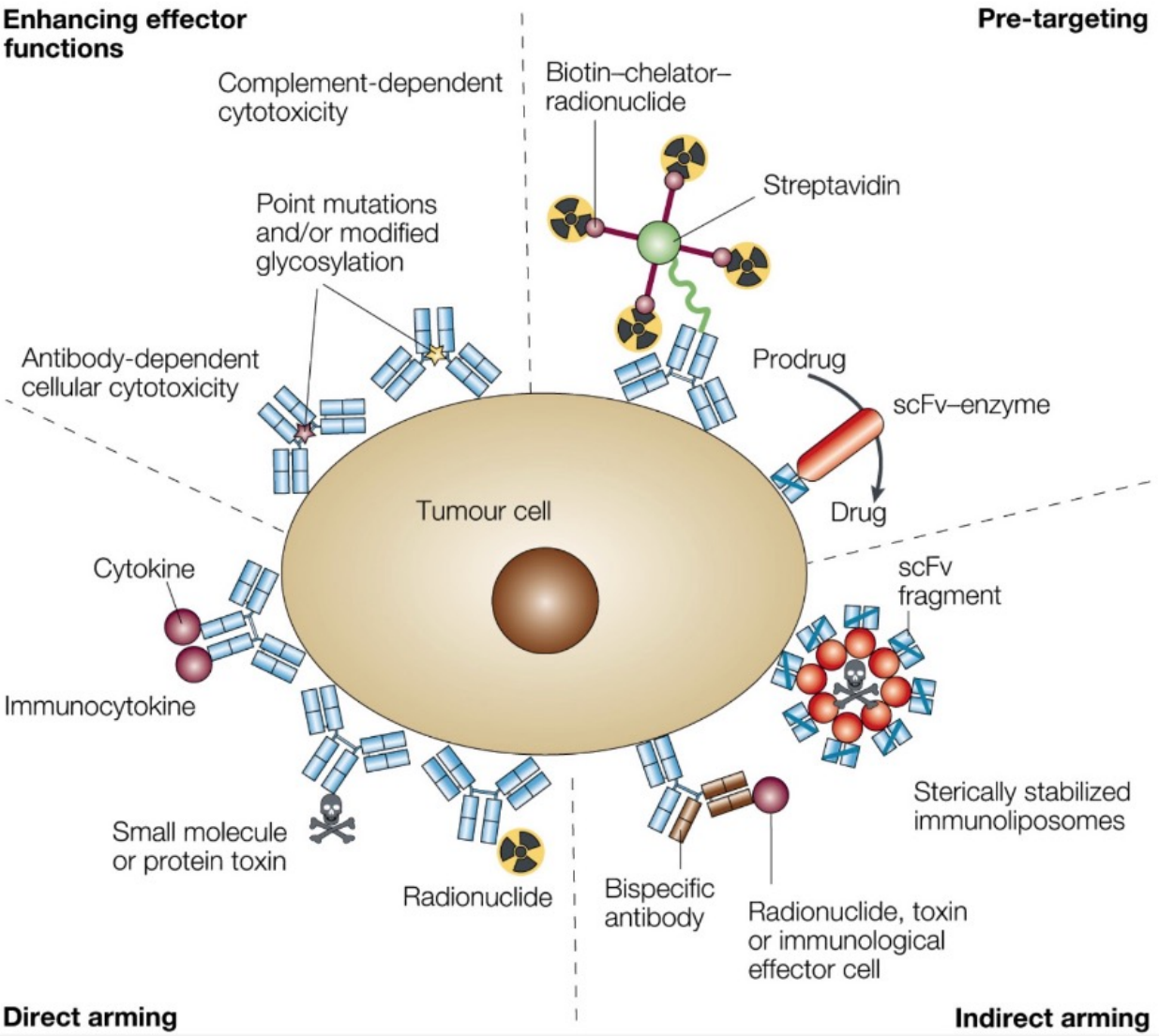
“armed” biologicals – “smart” biopharmaceuticals



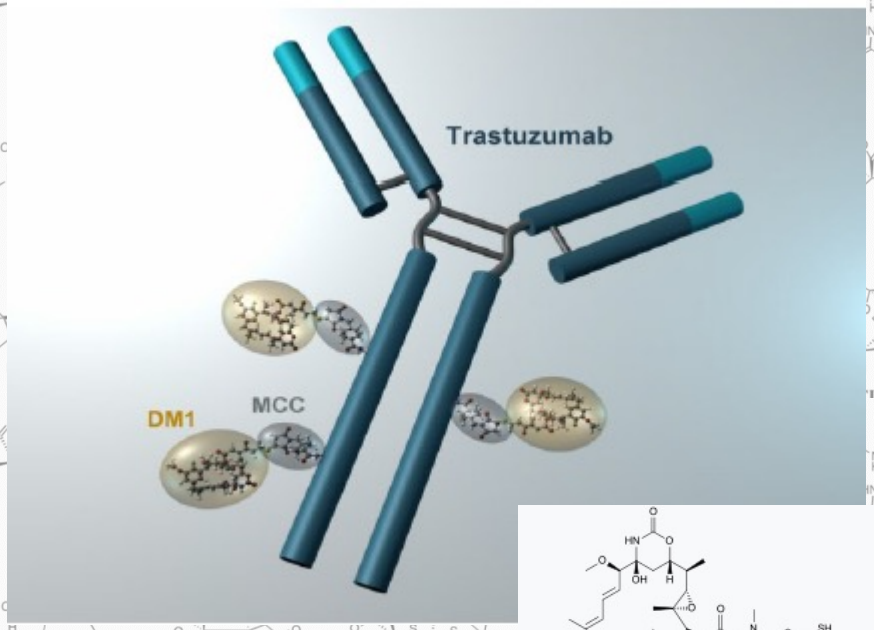
Hansel et al, Nature Rev, 2010

- **Monoclonal mAbs**
  - Majority of biotherapeutics in drug development
  - Murine, humanized or fully human mAbs
- **Fab-region**
  - **Variable domain: antigen binding**
  - Interaction with the target
    - membrane bound or soluble target
    - high specificity
  - Antagonistic or agonistic drug effect
  - → Associated with pharmacological/ toxicological activity (exaggerated pharmacology)
- **Fc-region**
  - **Constant domain: effector function, homeostasis**
  - → Relevant for pharmacological activity, half life, and adverse effects

“armed” biologicals – “smart” biopharmaceuticals
therapeutic antibodies with enhanced activities



“armed” biologicals – ADCs -combining precision medicine with chemo-radio-therapy



DM1

T-DM1 (trastuzumab emtansine) recently entered clinics for resistant HER2 positive breast cancer

Custom made antibody therapeutics armed with chemotherapy (eg alpha amanitin)

“smart biologicals” : tomorrow magic bullet in medicine ?

Cell type and therapeutic target - specific chemotherapy

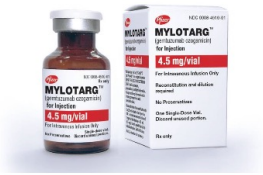
Antibody drug conjugates (ADC)- chemotherapy tubulin inhibitor

Bispecific antibodies: magic bullet in medicine or immunosafety nightmare ?

Emerging class of cancer therapeutics: mechanisms by which ADC are internalized and activated remain unclear « beaking up is hard to do »

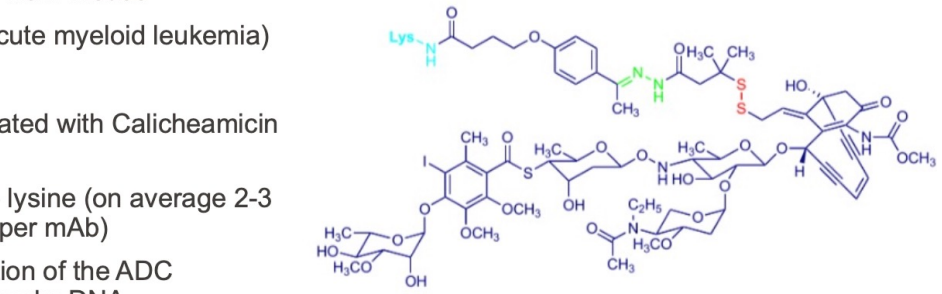
“armed” biologicals – ADCs -combining precision medicine with chemo-radio-therapy

Mylotarg: Toxin armed mAb



- mAb humanized from mouse
- Target: CD33 (acute myeloid leukemia)

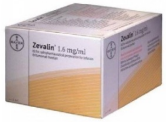
- Antibody conjugated with Calicheamicin (bacterial toxin)
- NHS coupling to lysine (on average 2-3 toxin molecules per mAb)
- After internalization of the ADC Calicheamicin breaks DNA → Apoptosis



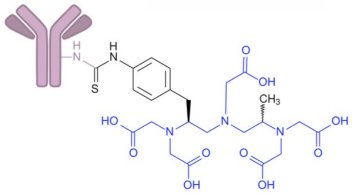
Biologically instable linker:
1) Disulfide bridge: reduction upon internalization
2) Hydrazone: hydrolysis under acidic conditions

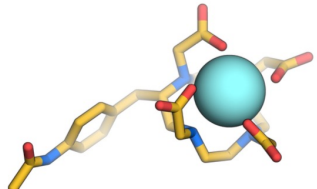
Antibody Engineering of armed antibodies involves intensive linker chemistry!

Zevalin: ^{90}Yttrium Ibritumomab Tiuxetan



- Coupling of a chelating agent (Tiuxetan, DTPA derivate) to Ibritumomab
- Tiuxetan can bind multiple isotopes:
  - $^{111}In^{3+}$ (diagnosis)
  - $^{90}Y^{3+}$ (therapy, $t_{1/2}$ = 64 h)
- $^{90}Y^{3+}$ will penetrate solid tissue (range of about 200 cells)
- Most successful therapeutic antibody
- Comparison to non-armed antibody equivalent: 80/56% overall response, 30/16% complete remission





Common metastatic sites in breast cancer

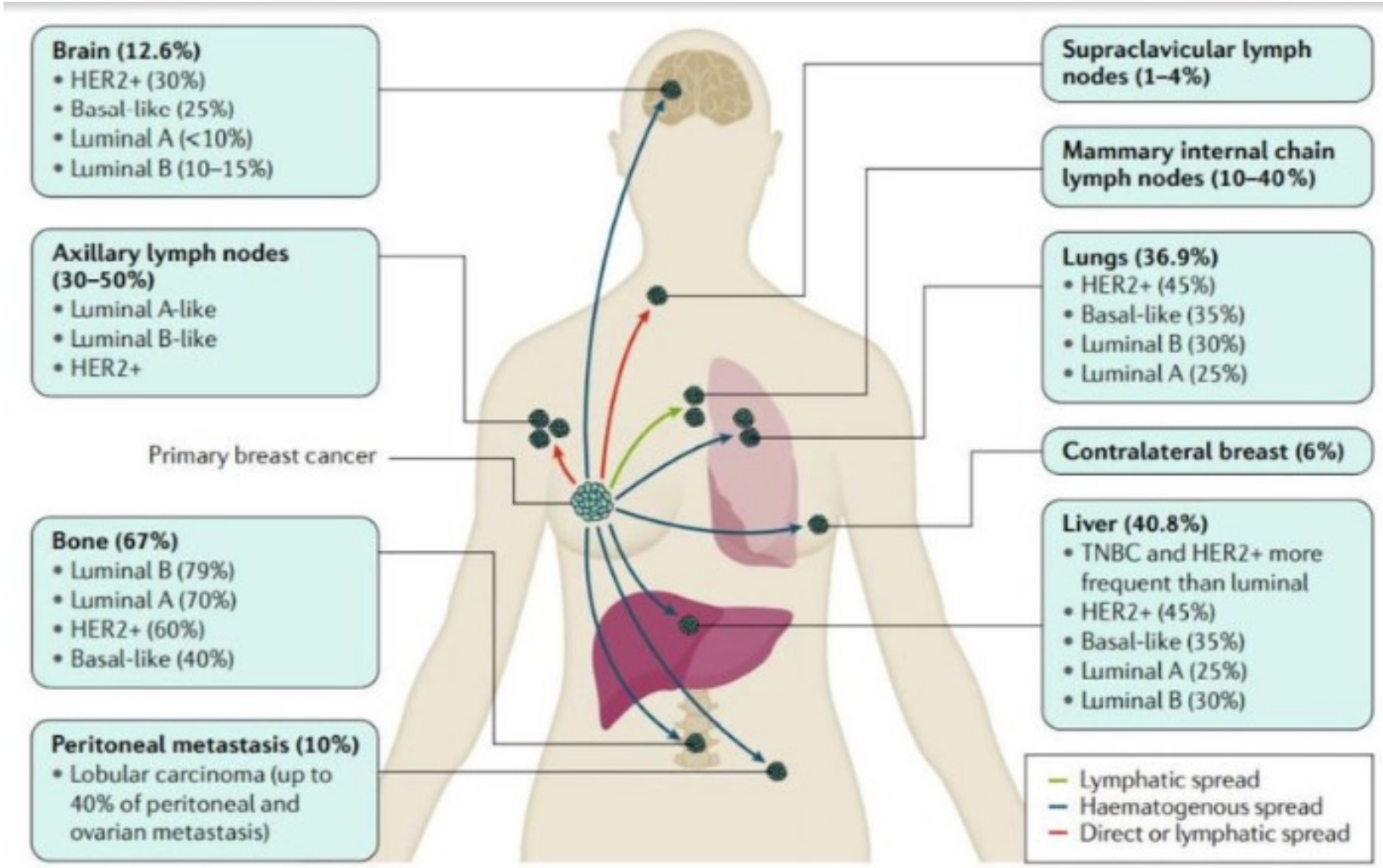
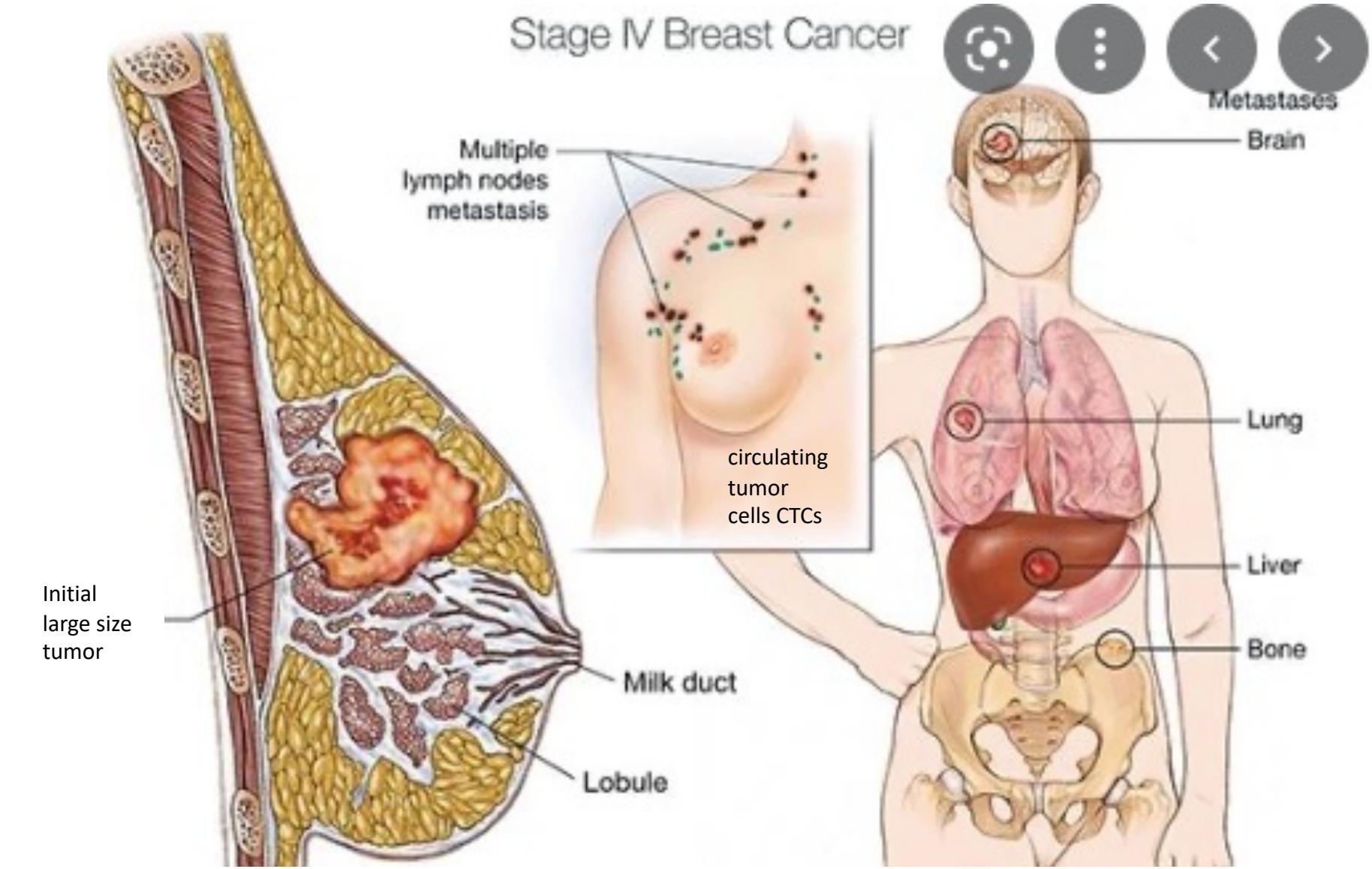


Illustration of common metastatic sites in breast cancer [2]. The most common metastatic sites for breast cancer are the bones, axillary lymph nodes, liver, and lungs. Approximately 10-40% of breast cancer tumors have

Most aggressive triple negative breast cancer TNBC fast track approval of sacituzumab (Trodelvy)



TNBC are negative for ER, PR and HER2 – represents 15-20% of invasive BC

“armed” biologicals – Sacituzumab
In non ER+, PR+ and Her2+ triple negative breast cancer

Immunomedics **Immunomedics Awarded Fast Track Designation by FDA for Sacituzumab Govitecan (IMMU-1...** SIGN IN

About triple-negative breast cancer (TNBC)

TNBC represents breast cancers that are negative for estrogen and progesterone receptors, as well as human epidermal growth factor receptor 2, or HER2. This type of breast cancer comprises about 15-20% of all invasive breast cancers and is more prevalent in young and African-American women. Despite the fact that initial responses with chemotherapy are high, TNBC characteristically has a high recurrence rate and is perhaps the most difficult type of breast cancer to treat successfully with current cytotoxic agents. According to a published report, the median survival for patients with metastatic triple-negative breast cancer is estimated to be 13 months.[1] Currently, there are no targeted treatments available for TNBC.

About Sacituzumab Govitecan

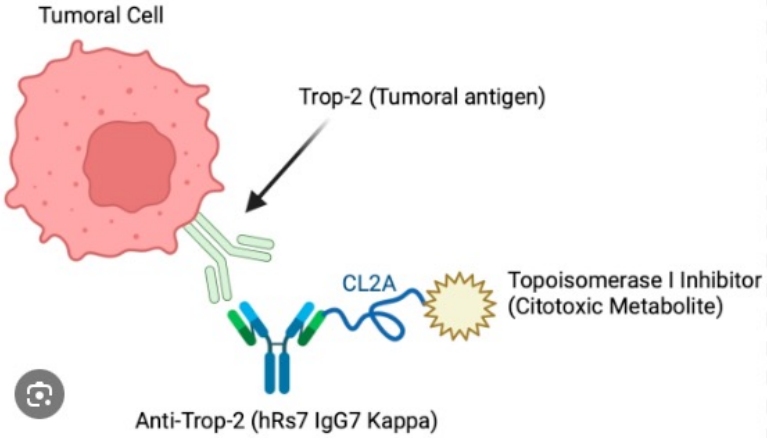
Sacituzumab govitecan is composed of hRS7, a humanized antibody that binds to the trophoblast cell-surface antigen (TROP-2), also known as the epithelial glycoprotein-1 antigen (EGP-1). TROP-2 is expressed by many human tumors, such as cancers of the breast, cervix, colon and rectum, kidney,

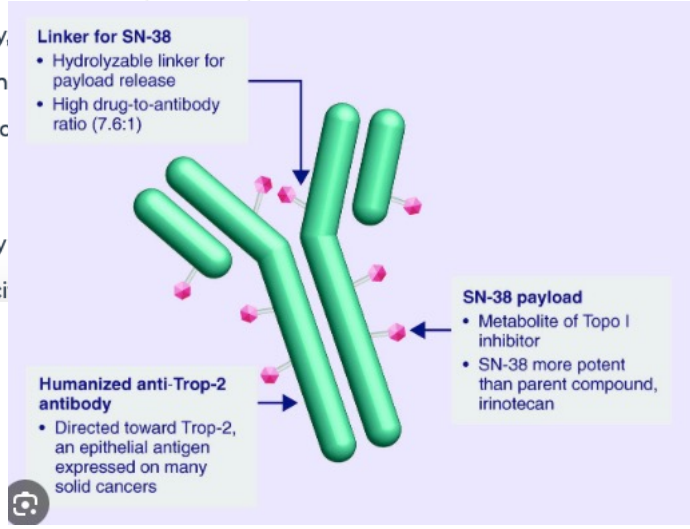
d expression in normal human tissues. Th

ng to TROP-2, making it a suitable candic

(Camptosar), which is a standard therapy

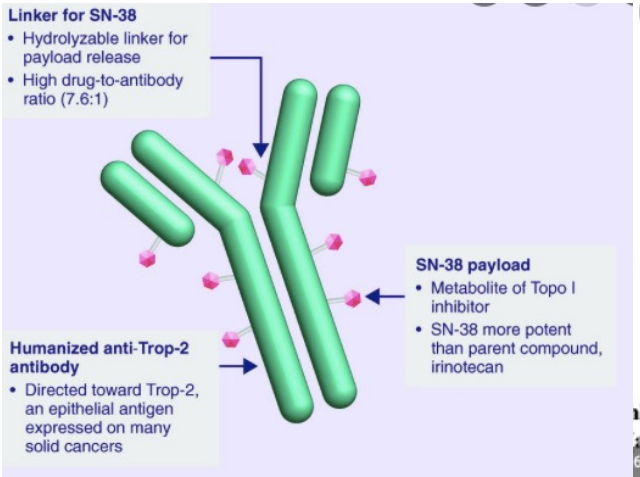
gastrointestinal and hematological toxici

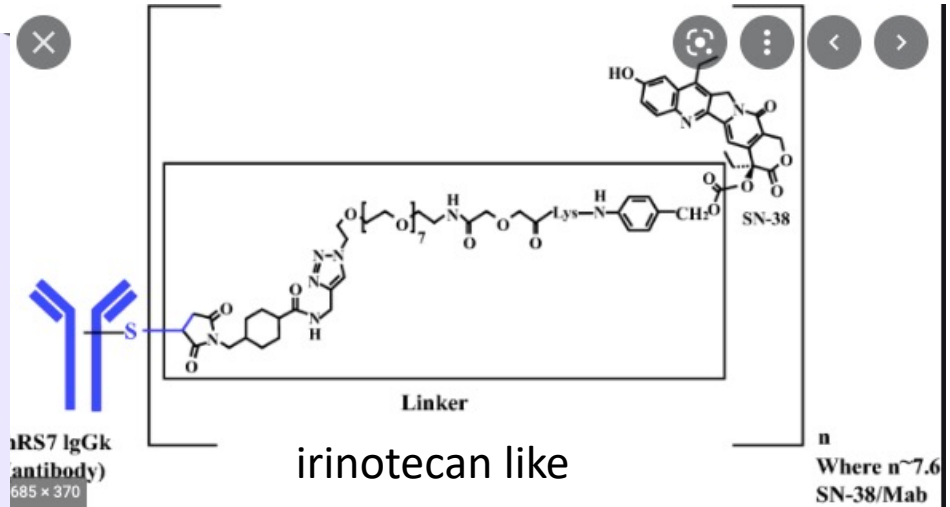




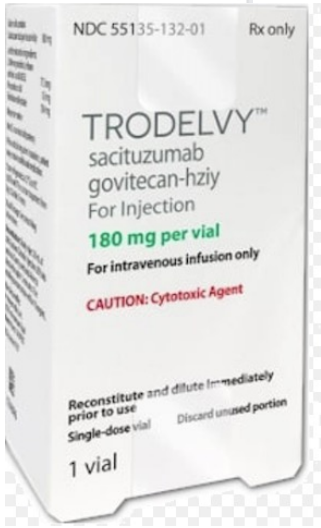
“armed” biologicals – eg. in triple negative breast cancer TNBC (FDA fast track sacizutumab 2021 when Kadcyla fails)

7 molecules of highly toxic topoisomerase inhibitor irinotecan/hu IgG1

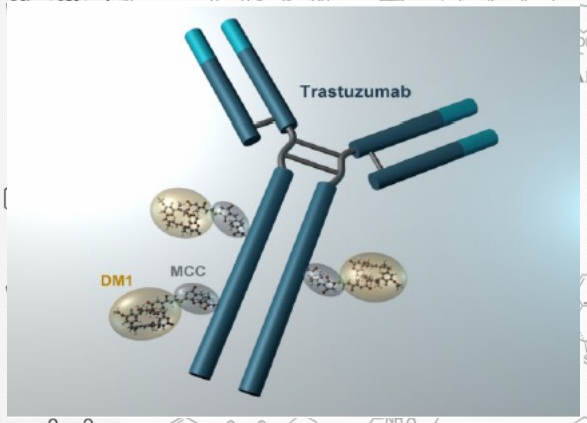




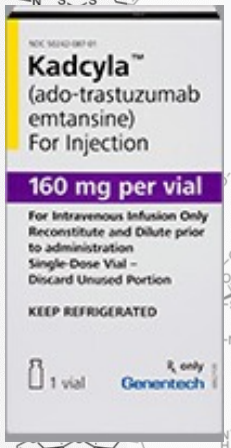
irinotecan like



“armed” biologicals – ADCs -combining precision medicine with chemo-radio-therapy



DM1



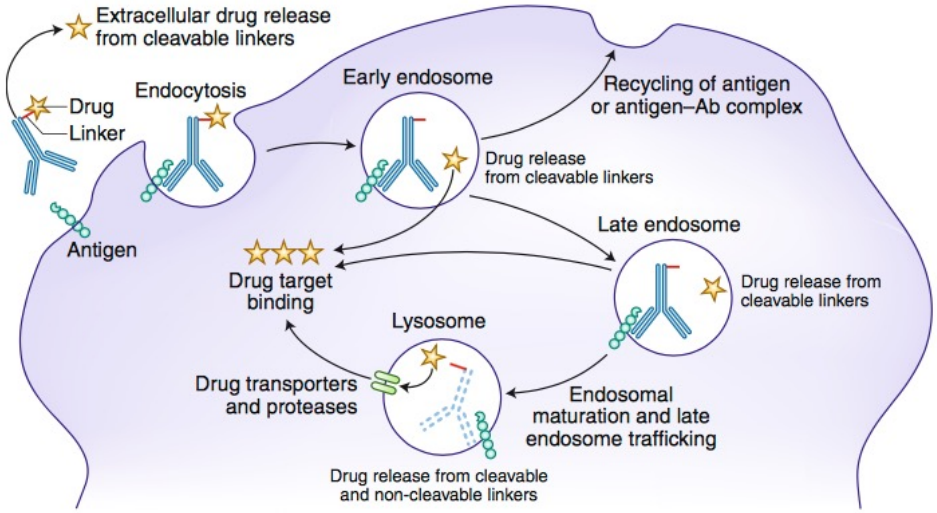


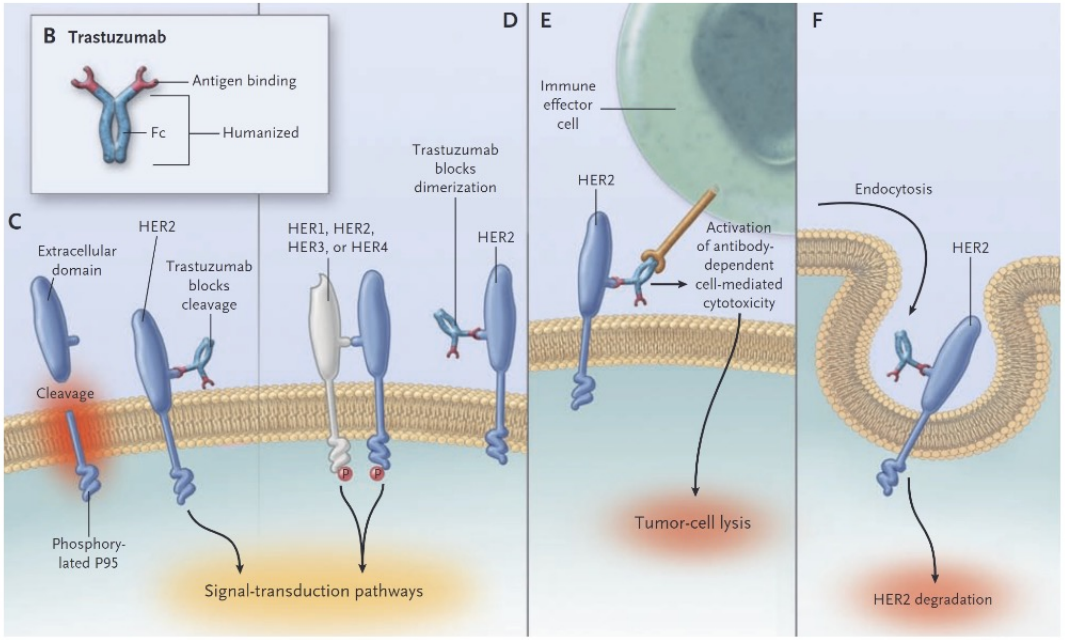
Fig. 1 | Mechanisms for drug release from antibody–drug conjugates. Depending on the linker used,

Van Epps and Senter PD. 2019. Nature Chemical Biology 15:934-

Bioactive compound
Oxaliplatin
p53 DNA
RB94 DNA
RRM2 siRNA
Doxorubicin
Paclitaxel
Doxorubicin
Docetaxel
Melanoma antigens + IFNγ

ADCs can be conjugated to other types of molecules, such as oligonucleotides,radionuclides,protein toxins.

Her2 (EGFR) is amplified in 10% of breast and gastro oesophagal cancer

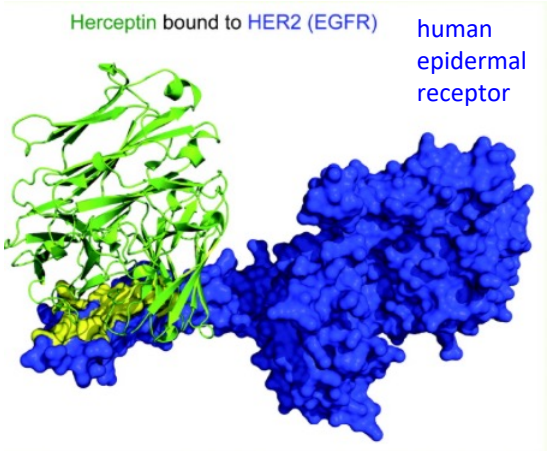


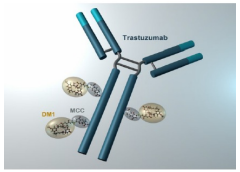
- Humanized through CDR grafting
- First approved humanized antibody (Genentech)
- Plasma half-life ~5.8 days
- Only 1 case of HAHA (human-anti-humanized-antibody) response in 903 patients

Mechanism of Trastuzumab

- Prevents shedding of HER2
- Blocks dimerization
- Induction of ADCC
- Clustering induces receptor internalization via endocytosis

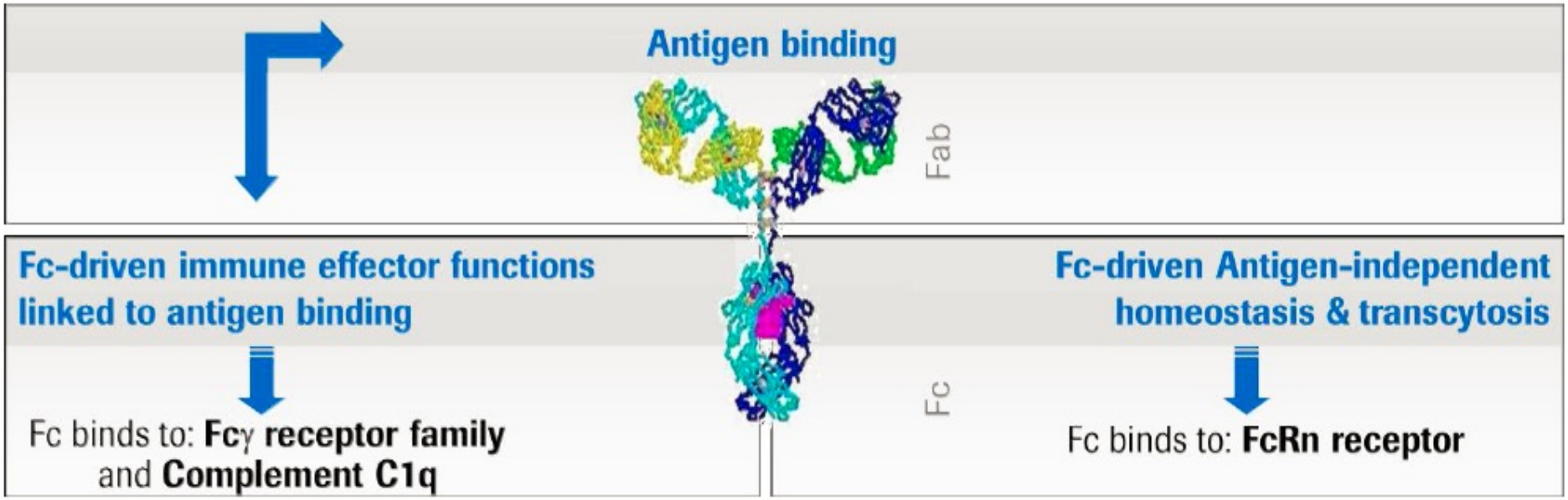
New England Journal of Medicine, (2007), 39-51, 357(1)



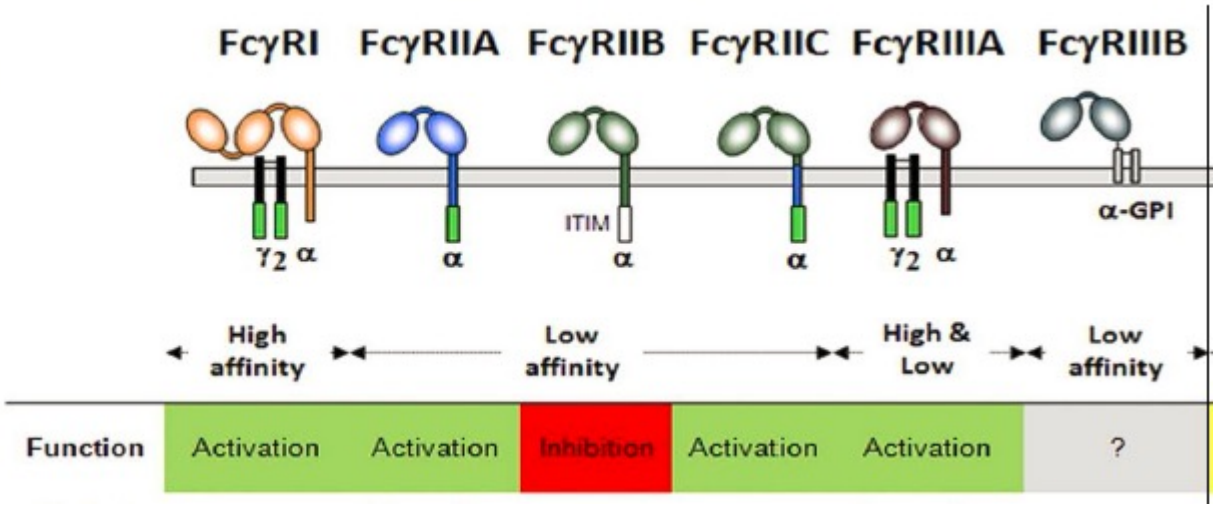


trastuzumab (Herceptin)
trastuzumab-DM1 (Kadcyla)

Engineering IgG can be used to modulate FcR interaction

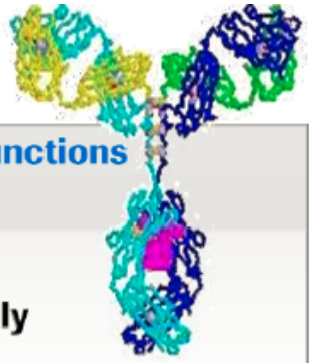


FcγR = Fc gamma-receptor family



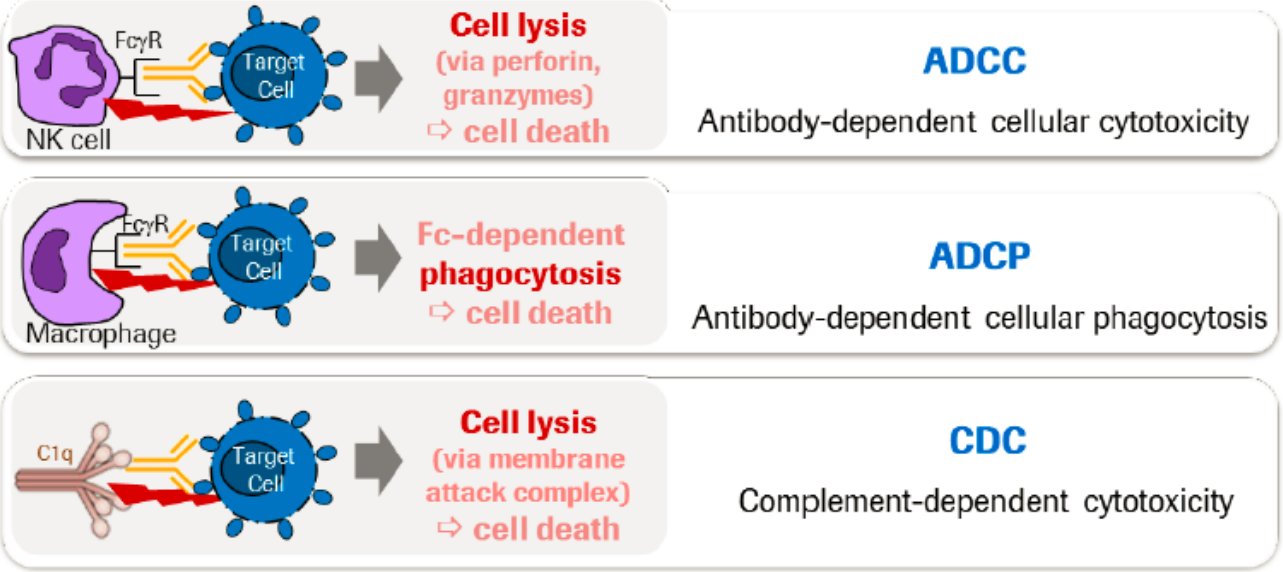
Silencing of the Fc part (mutagenesis) in situation where you do not want to eg activate NK cells

Fc receptor driven effector functions linked to antigen binding



Fc-driven immune effector functions linked to antigen binding

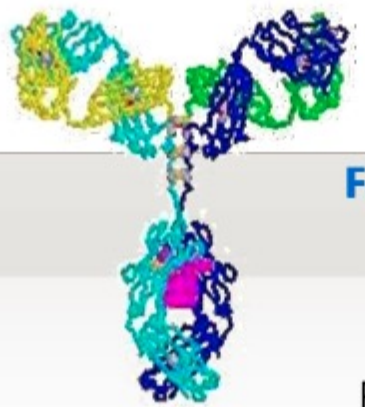
Fc binds to: **Fcγ receptor family** and **Complement C1q**



The effector function is exerted by complement or by immune cells that bind to the Fc part of a mAb

Fc part has two complement binding sites

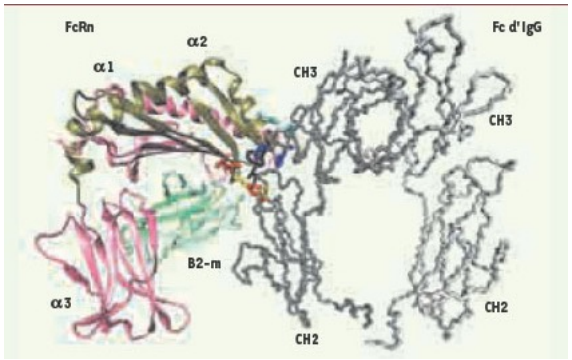
Fc receptor driven effector functions independent to antigen binding



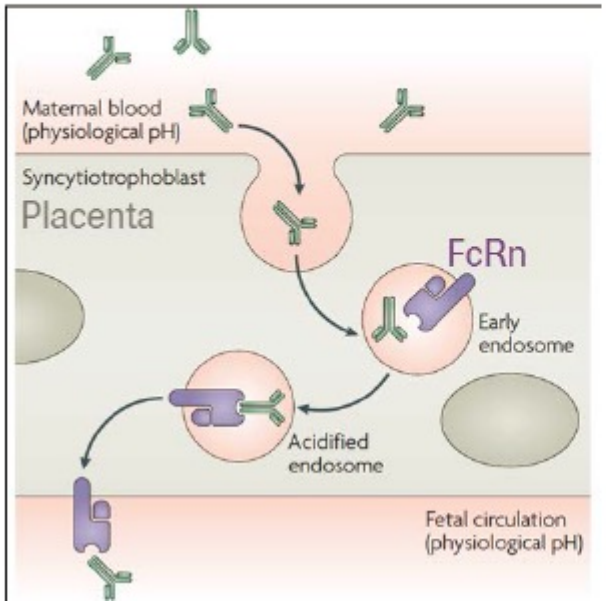
contribute to host defense : long half life of IgG

Fc-driven Antigen-independent homeostasis & transcytosis

Fc binds to: **FcRn receptor**

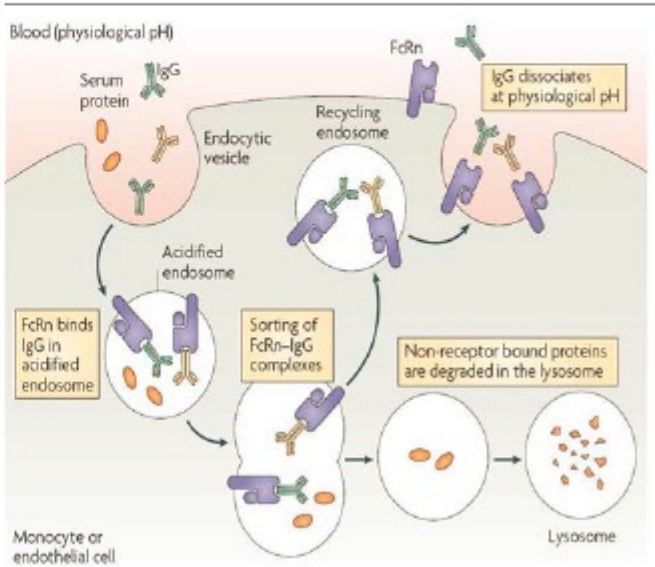


- **FcRn mediates transcytosis** of IgG (via epithial gut cells; placenta)



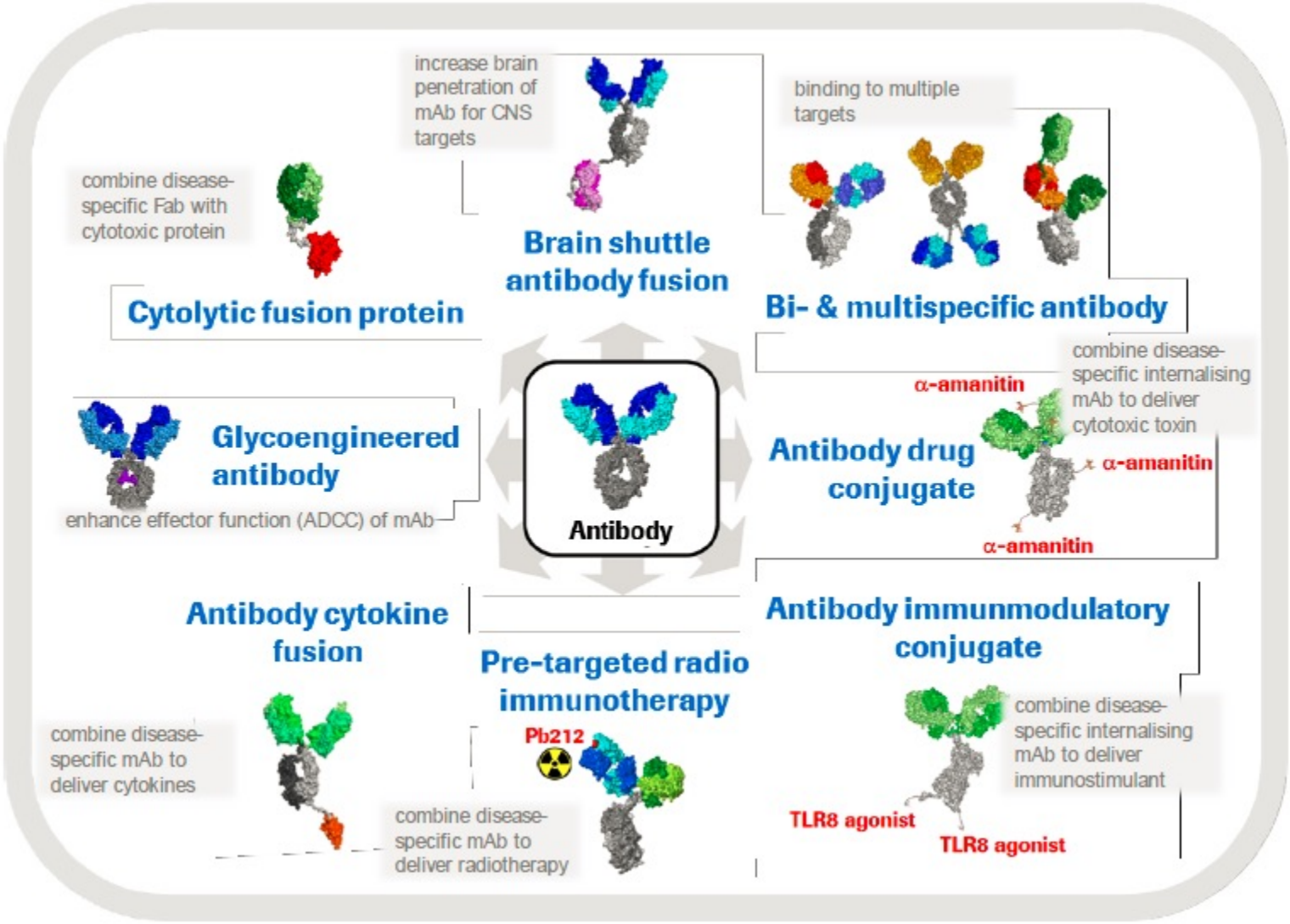
Roopenian D, Akilesh S (2007) Nature Rev Immunology 7:715-725

- **FcRn prevents lysosomal catabolism** of IgG by recycling via endosomal pathway (confers long plasma half life of IgG)



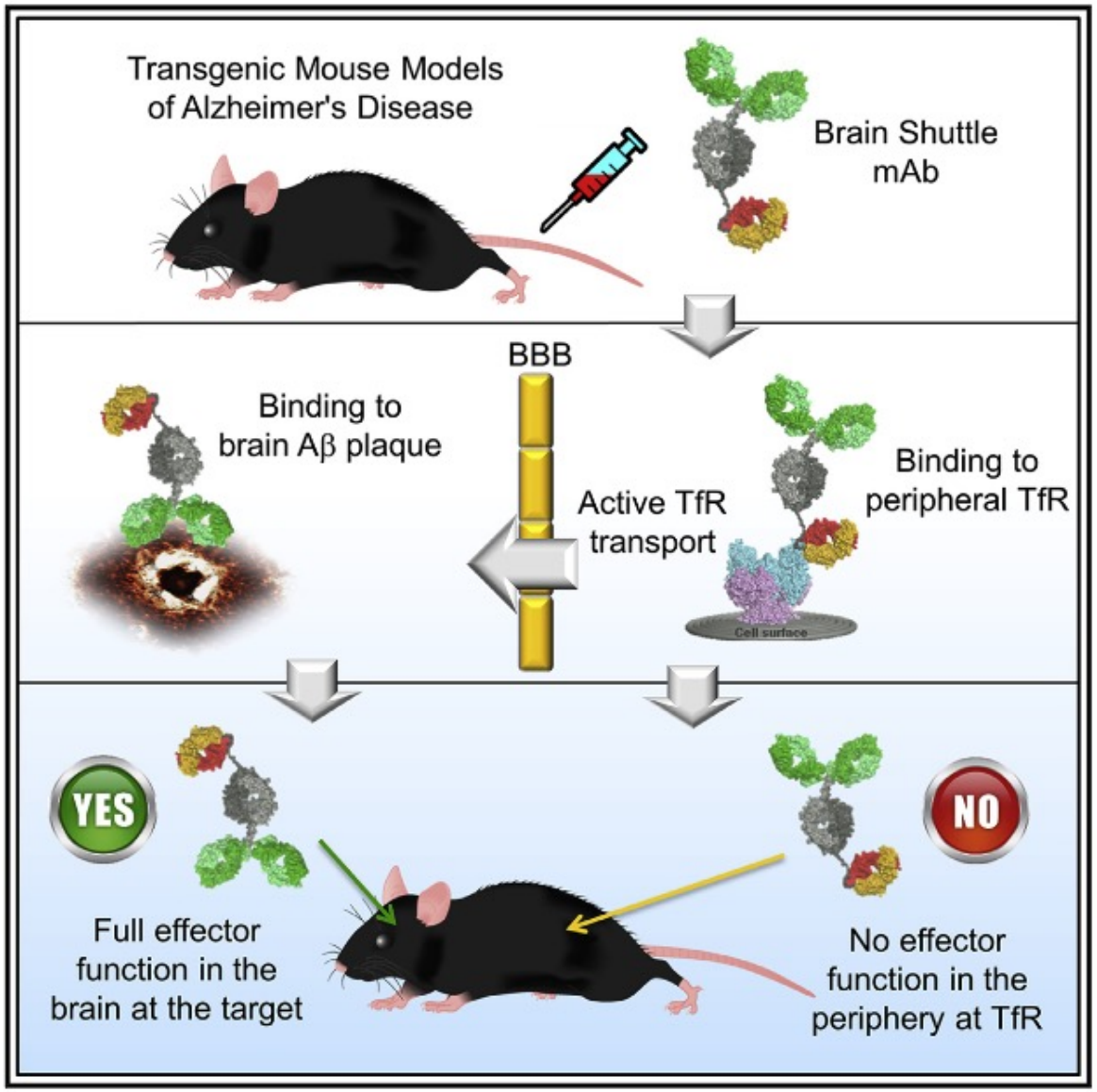
Fc receptor driven effector functions independent to antigen binding

GOAL: COMBINE DIFFERENT MODE OF ACTION TO ENHANCE EFFICACY OF mABS



Glycosylation pattern may influence the biology and therapeutic index of mABs

Brain shuttle mAbs for Alzheimer's disease with attenuated peripheral effector function due to an inverted binding mode

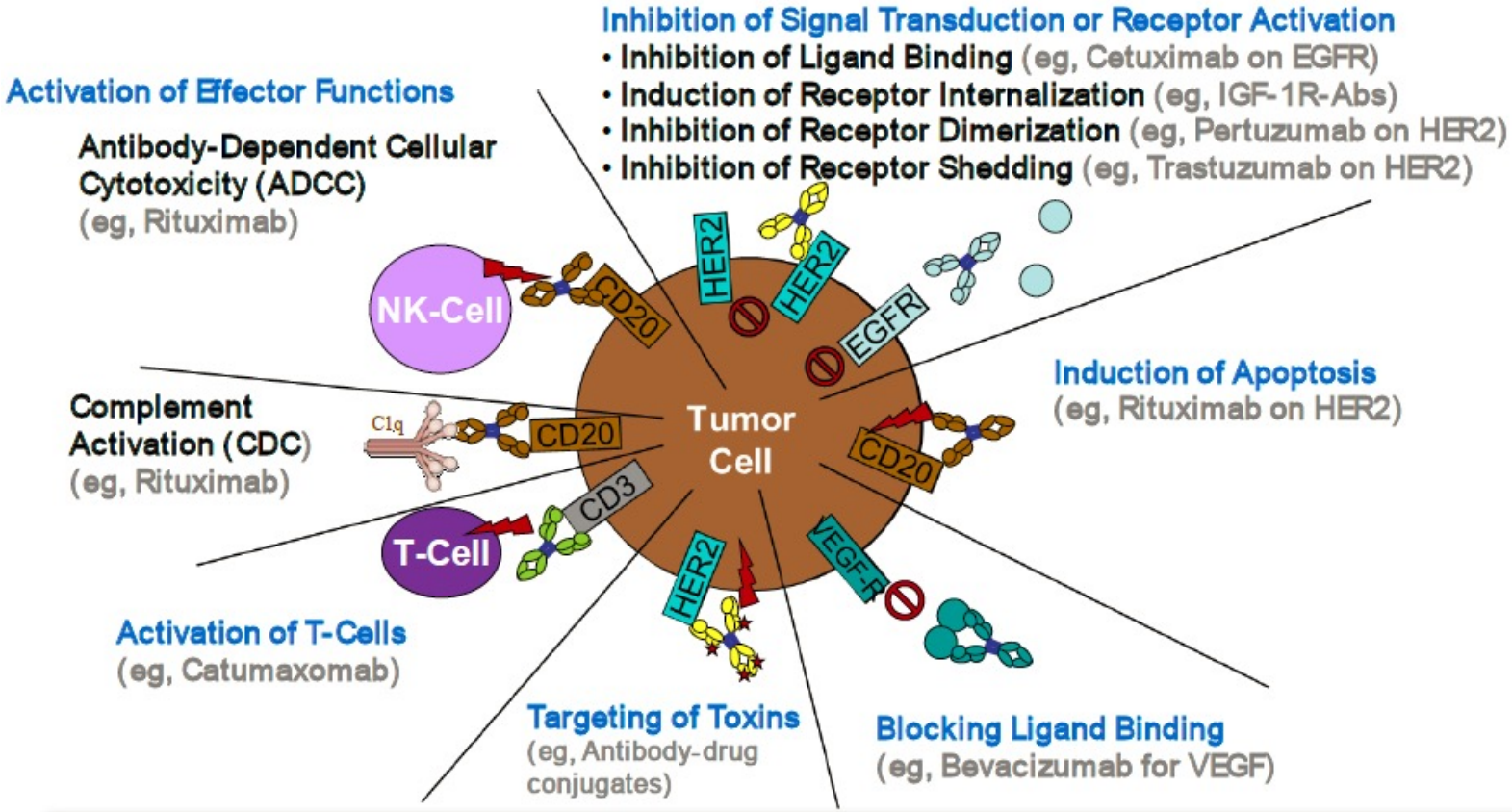


No first infusion Fc portion dependent adverse reaction in periphery, yet BBB transport via Ferritin receptor and clearing of brain Aβ plaques in mouse model by active Fc dependent effector function (eg cellular cytotoxicity)

Weber F et al. 2018 Cell Reports 22, 149–162

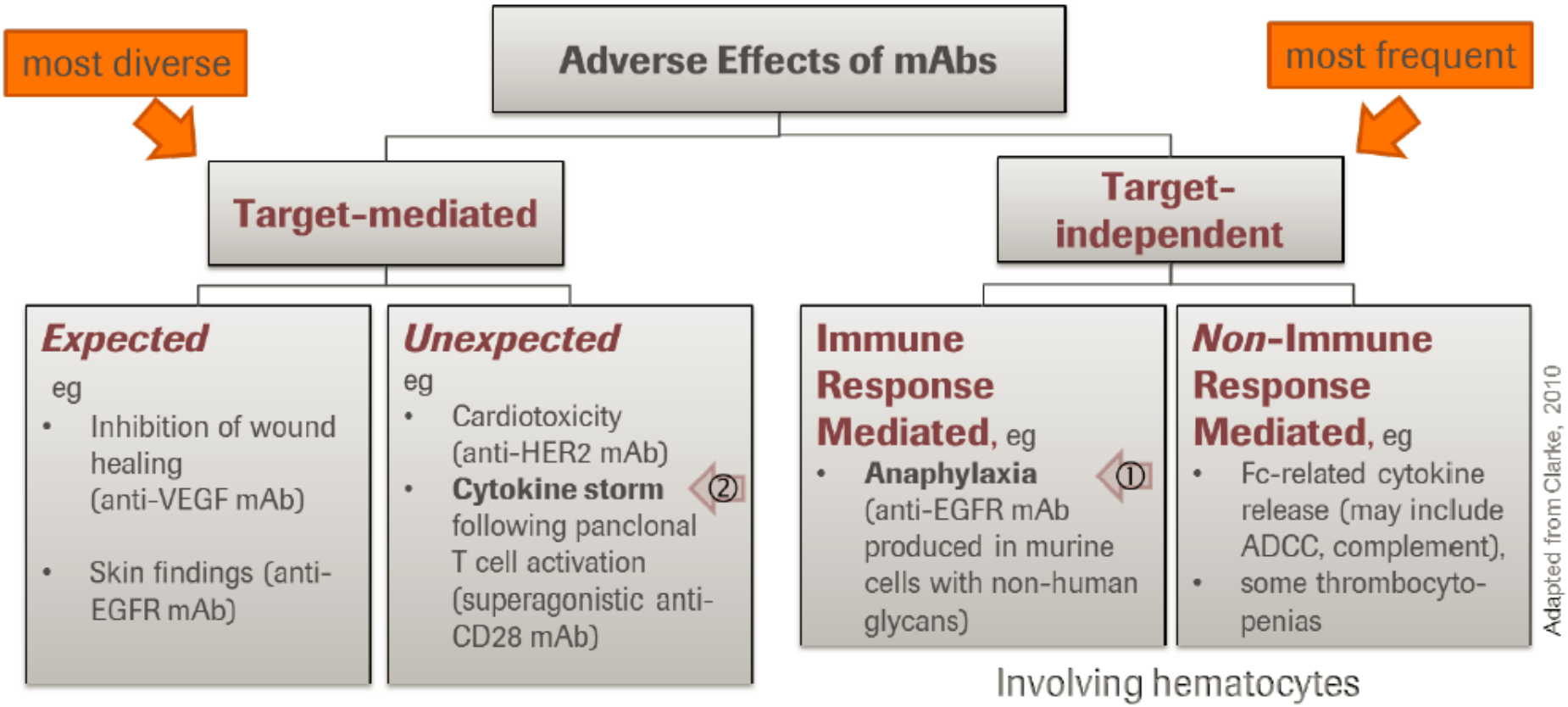
mABs in drug development

The Pharmacological Mode of Action of mAbs is Complex and may Involve Contributions from Multiple Mechanisms



The *in-vivo* net contribution of different modes of action described for one mAb is often incompletely understood and may also be different in different indications.

Mechanisms of adverse effects with mABs



MABs are subjected to intense scrutiny in drug safety and development

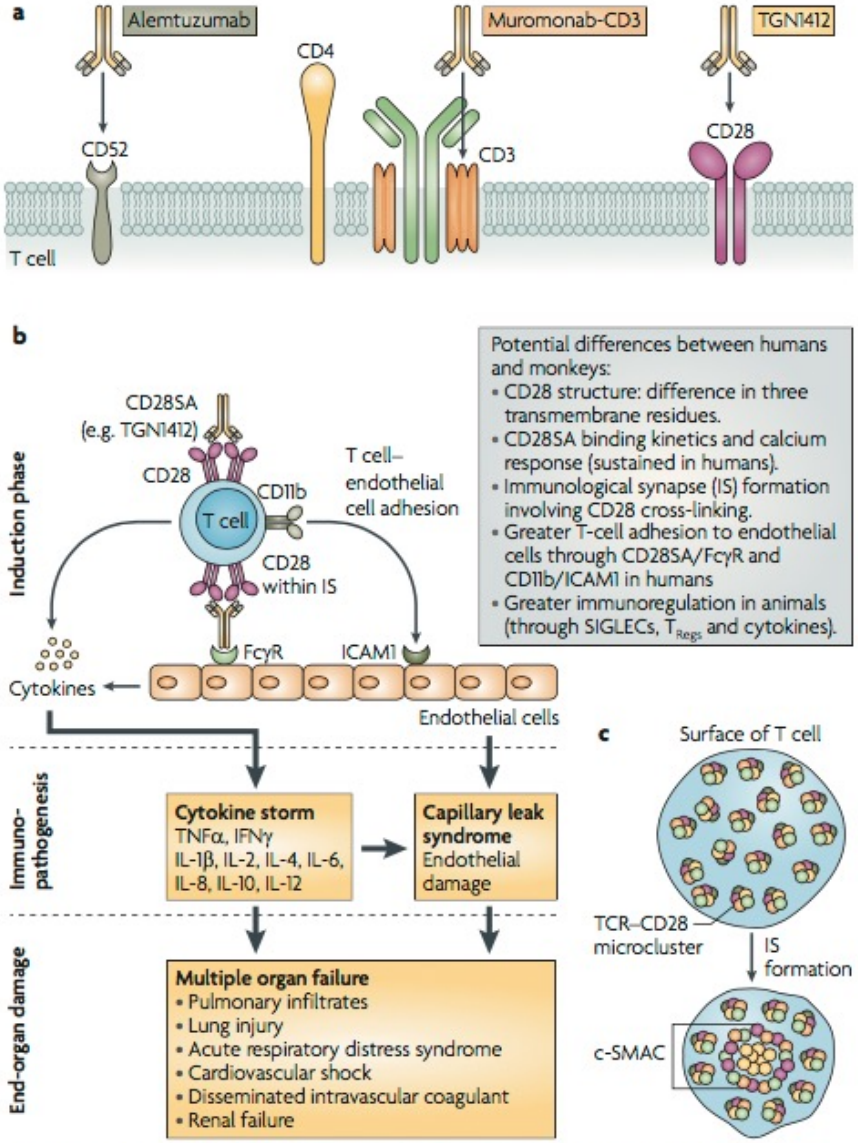


Figure 3 | **Monoclonal antibodies and the cytokine storm. a** | Surface receptors on

Mechanisms of adverse effects with mABs eg TGN1412

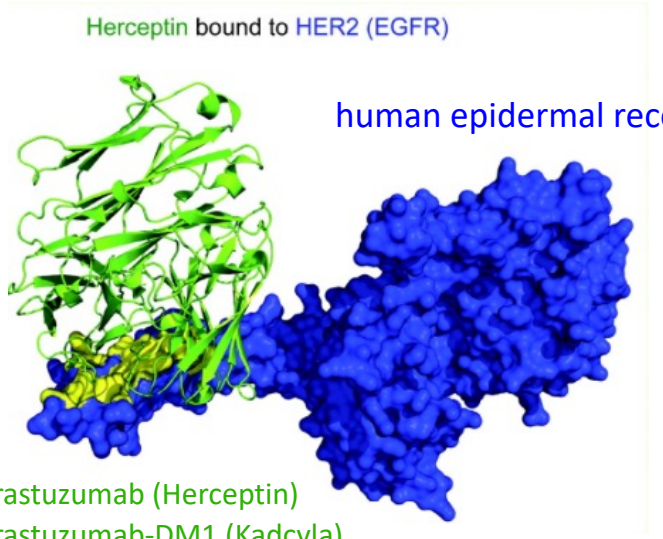
◆ **Clinical signs of cytokine storm**

- Early onset after 1st infusion with a mAb
- Signs and symptoms
  - Nausea, headache, fever, hypotension, hypoactivity, rash, shock, lower body temp., multiorgan failure
  - Neurological findings (encephalopathy, tremors, dizziness, seizures)
- Huge increases in cytokines and other mediators

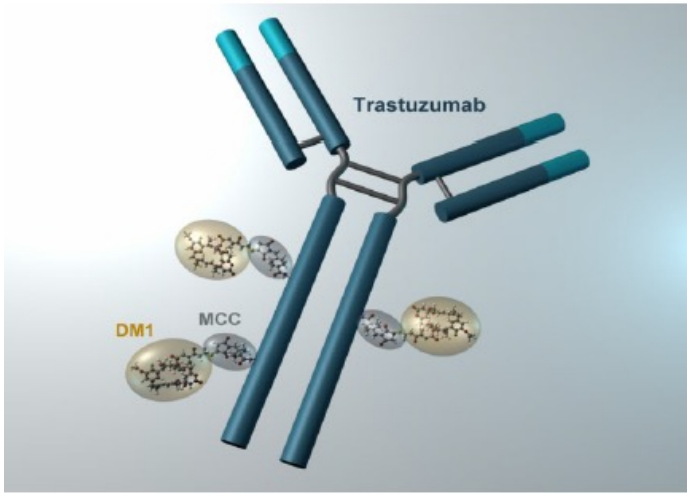
◆ **Cytokine storm in patients first time treated with anti-CD28 mAb TGN1412**

- Clinical signs: Severe symptoms, tissue damage, multiorgan failure
- Retrospectively, the mAb was shown to be a superagonist to CD28 with activation of all CD28⁺ immune cells

MABs are subjected to intense scrutiny in drug safety and development



trastuzumab (Herceptin)
trastuzumab-DM1 (Kadcyla)



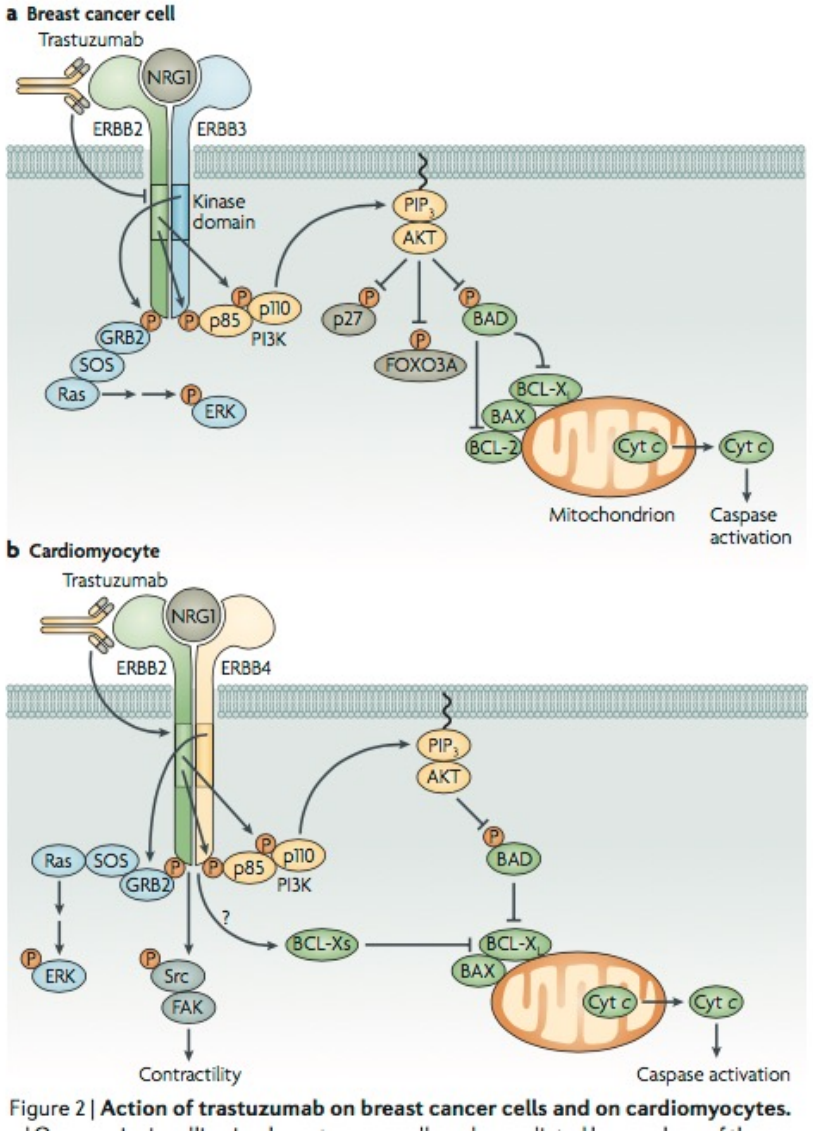


Figure 2 | **Action of trastuzumab on breast cancer cells and on cardiomyocytes.**

MABs are subjected to intense scrutiny in drug safety and development

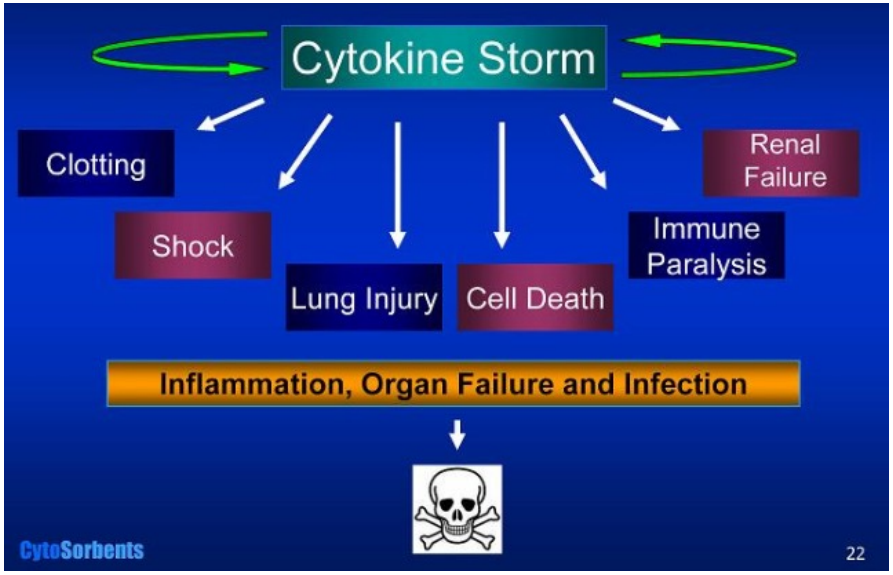
Clinical signs of cytokine storm

MOLECULAR BIOLOGY COMPLEX INCLUDING PATIENT HETEROGENEITY SAFETY HURDLE !

- Early onset after 1st infusion with a mAb
- Signs and symptoms
  - Nausea, headache, fever, hypotension, hypoactivity, rash, shock, lower body temp., multiorgan failure
  - Neurological findings (encephalopathy, tremors, dizziness, seizures)
- Huge increases in cytokines and other mediators

Cytokine storm in patients first time treated with anti-CD28 mAb TGN1412

- Clinical signs: Severe symptoms, tissue damage, multiorgan failure
- Retrospectively, the mAb was shown to be a superagonist to CD28 with activation of all $CD28^+$ immune cells



Peptides vs small M_R compounds

Table I. Comparison Between Peptides and Small Molecules

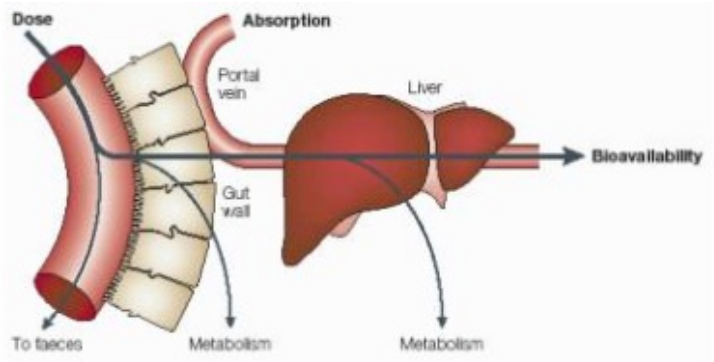
Small molecules	Peptides
• ~80% drug market	• ~2% drug market
• Low cost	• High cost
• Permeable	• Low permeability
• Stable	• Limited stability
• Good oral bioavailability	• Poor oral bioavailability
• Easier synthesis	• More challenging synthesis
• 95% current medicines	• Short half-life
	• Limited to extracellular targets
	• High binding affinity
	• Easier to identify
	• Excellent target specificity
	• Broad disease targets
	• Mechanism of action well understood
	• Low toxicity and immunogenicity
	• Low risk of drug–drug interaction potential
	• Lower impact from generics

Peptides are increasingly being developed as innovative medicine !
Pharmacological intervention on protein:protein interactions !

MDO (multiparameter drug optimization) for small MW cpds

MDO simplified (drug likeliness or Pfizer's "rule of five")

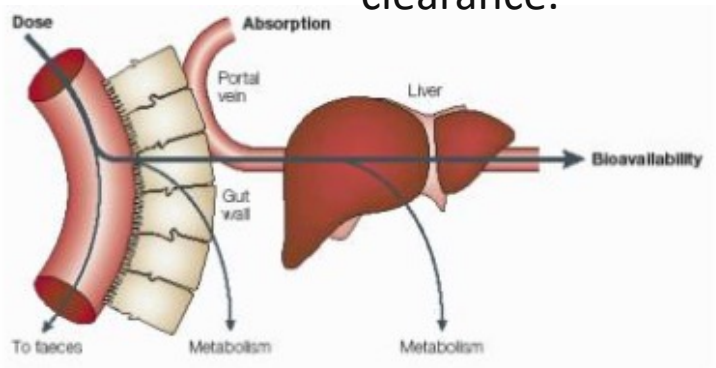
- Solubility
- Lipophilicity (clogP) octanol water partition <5) (partition coefficient)
- Number of H donors (<5)
- Number of H acceptors (<10)
- Molecular weight (generally <500 Da
- Permeability (PAMPA assay)
- CYP450s screen (eg. adverse drug metabolites
- Metabolic stability (PK, PD, ADME)
- Cardiac ion channels (hERG, "torsade de pointes"
- CEREP screens, kinase panels, GPCR panels



C.A. Lipinski et al. *Adv. Drug. Delivery Reviews* **23** (1997) 3.

Challenging MDO (multiparameter drug optimization) for peptides

- Several blockbuster peptide drugs are currently on the market
- Although peptides only represent 2% of the drug market, the market is growing twice as quickly and might soon occupy a larger niche
- Natural peptides typically have poor absorption, distribution, metabolism, and excretion (ADME) properties with rapid clearance, short half-life, low permeability, and sometimes low solubility.
- Strategies have been developed to improve peptide drugability through enhancing permeability, reducing proteolysis and renal clearance, and prolonging half-life. In vivo, in vitro, and in silico tools are available to evaluate ADME properties of peptides, and structural modification strategies are in place to improve peptide developability.
- KEY WORDS: ADME; peptides; pharmacokinetics; proteolysis; renal clearance.



C.A. Lipinski et al. *Adv. Drug. Delivery Reviews* **23** (1997) 3.

Challenging MDO (multiparameter drug optimization) for peptides

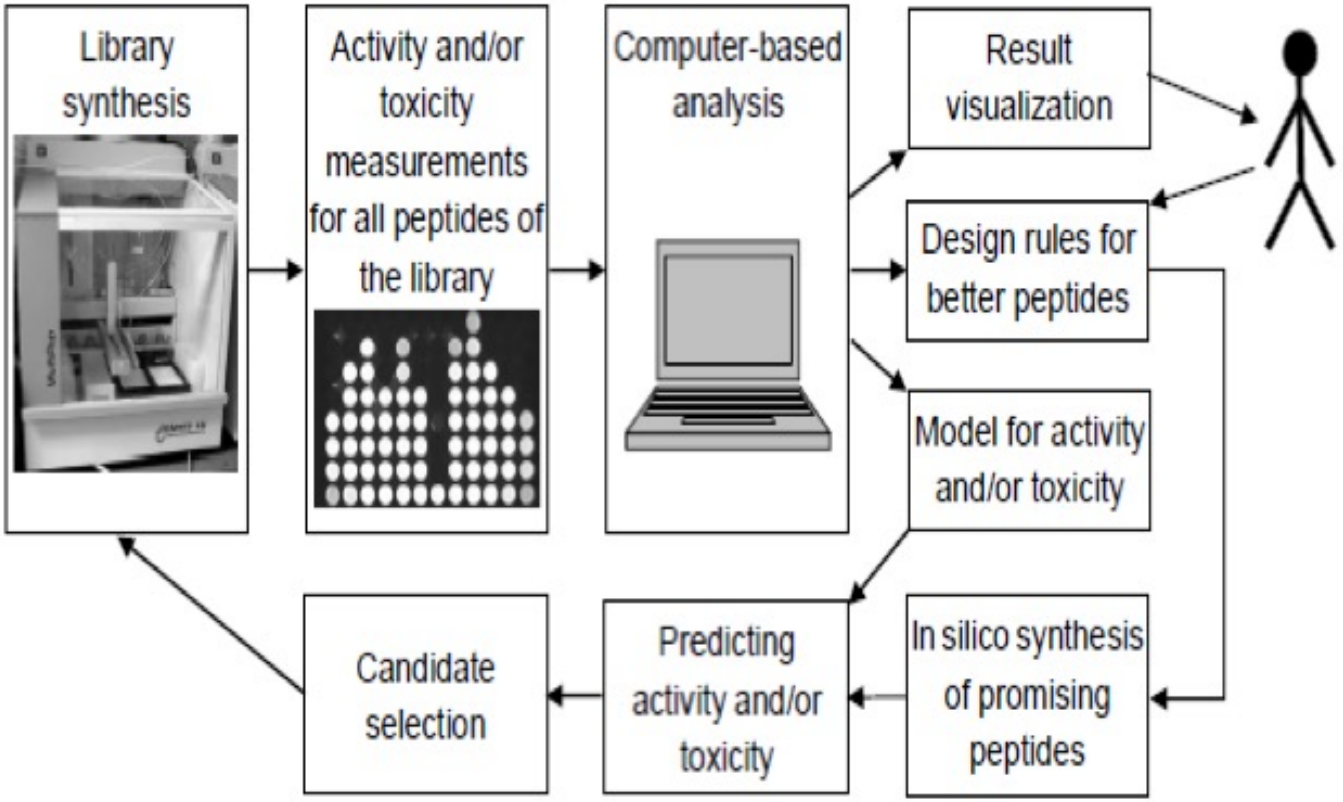


Figure 5. Optimization strategy for peptide libraries (modified from [134])

INSULIN – FIRST BIOLOGICAL

- **A century old peptide medicine that revolutionized therapies of type I diabetic patients and later challenged biotechnology**
- **A far reaching clinical revolution without precedent**

(average life expectancy of a 10y old type I diabetic patient was 1 y, today is >60 y !)

1869 P. Langerhans describes islets from pancreas
1889 Von Mehring : total pancreatectomy in dogs is followed by severe diabetes

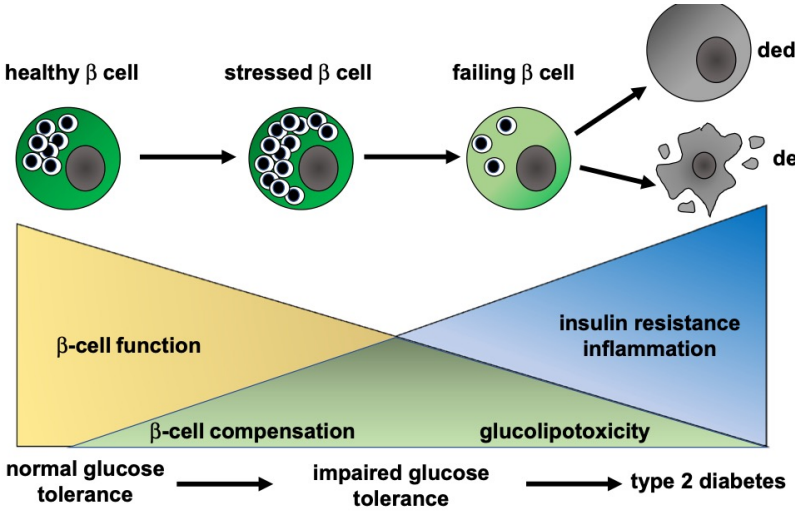
1921
F Banting-C.Best Beagle dogs

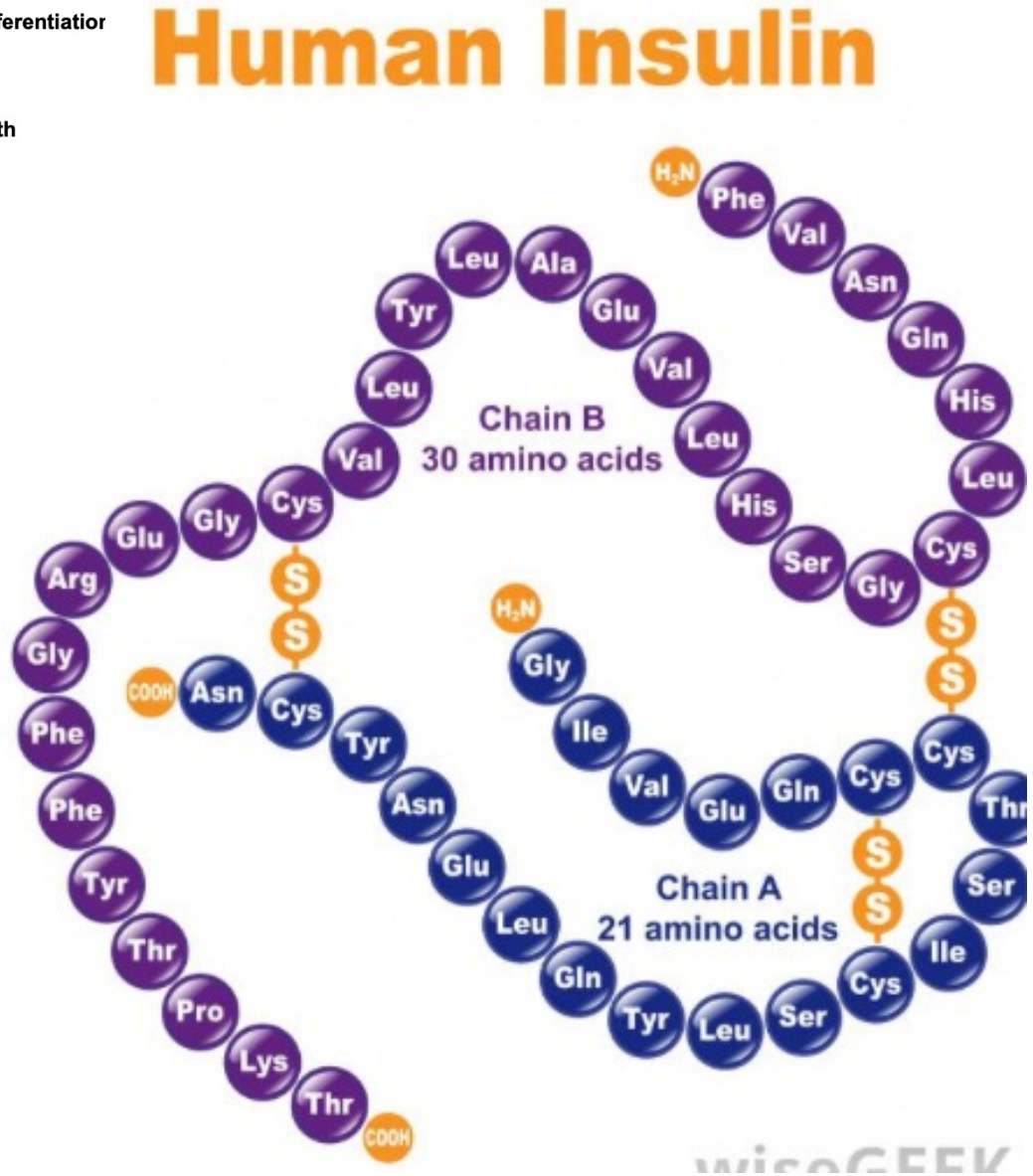
Discovery of the first biological (peptide) in Toronto: July 1921 - Banting and Best cracking 1000 y old mystery !

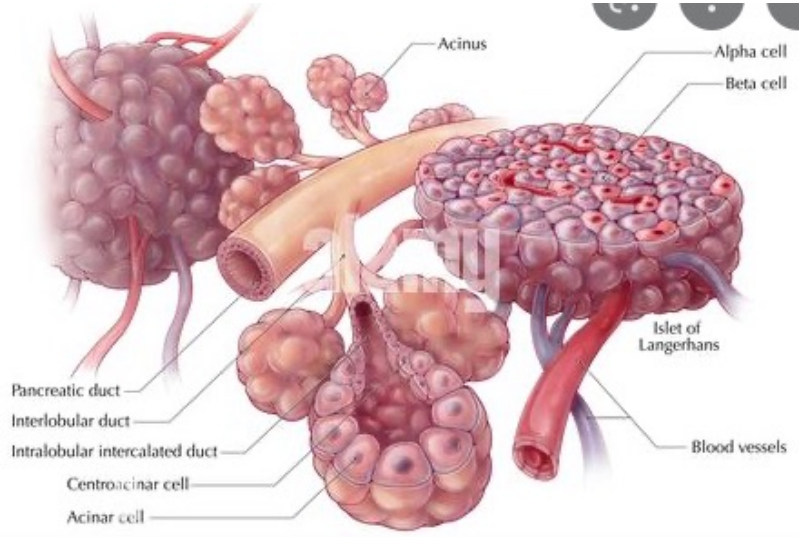
Prof Banting and McLoad 1929

« Inventas vitam iuvat excoluisse per artes »

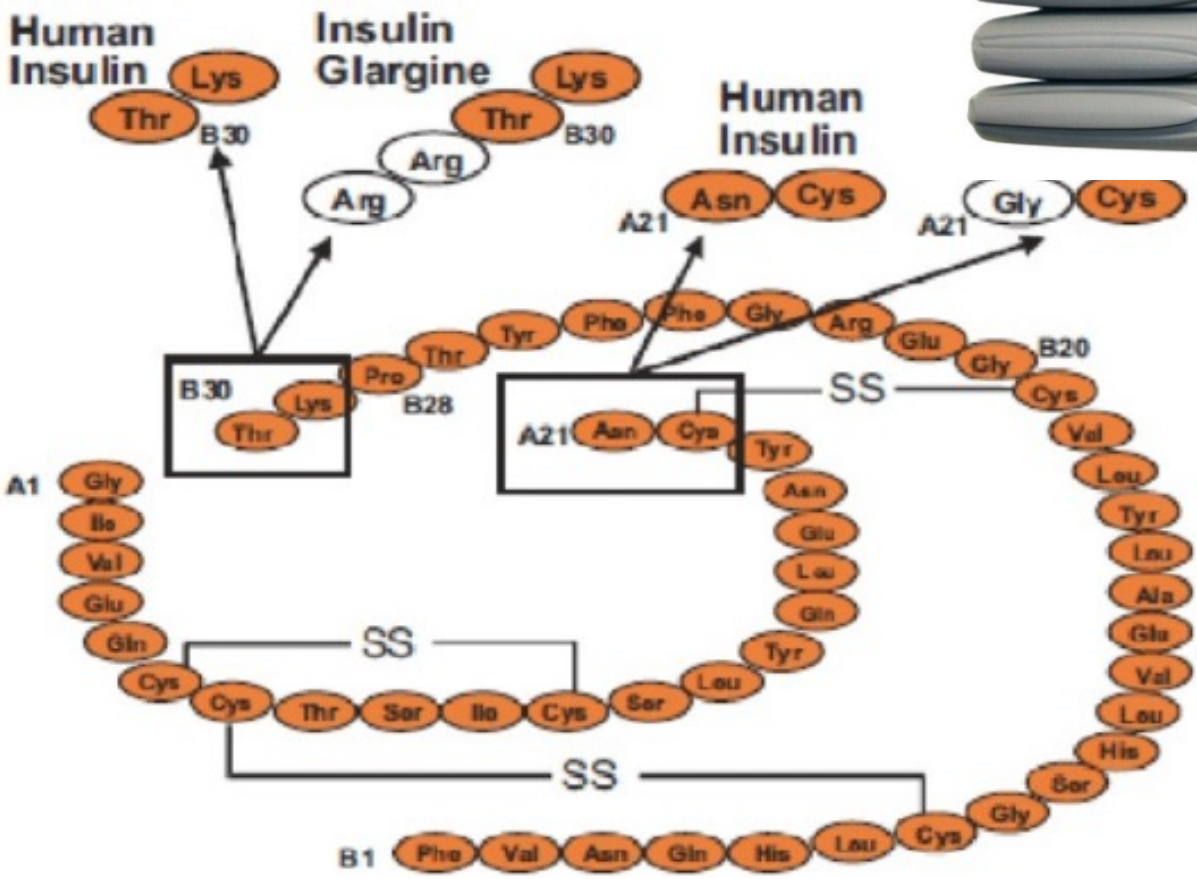
First biological (peptide) ever described :
Toronto F.Banting and C.Best cracking a 1000 y old mystery !



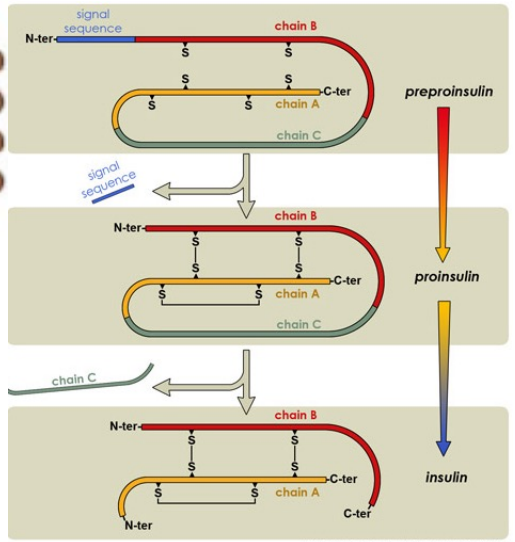




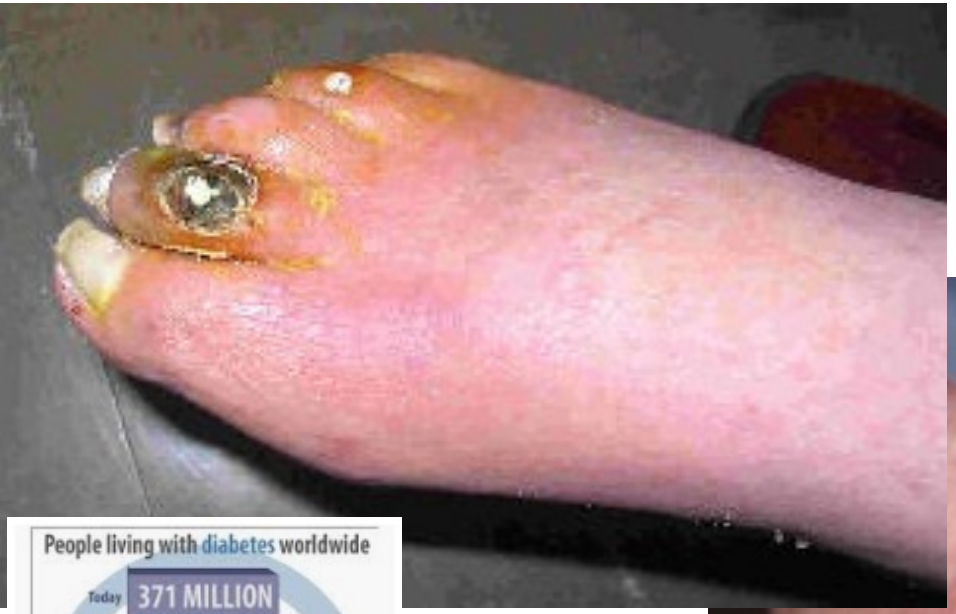
Insulin glargine differs by replacing Asp and Gly positon 21 and Cterm ext of chain B
Medical need ; shifting isoelectric point pH5.4 to 6.7 makes more soluble at pH7 and pharmacodynamic peakless level for 24 hours



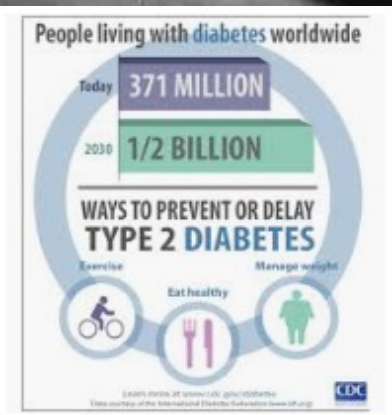
Modifications of the Insulin Sequence in Insulin Glargine.

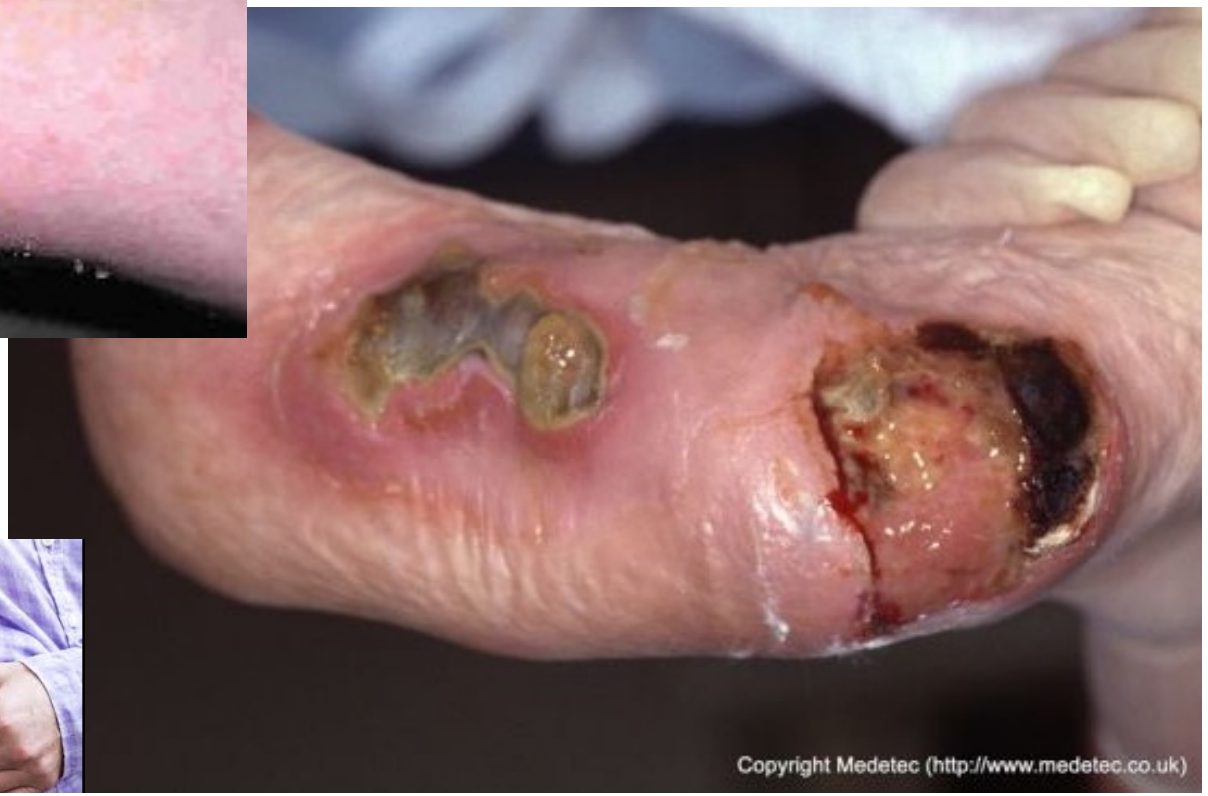


Advanced type II diabetes : peripheral ischaemia-necrosis



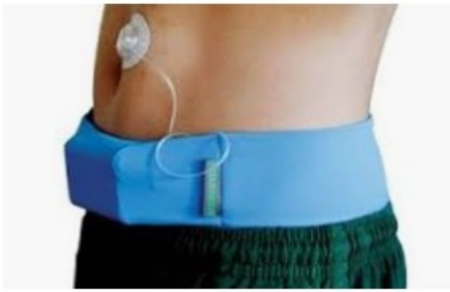
MEDICAL NEED :
IMPROVE INSULIN
SENSITIVITY ASAP !





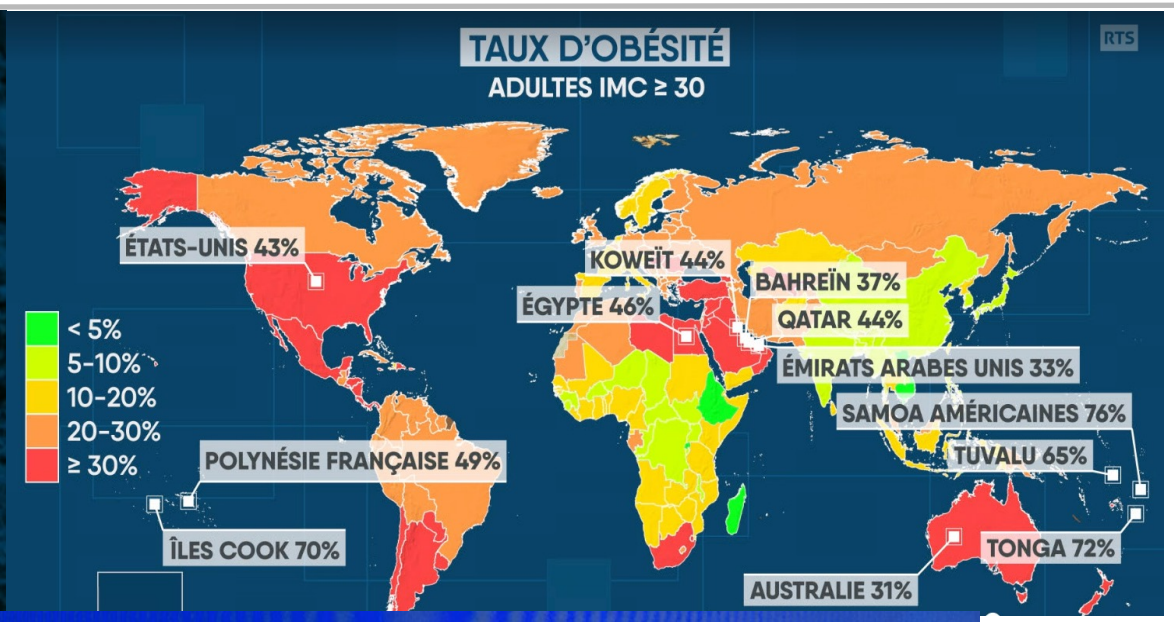
MACRO- AND MICROVASCULARIZATION IMPAIREMENTS

Insulin seringes, automatic pumps connected to acucheck today‘s reality in clinical practice with both type I and II diabetics



Global Insulin pump Accessories Market 2017 ...

ww pandemia – 4 fold prevalence increase in young adults !



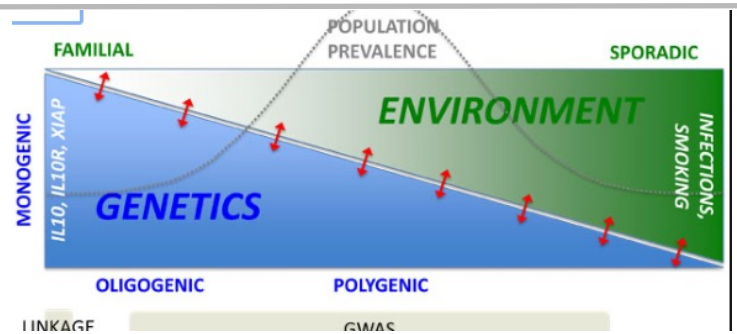
Metabolic diabesity syndrome : a public health challenge

#	Country	Income group	% obesity
19	United States	High income	42.74
20	Saudi Arabia	High income	42.45
21	Palau	High income	42.08
22	Puerto Rico	High income	42.05
23	Iraq	Upper-middle income	41.41
24	Jordan	Lower-middle income	39.93
25	Chile	High income	39.67
26	Barbados	High income	38.90
27	Palestine	Lower-middle income	38.52
28	Libya	Upper-middle income	37.91
29	Bahrain	High income	37.25
30	Panama	High income	37.06
31	Mexico	Upper-middle income	36.86
74	Poland	High income	28.28
75	Peru	Upper-middle income	28.12
76	Tunisia	Lower-middle income	27.71
77	United Kingdom	High income	27.63
78	Greenland	High income	27.58
79	Guatemala	Upper-middle income	27.57
80	Slovakia	High income	27.57
81	Azerbaijan	Upper-middle income	27.33
82	Czechia	High income	26.85
83	Kyrgyzstan	Lower-middle income	26.83
84	Canada	High income	26.73
85	Lithuania	High income	26.02
152	Tanzania	Lower-middle income	12.95
153	Kenya	Lower-middle income	12.87
154	Nigeria	Lower-middle income	12.84
155	Bhutan	Lower-middle income	12.68
156	Switzerland	High income	12.47

...er-middle ...me		15.44
...income		15.33
...h income		14.98
...income		14.93
...ver-middle ...me		14.21
...income		14.06
...h income		13.97
...h income		13.55
...ver-middle ...me		13.28

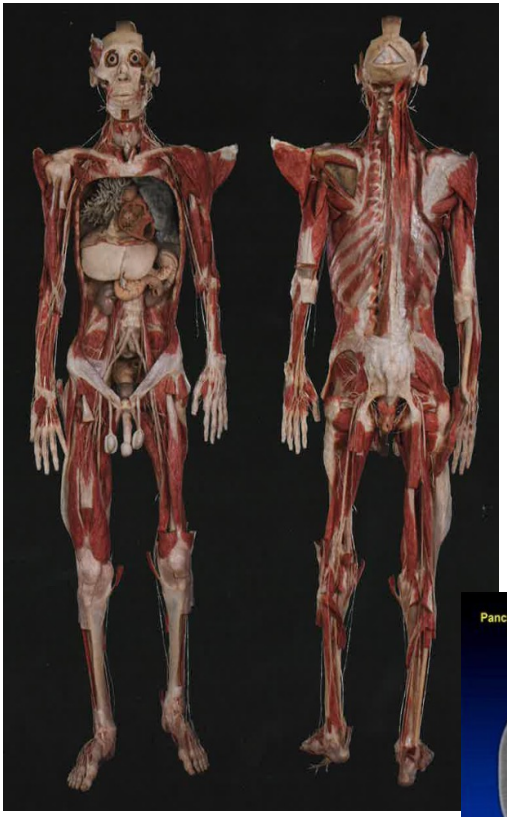
Metabolic syndrome : a public health challenge

Metabolic syndrome : when patients become insulin resistant

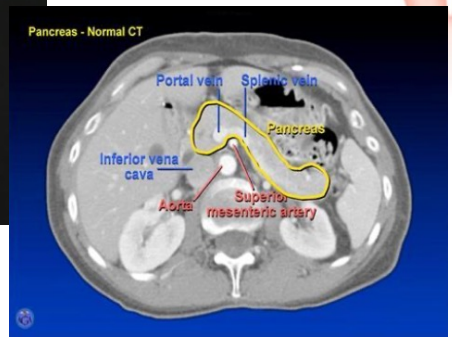


Metabolic Sydrome and Tissue-Tissue Cross Talk

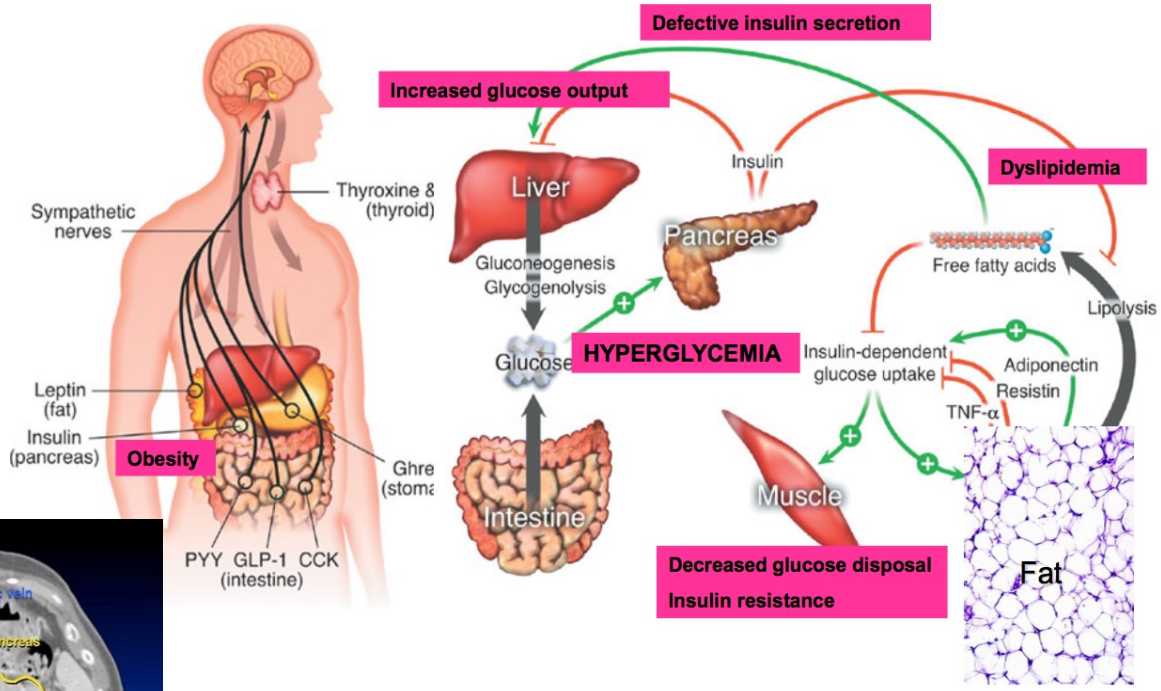
MEDICAL CHALLENGE : COMPLEX TRAITS DISEASE : GENETICS AND ENVIRONEMENT PLAY A COMBINED ROLE



Gubener Plastinate GmbH
Guben Germany

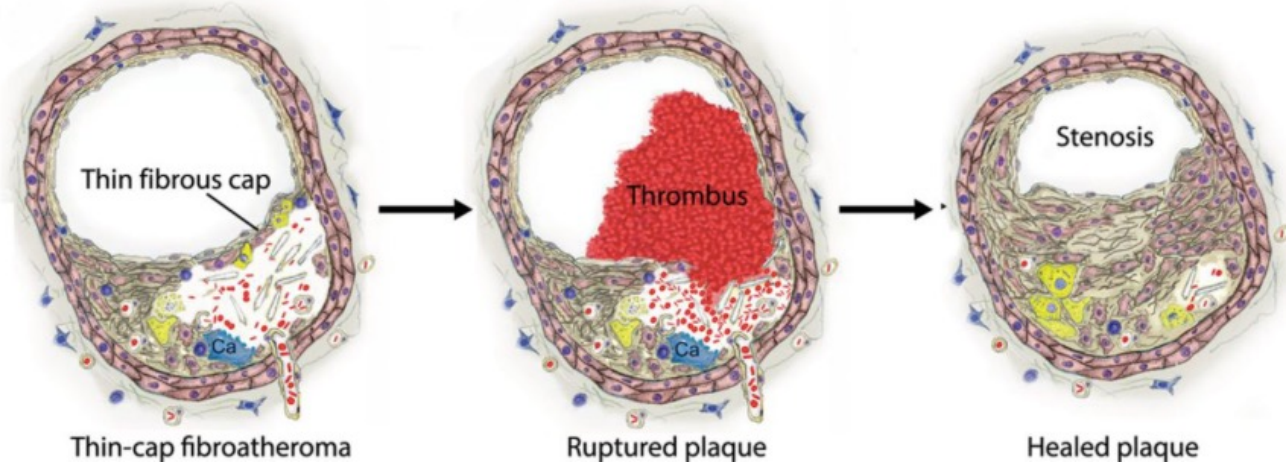


Metabolic Syndrome and Tissue-Tissue Cross Talk

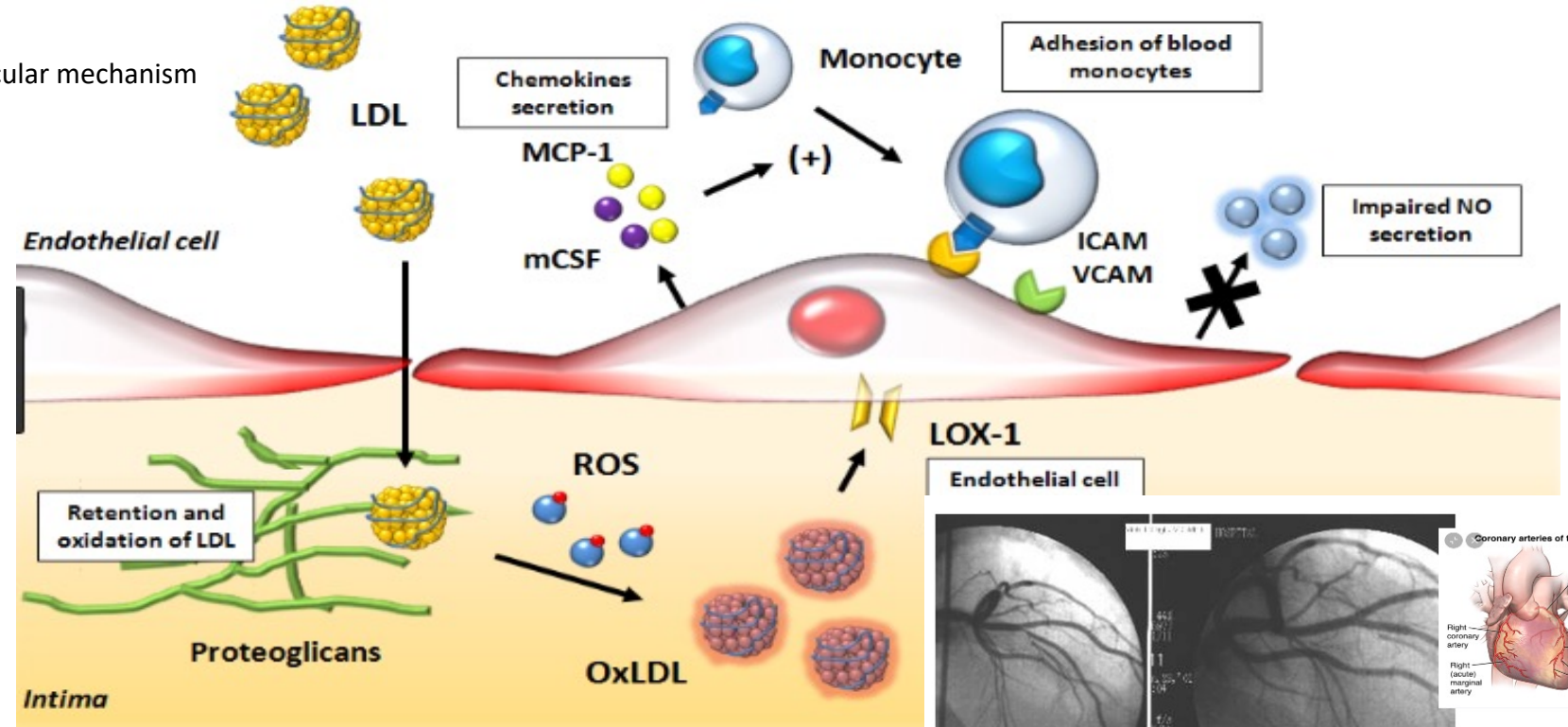


Stroke and acute heart failure : the unstable atheroma plaque

at histopathological level



at molecular mechanism



University sport activities : my contribution - bike tour 800 km 5 days on my bike !

NUMBER ONE THERAPY

Mit dem Rad auf Eucor-Tour

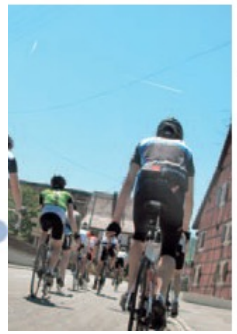
Jedes Jahr radeln Eucor-Studierende die fünf Unistädte am Oberrhein ab

FOTOS: TEAM TOUR EUCOR

EUCOR KIT

…rie Schaub

…uhe, Straßburg, Mulhouse, …el, Freiburg. Fünf Eucor-Unis, …pen, fünf Tage auf dem Sattel, …cke von 600 bis 800 Kilome… …lieber in die Pedale tritt, als …Bänken im Hörsaal zu sitzen, …Eucor-Unis mit dem Fahrrad …. Jeden Frühling organisieren …de an dem Karlsruher Institut …ologie (KIT) die „Tour Eucor", …tour, bei der jede Eucor-Uni …ufziel ist. „Die Initial-Idee für …Tour Eucor 1998 war relativ …erklärt Paul Knipper aus dem …tionsteam. Damit der Eucor- …nicht nur auf dem Papier steht, endet nach wie vor zwar in Karlsruhe, aber teilnehmen können alle Mitarbeiterinnen und Mitarbeiter, Studierende und Alumni der fünf oberrheinischen Universitäten.



„Keine Hobbybastler"

Die Pioniere gründeten einen Verein, der mittlerweile zu einem professionellen Organisationsteam herangewachsen ist. Neben Streckenführerinnen und Streckenführern stellt das Team zehn Begleiterinnen und Begleiter zusammen, die mit den Tourmobilen die Radsportlerinnen und Radsportler verpflegen und sich um den Gepäcktransport kümmern. Diese logistische Höchstleistung begeistert so manchen Teilnehmenden. „Das sind keine Hobbybastler, die kommen mit dem Mercedesbastler auf die Minute genau an. Es ist beeindruckend, wie die das organisieren", so Roger Clerc. Der Professor für Biomedizin und Molekularbiologie an der Universität Basel fährt selbst seit drei Jahren mit und befürchtet, der einzige Dozent zu sein, der sich für die Eucor-Strecke auf den Sattel schwingt. Denn die Lehrenden machen sich auf den circa 700 Kilometern rar. „Viele haben Bedenken, weil sie dieselben Studenten ja bei den Prüfungen wiedertreffen könnten", sagt Clerc, der als Damit jeder auf seine Kosten kommt, gibt es unterschiedliche Routen, die nach Schwierigkeitsgraden eingeteilt sind. In der Kategorie „blau" fahren gemütliche Stadtradfahrer immer den direktesten Weg zwischen den Etappen. Steigungen werden vermieden, heißt es auf der Homepage. Die „Hellroten" haben eine ähnliche Strecke zu bewältigen, nehmen aber kleine Steigungen für einen schönen Ausblick in Kauf. Gruppe „mittelrot" hat etwas mehr Geschwindigkeit und Berge auf der Strecke. „Dunkelrot" setzt noch eins drauf, aber die Profis und Rennradler befinden sich meist in den schwarzen Gruppen. „Hellschwarz" eignet sich für Radsportler - die „Dunkelschwarzen" suchen die ultimative Herausforderung, nehmen jeden Berg mit und legen die längste Strecke zurück.

Einzige Bedingung für die Teilnehmer: Das Rad muss fahrtüchtig sein und jeder sollte einen Ersatzschlauch

im Gepäck haben. Denn Pannen passieren immer wieder. Manchmal aber auch, weil die Räder nicht ausreichend auf solche Strecken vorbereitet wurden. Da wetzen sich schnell Bremsen ab oder alte Schläuche platzen. Deshalb sollte jeder Teilnehmer seinen Drahtesel vor der Tour nochmal gründlich unter die Lupe nehmen, raten die Radprofis vom Team.

Eucor an der frischen Luft erleben

Und dann kann's losgehen. Ob sportliche Herausforderung, Gruppenerlebnis oder Rheinidylle, den persönlichen Anspruch kann sich jeder Teilnehmer selbst aussuchen. Das Ziel für Michael Winz, Student in Freiburg, war es, fünf Tage auf dem Fahrrad zu sitzen und für die „Vaude Transschwarzwald" zu trainieren, ein Etappenrennen für Freizeitsportlerinnen und Freizeitsportler und Profis. Auch er findet die Radtour „eine tolle Sache". Die gemeinsamen Erlebnisse auf der Strecke und das Abendprogramm in den Eucor-Städten schweißen zusammen. Auch wenn die Kontakte nicht immer von Dauer sind, haben sich während der Tour schon interessante Bekanntschaften ergeben. Roger Clerc profitiert gerne von seinen jüngeren Weggefährten, um mit ihnen

metabolic syndrome pandemia :
best preventive medicine : exercise every day !

VORWORT

NUMBER ONE THERAPY

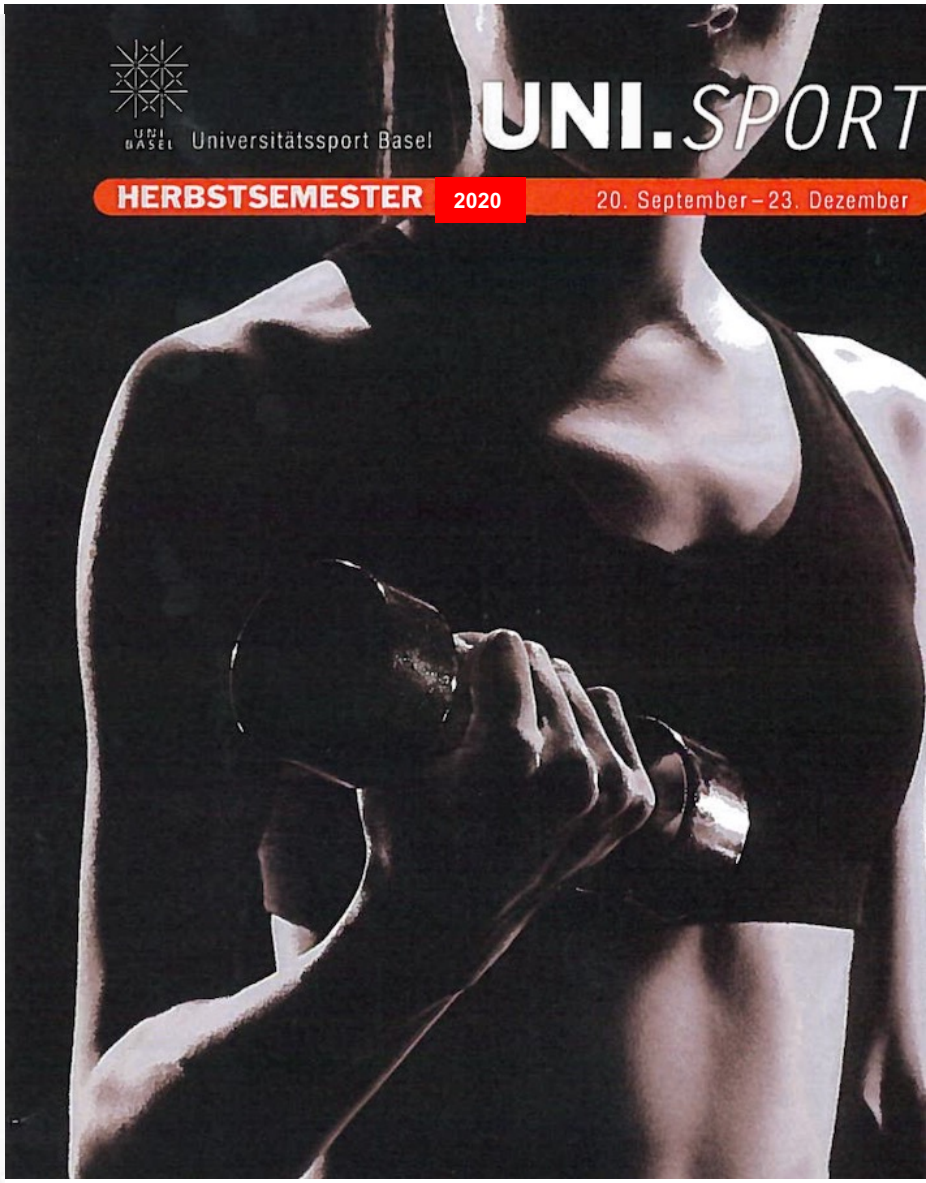
«Sparsame Gene»: Trainiere jeden Tag beim Unisport!

Das Universitätsstudium und insbesondere seine Prüfungen innerhalb bestimmter Fristen sind stressig. Wir sind so mit einem Lebensstil konfrontiert, welcher uns - nicht nur an der Universität - immer mehr geistige Leistungen in immer kurzfristigeren Zeithorizonten abverlangt, wobei wir uns oft nicht mehr ausreichend körperlich aktiv bewegen. Zu dieser Bewegungsarmut gesellt sich oft eine ungesunde Ernährung. Viele Menschen sind daher übergewichtig und entwickeln metabolisches Syndrom mit u.a. erhöhtem Blutzucker, Bluthochdruck und erhöhten Blutfettwerten.

Unser Genom hat sich in Millionen von Jahren entwickelt, in denen Nahrungsmangel herrschte. Der Mensch der Urzeit war ein hoch beweglicher Jäger, der nur selten etwas zum Essen fand. Sein Körper passte sich evolutiv daran an, diese rare kostbare Energie in seinen Geweben dank der "sparsamen Gene" zu speichern. An den erst seit wenigen Jahrzehnten herrschenden „plötzlichen" Überschuss an sehr kalorienreicher Nahrung und die mangelnde körperliche Bewegung heutzutage, konnte sich unser Genom noch nicht anpassen. Um das empfindliche Gleichgewicht unserer körperlichen und geistigen Gesundheit zu erhalten, ist es deshalb notwendig, regelmässig sportliche Aktivitäten, die Spass machen, zu betreiben. Auch eine intelligente Ernährung, mit wenig Zucker und gesättigten Fetten, dafür aber reich an Ballaststoffen und Eiweiss, und ein nur moderater Alkoholkonsum, sind notwendig, um unsere "sparsamen Gene" so zu trainieren, dass wir kein metabolisches Syndrom entwickeln. Deswegen gibt es Unisport: um die Balance zwischen Körper und Gehirn während dem Studium beizubehalten

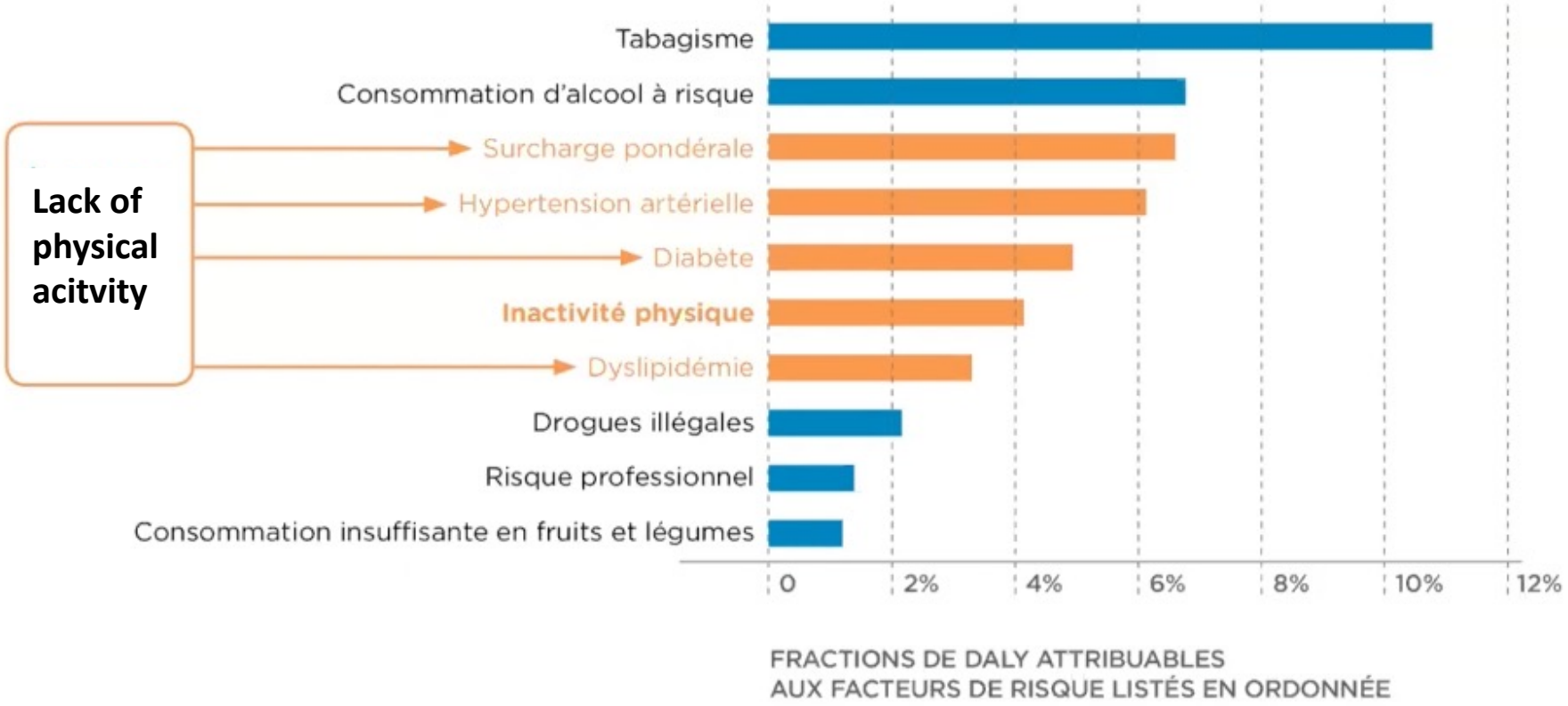
Buchen Sie daher jeden Tag einen Sport-Termin im Kalender und geniessen Sie das aussergewöhnliche Sportangebot der Universität! Über 80 verschiedene Sportarten werden im neuen Unisport Programm angeboten. Beteiligen Sie sich aktiv am Unisport. „A day without sport is not a successful day!"

Prof. Dr. Roger G. Clerc



Type II diabetes and metabolic syndrome pandemia : best preventive medicine : exercise every day !

FIGURE 3
CLASSEMENT DES PRINCIPAUX FACTEURS DE RISQUES RESPONSABLES
DES ANNÉES DE VIE PERDUES DANS LES PAYS DÉVELOPPÉS, 2004 (ADAPTÉ DE WHO 2009)



Risk factors involved in life expectancy reduction in developed countries (loss of lived years)

(adapted from WHO 2009 from Prof V Gremaux CHUV UNIL)

GLOBAL HEALTH METRICS | VOLUME 396, ISSUE 10258, P1223-1249, OCTOBER 17, 2020

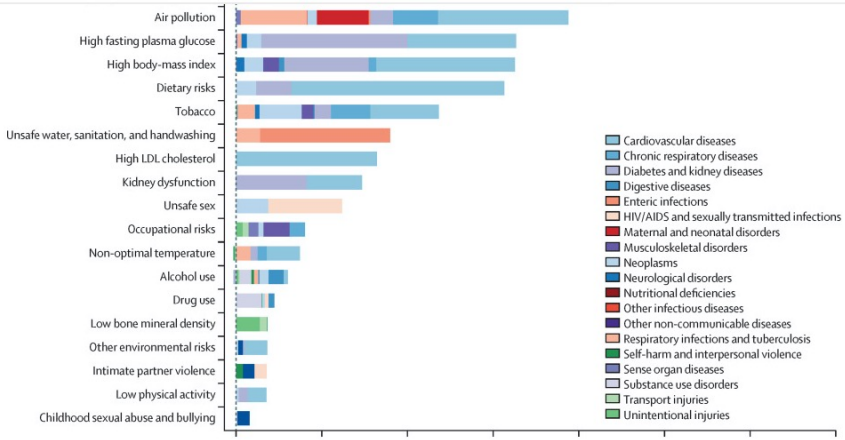
Global burden of 87 risk factors in 204 countries and territories, 1990–2019: a systematic analysis for the Global Burden of Disease Study 2019

GBD 2019 Risk Factors Collaborators [†] • Show footnotes

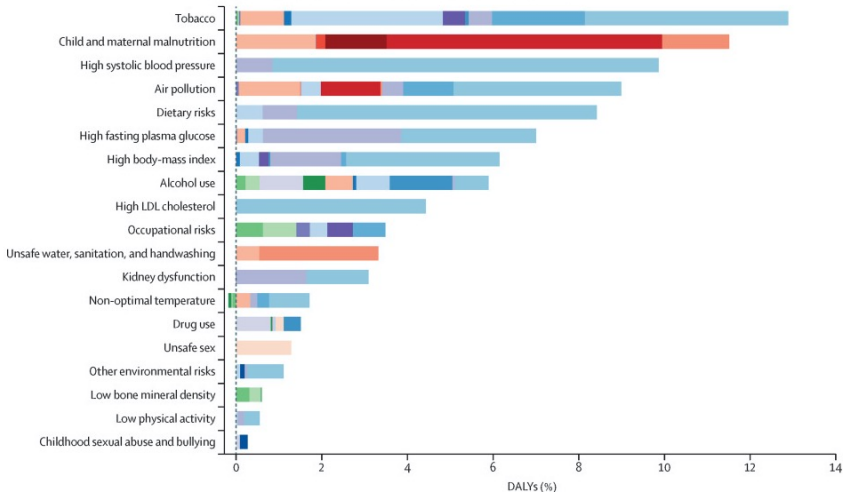
Open Access • Published: October 17, 2020 • DOI: https://doi.org/10.1016/S0140-6736(20)30752-2 •

THE LANCET

Global burden of 87 risk factors in 204 countries and territorie...



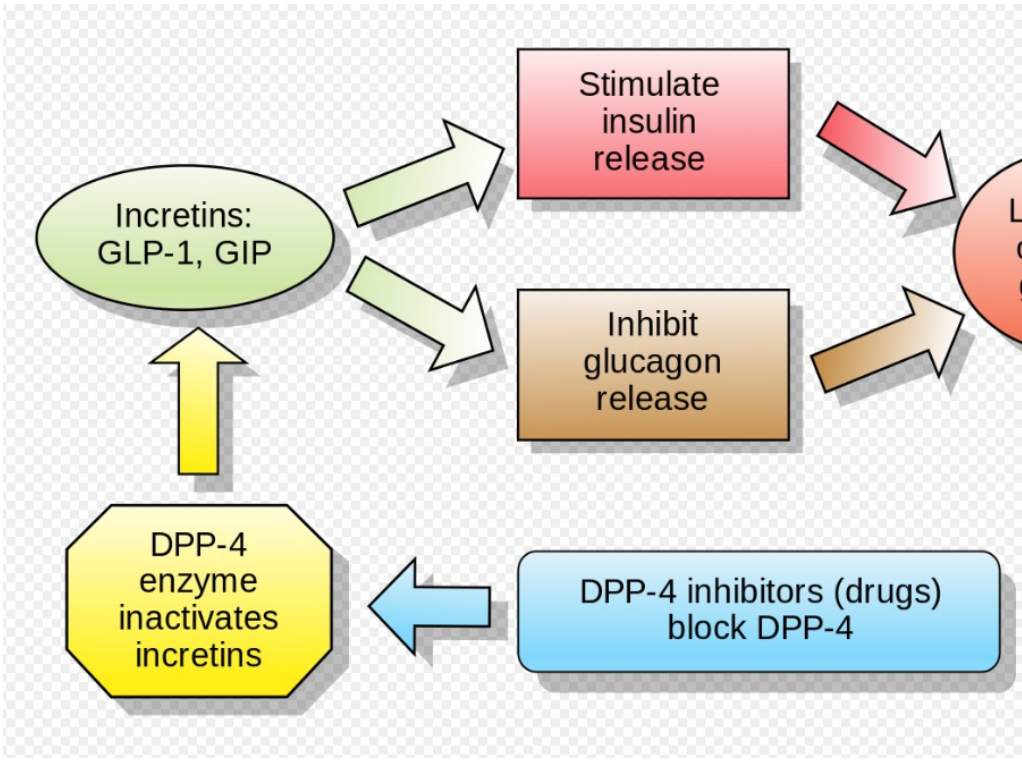
D Global attributable DALYs from Level 2 risk factors for males in 2019

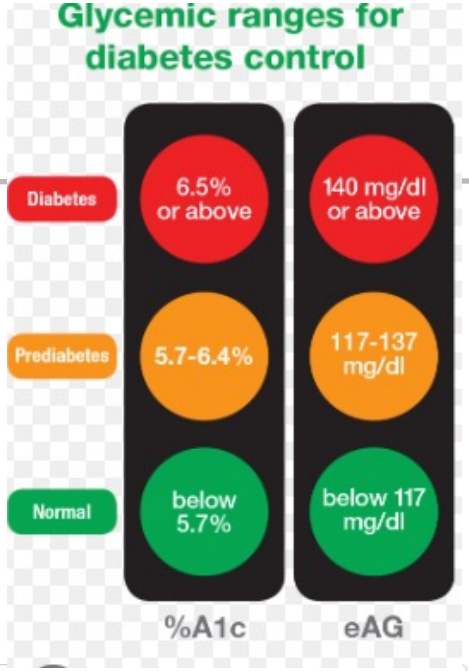


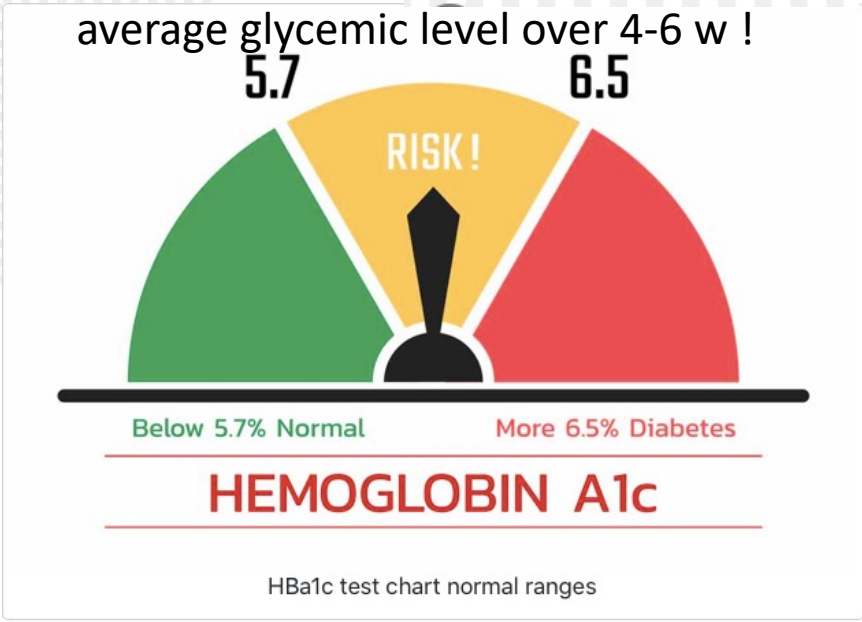
BENEFITS OF PHYSICAL ACTIVITY : THE SEDENTARITY DISEASES

	ADULTES, TOUS ÂGES
MORTALITÉ, TOUTE CAUSE	Diminution du risque
SANTÉ CARDIOMÉTABOLIQUE	Diminution du risque de maladie cardiovasculaire et de mortalité cardiovasculaire Diminution du risque d'hypertension Diminution du risque de diabète de type 2
CANCER	Diminution du risque de cancer de la vessie, du sein, du colon, de l'endomètre, de l'œsophage, du rein, de l'estomac et du poumon
SANTÉ MENTALE	Réduction du risque de démence Amélioration des fonctions cognitives Amélioration de la qualité de vie Amélioration du sommeil Réduction du risque de dépression
STATUT PONDÉRAL	Effet supplémentaire sur la perte de poids lorsque l'activité physique est combinée avec une restriction alimentaire modérée Perte de poids et prévention de la reprise de poids lorsqu'une quantité suffisante d'activité physique d'intensité modérée à élevée est atteinte.
	PERSONNES ÂGÉES
CHUTES	Réduction du risque de chute Réduction du risque de blessure liée à une chute
CAPACITÉ PHYSIQUE	Amélioration de la capacité physique chez la personne âgée avec et sans fragilités.
	FEMMES ENCEINTES OU EN POST-PARTUM
DURANT LA GROSSESSE	Réduction du risque d'une prise de poids excessive Réduction du risque de diabète gestationnel Aucun risque pour le fœtus lié à une activité physique d'intensité modérée
DURANT LA PÉRIODE DU POST-PARTUM	Réduction du risque de dépression post-partum

Peptide drugs – GLP1r and incretins

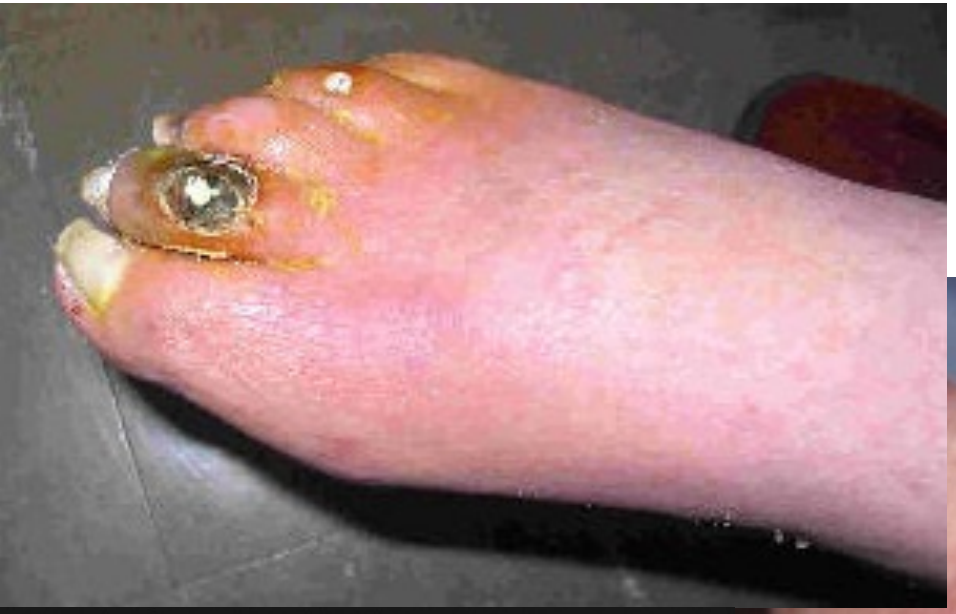




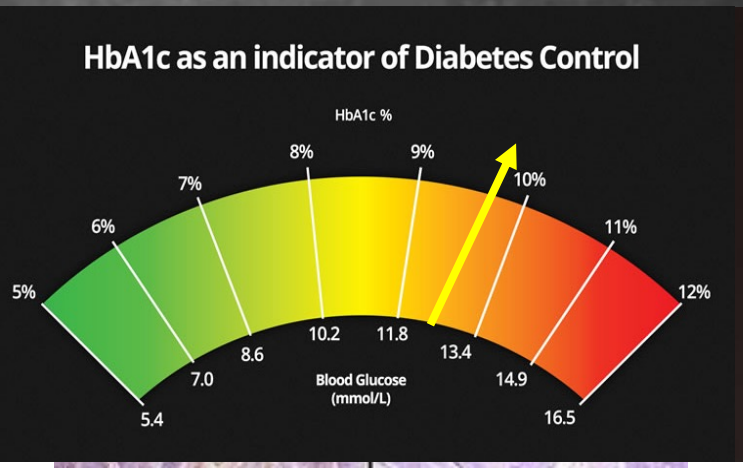


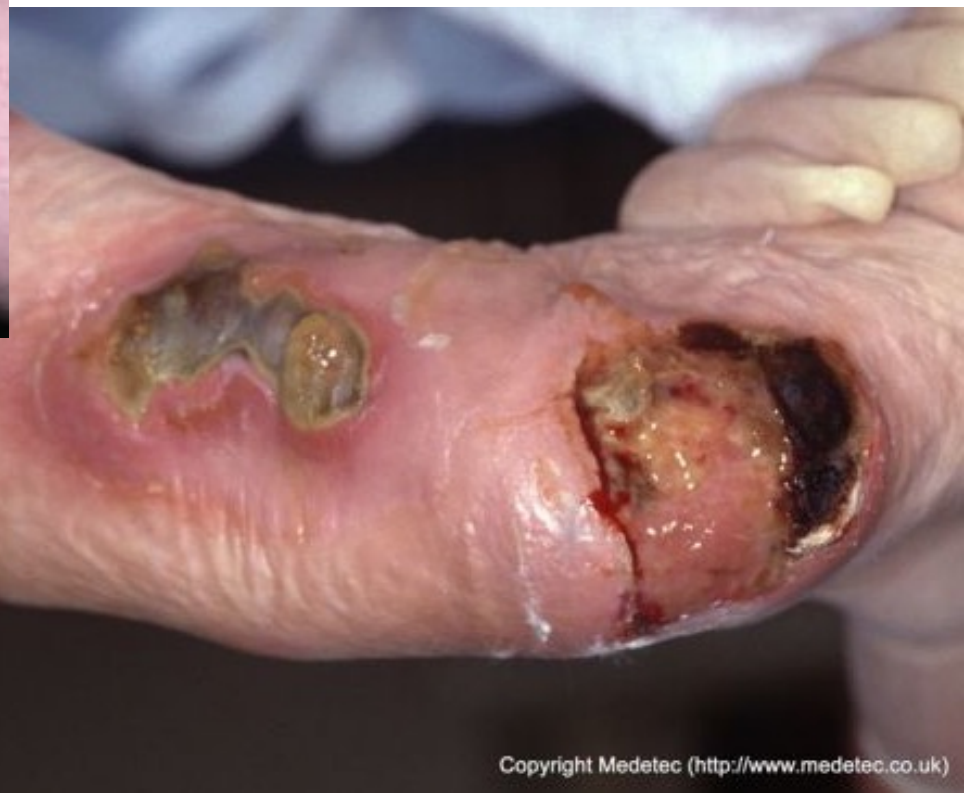
- **GLP1 receptor agonists (incretins) from gut L cells have entered the clinics in the last decade**
- **for management of diabetes type II and obesity - drawback: short half life, recurrent injections**

Advanced type II diabetes : peripheral ischaemia-necrosis
Insulin resistance as a consequence of obesity



MEDICAL NEED : IMPROVE TYPE II DIABETES **USING A MEDICINAL CHEMISTRY DESIGN**



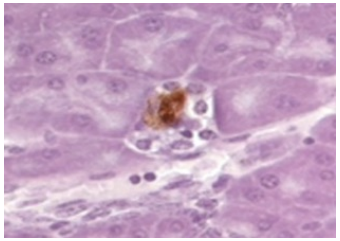


MACRO- AND MICROVASCULARIZATION IMPAIREMENTS

Progression to type II diabetes : pancreatic beta cells exhaustion

pathophysiology of an insidious disease

Stage I II III IV



Obese
Peripheral insulin resistance

Pre-diabetic/obese
Impaired glucose tolerance

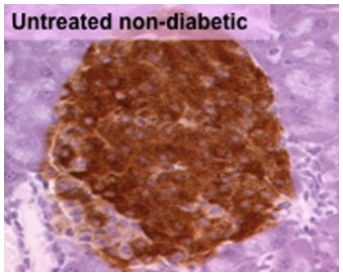
β-cell failure and insulin resistance

diet

Early diabetes

Late diabetes hyperglycaemia

Prevention
- insulin sensitization
- organoprotection ?
- decrease vascular risk?



Intervention
- Normalize glucose
- Preserve β-cell insulin?
- decrease vascular risk?

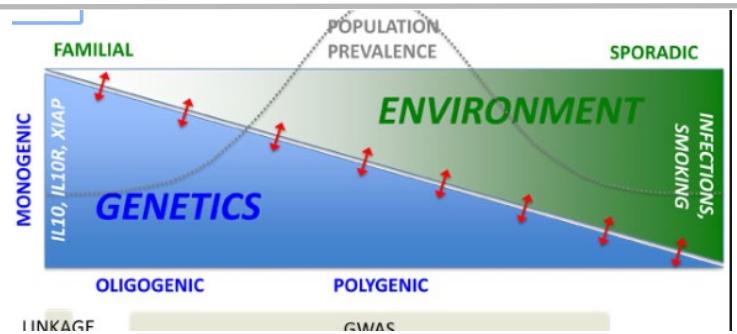
Vascular complications

insulin

← Environment + "obesigenes" →

← "+diabetogenes" →

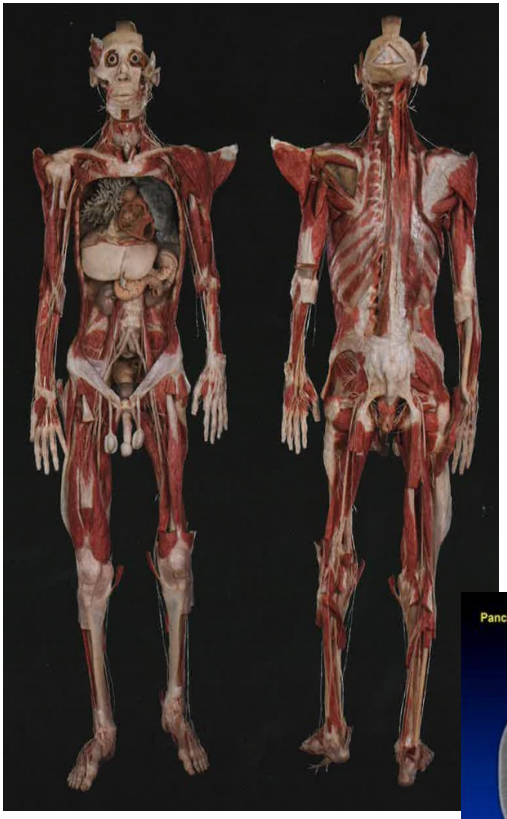
Physiopathology of metabolic syndrome : when patients become insulin resistant



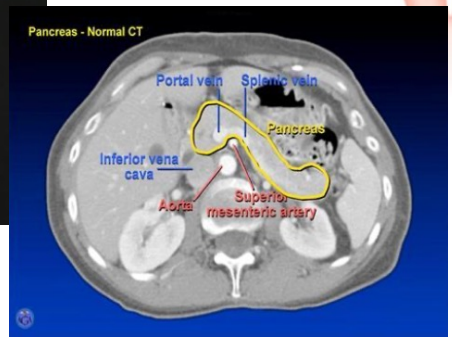
Metabolic Sydrome and Tissue-Tissue Cross Talk

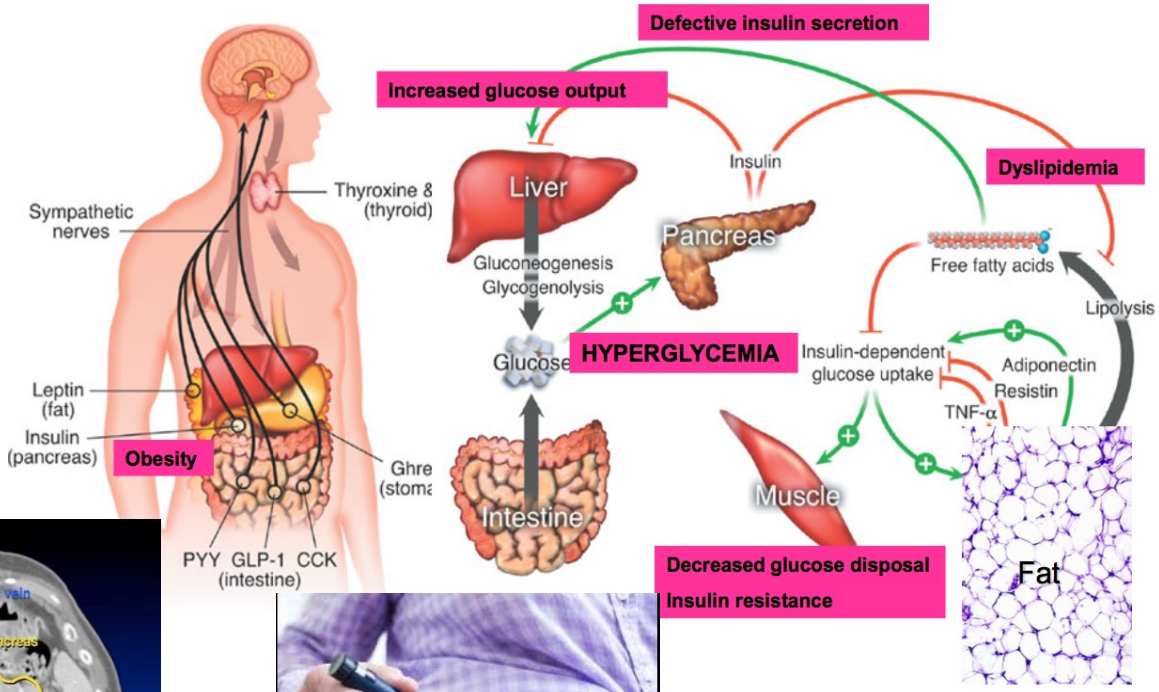
MEDICAL CHALLENGE : COMPLEX TRAITS DISEASE : GENETICS AND ENVIRONEMENT PLAY A COMBINED ROLE

Metabolic Syndrome and Tissue-Tissue Cross Talk



Gubener Plastinate GmbH
Guben Germany





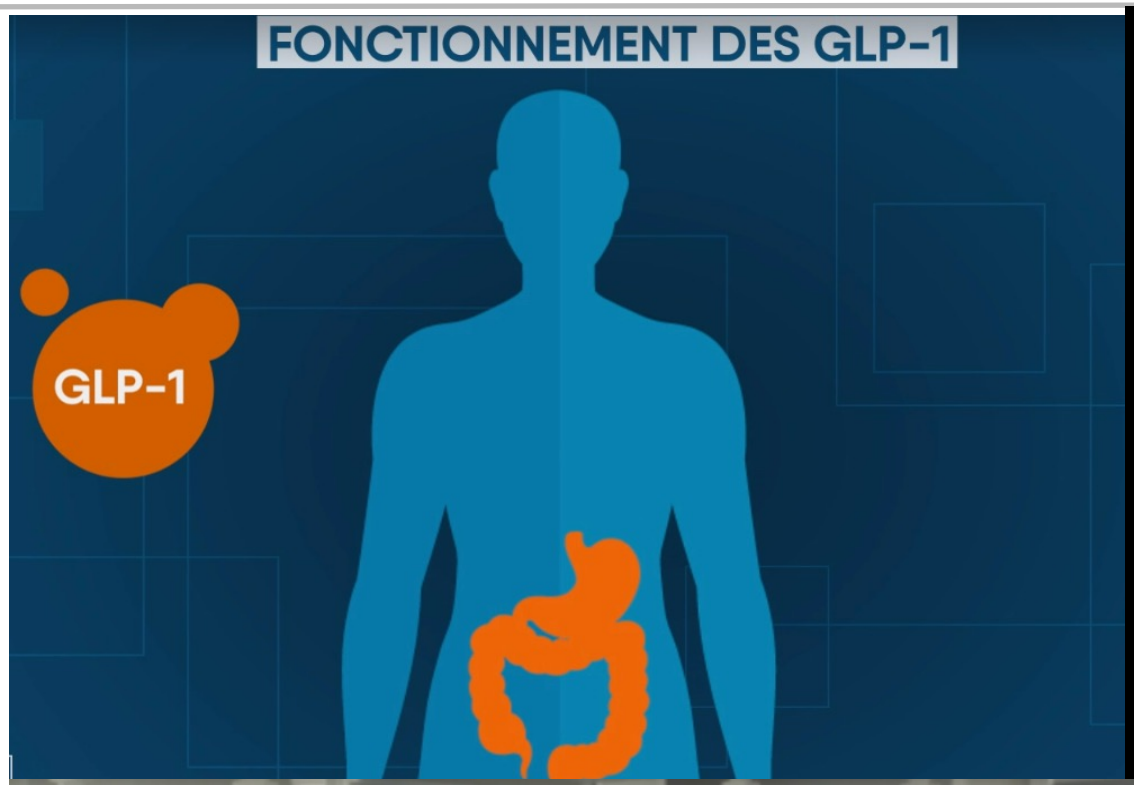
Type I diabetes develops as an auto immune disease

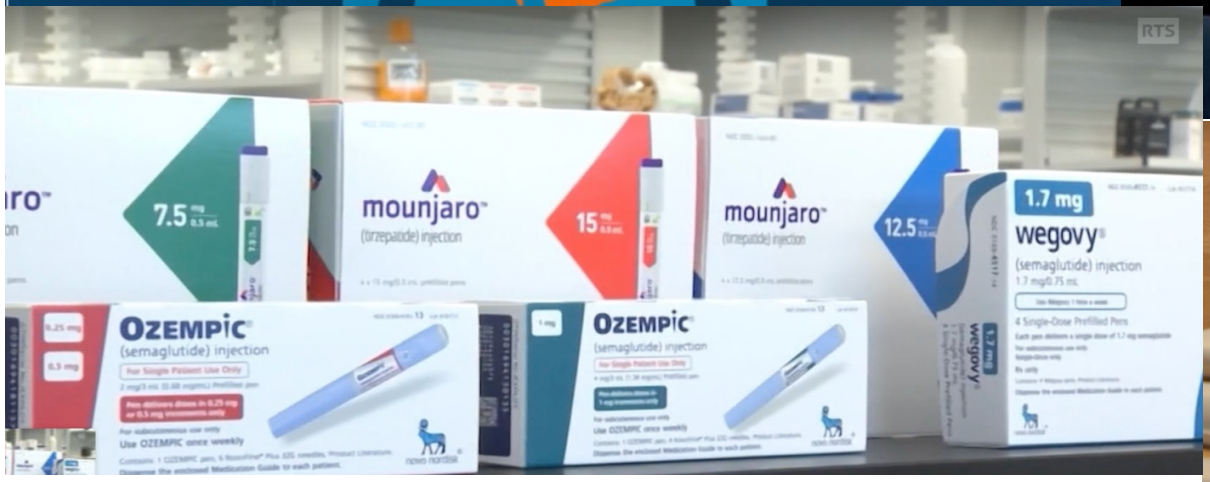
(prevalence 5-10% of all diabetic patients)

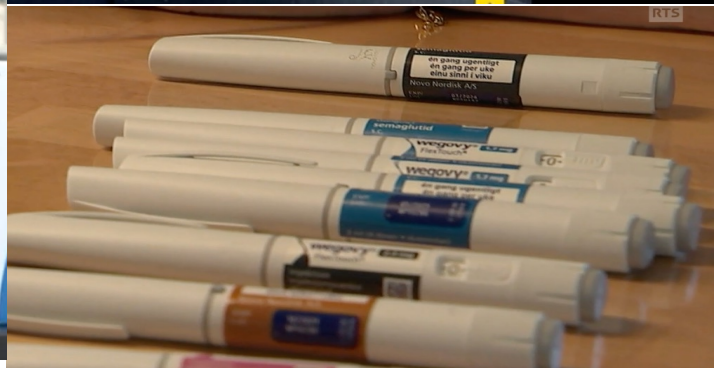
Cases of type 1 diabetes, which is caused by the body's own immune system attacking pancreatic cells, rose in young people during the COVID pandemic Credit: BSIP/Universal Images Group via Getty

Nature News July 21. 2023

GLP1-2 agonists - originally developed in type 2 diabetes insulin resistance pathologies !







The advent of the glucagon peptide superfamily – 1980-1990s

THE JOURNAL OF BIOLOGICAL CHEMISTRY

Vol. 265, No. 33, Issue of November 25, pp. 20259–20262, 1990
Printed in U.S.A.

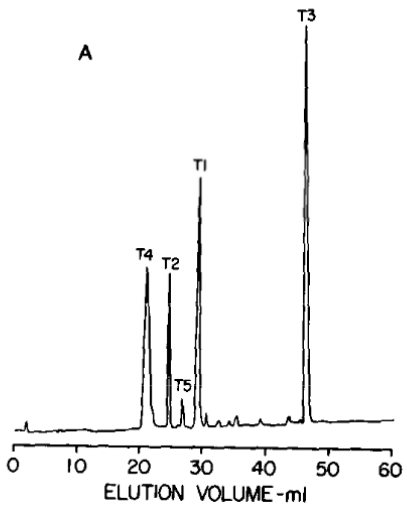
Purification and Structure of Exendin-3, a New Pancreatic Secretagogue Isolated from *Heloderma horridum* Venom*

(Received for publication, April 10, 1990)

John Eng‡§¶, P. C. Andrews‖, Wayne A. Kleinman‡, Latika Singh, and Jean-Pierre Raufman****

*From the ‡Solomon A. Berson Research Laboratory, Veterans Affairs Medical Center, Bronx, New York 10468, the §Department of Medicine, Mount Sinai School of Medicine, New York, New York 10029, the ‖Department of Biological Chemistry, University of Michigan Medical School, Ann Arbor, Michigan 48109, and the **Department of Medicine, State University of New York-Health Science Center, Brooklyn, New York 11203*

An amino-terminal histidyl structure (His[1]) is characteristic of most peptides in the glucagon superfamily. minal sequencing was used to screen for new His[1] peptides that might be members of the glucagon superfamily. Gila



% HOMOLOGY		5 10 15 20 25 30 35 40 45
100	EXENDIN-3	H S D G T F T S D L S K Q M E E E A V R L F I E W L K N G G P S S G A P P P S #
32	HELOSPECTIN	H S D A T F T A E Y S K L L A K L A L Q K Y L E S I L G S S T S P R P P S S
26	HELODERMIN	H S D A I F T E E Y S K L L A K L A L Q K Y L A S I L G S R T S P P P #
41	SECRETIN	H S D G T F T S E L S R L R D S A R L Q R L L Q G L V #
48	GLUCAGON	H S Q G T F T S D Y S K Y L D S R R A Q D F V Q W L M N T
50	GLP-1	H A E G T F T S D V S S Y L E G Q A A K E F I A W L V K G R #
29	GLP-2	H A D G S F S D E M N T I L D N L A A R D F I N W L I Q T K I T D R
37	PHI	H A D G V F T S D F S R L L G Q L S A K K Y L E S L I #
29	VIP	H S D A V F T D N Y T R L R K Q M A V K K Y L N S I L N #
26	GIP	Y A E G T F I S D Y S I A M D K I R Q Q D F V N W L L A Q K G K K S D W K H N I T Q
13	GRF	Y A D A I F T N S Y R K V L G Q L S A R K L L Q D I M S R Q Q G E S N Q E R G A R A R L #
24	PACAP38	H S D G I F T D S Y S R Y R K Q M A V K K Y L A A V L G K R Y K Q R V K N K #

Papers : the advent of the glucagon peptide superfamily - 1990s

THE JOURNAL OF BIOLOGICAL CHEMISTRY

Vol. 265, No. 33, Issue of November 25, pp. 20259–20262, 1990
Printed in U.S.A.

Purification and Structure of Exendin-3, a New Pancreatic Secretagogue Isolated from *Heloderma horridum* Venom*

(Received for publication, April 10, 1990)

John Eng‡§¶, P. C. Andrews‖, Wayne A. Kleinman‡, Latika Singh, and Jean-Pierre Raufman****

*From the ‡Solomon A. Berson Research Laboratory, Veterans Affairs Medical Center, Bronx, New York 10468, the §Department of Medicine, Mount Sinai School of Medicine, New York, New York 10029, the ‖Department of Biological Chemistry, University of Michigan Medical School, Ann Arbor, Michigan 48109, and the **Department of Medicine, State University of New York-Health Science Center, Brooklyn, New York 11203*

An amino-terminal histidyl structure (His[1]) is characteristic of most peptides in the glucagon superfamily. An assay for His[1] peptides performed by amino-terminal amino acid sequencing was used to screen venom from the Gila monster lizard, *Heloderma horridum*. Two His[1] peptides were identified: helospectin and a new His[1] peptide that has been named exendin-3 to indicate that it is the third peptide to be found in an exocrine secretion of *Heloderma* lizards which has endocrine activity, the first two being helospectin (exendin-1) and helodermin (exendin-2). In the lot of *H. horridum* venom tested, exendin-3 was 5–10-fold more abundant in molar concentration than helospectin. The structure of exendin-3 was analyzed by amino acid sequencing and mass spectrometry. Exendin-3 is a 39-amino acid peptide with a mass of 4200. It contains a carboxyl-terminal amide and has a strong homology with secretin at its amino-terminal 12 amino acids. The complete structure of exendin-3 is His-Ser-Asp-Gly-Thr-Phe-Thr-Ser-Asp-Leu-Ser-Lys-Gln-Met-Glu-Glu-Glu-Ala-Val-Arg-Leu-Phe-Ile-Glu-Trp-Leu-Lys-Asn-Gly-Gly-Pro-Ser-Ser-Gly-Ala-Pro-Pro-Pro-Ser-amide. It is 32 and 26% homologous with helospectin and helodermin, respectively. It has greatest homology with glucagon (48%) and human glucagon-like peptide-1 (50%). Exendin-3 (3 μM) stimulated increases in cellular cAMP and amylase release from dispersed guinea pig pancreatic acini.

The glucagon superfamily consists of a diverse group of biologically active peptides that are structurally related by having an amino-terminal histidine residue (His[1]) and a phenylalanine residue at position 6 (Phe[6]) (1) or one of several variant structures such as Tyr[1]-Phe[6] (2, 3), His[1]-Tyr[6] (4), or His[1]-Leu[6] (5). An assay for His[1] can potentially be used to identify peptides belonging to this family. Although special chemical methods have been reported for the detection of His[1] peptides (6, 7), a more general method of assaying for His[1] is amino-terminal amino acid sequencing. This assay was used recently to monitor the purification of glucagon from chinchilla pancreas (8). To test the hypothesis that His[1] is a chemical marker for biologically active peptides, amino-terminal sequencing was used to screen for new His[1] peptides that might be members of the glucagon superfamily. Gila monster venom was chosen for screening because venoms from *Heloderma suspectum* and *Heloderma horridum* had been shown previously to contain two biologically active His[1]-Phe[6] peptides, helospectin (9) and helodermin (10). When *H. horridum* venom was examined with the IHis[1] assay, a previously unrecognized His[1]-Phe[6] peptide was detected. In this study, we report the purification and structural characterization of this new peptide. It is named exendin-3 to identify it as the third peptide found in an exocrine secretion of *Heloderma* lizards which has endocrine activity. Like the earlier exendins, it is a pancreatic secretagogue.

MATERIALS AND METHODS

H. horridum venom (lot 74F-0240), diphenylcarbamyl chloride-treated trypsin, carboxypeptidase Y, and carboxypeptidase P were purchased from Sigma. Endoproteinase Glu-C (V8 protease) was purchased from Boehringer Mannheim.

His[1] Assay and Amino Acid Sequencing—Amino-terminal amino acid analysis was performed by a single cycle of Edman degradation using an automated gas-phase protein sequenator in combination with an on-line PTH[1]-derivative analyzer (Applied Biosystems). The molarity of the analyzer's weak solvent was adjusted to position PTH-His to elute between PTH-Ala and PTH-dehydroSer. Purified peptides and peptide fragments were sequenced with the gas-phase sequenator.

Isolation of His[1] Peptides from Heloderma Venom—Venom (25 mg) was dissolved in water to a concentration of 10 mg/ml and passed through a C_{18} Sep-Pak cartridge (Waters Associates, Milford, MA). The C_{18} cartridge was washed with 5 ml of water and eluted with 2 ml of 0.1% trifluoroacetic acid, 60% acetonitrile. Peptides in the eluate were separated by HPLC on an 8-mm × 10-cm MB C_{18} Radial-Pak column (Waters Associates). The column was eluted with a linear gradient from 20 to 60% acetonitrile in 0.13% heptafluorobutyric acid at a flow rate of 2 ml/min. One-minute fractions were collected. Aliquots from each fraction were assayed for His[1] content.

Enzyme Cleavages—Purified exendin-3 (10 nmol) was dried and digested separately with 0.2 μg of trypsin or 2 μg of V8 protease in 0.1 ml of 0.1 M ammonium bicarbonate overnight at room temperature. Peptide fragments were purified by HPLC on a Nova C_{18} Radial-Pak column (Waters Associates). The elutions were performed with a linear gradient from 0 to 40% acetonitrile in 0.1% trifluoroacetic acid at a flow rate of 1 ml/min.

Exendin-3 (1 nmol) was incubated with carboxypeptidase Y or carboxypeptidase P (1 μg each) in 0.05 ml of 0.05 M sodium acetate, pH 5.5, at room temperature for timed intervals up to 6 h. Aliquots from the mixtures were analyzed for release of free amino acids.

Amino Acid Analysis—Peptides were dried and hydrolyzed with gas-phase 6 M HCl at 150 °C for 60 min. Amino acids were analyzed with an automated amino acid derivatizer (Applied Biosystems) connected to an on-line phenylthiocarbamyl-derivative analyzer.

* This work was supported in part by the Department of Veterans Affairs. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

¶ To whom correspondence should be sent: Solomon A. Berson Research Laboratory, Veterans Affairs Medical Center, Bronx, New York 10468. Tel.: 212-584-9000 (ext. 1710).

[1] The abbreviations used are: PTH, phenylthiohydantoin; HPLC, high pressure liquid chromatography; FAB-MS, fast atom bombardment-mass spectrometry; VIP, vasoactive intestinal peptide.

THE JOURNAL OF BIOLOGICAL CHEMISTRY

Vol. 267, No. 11, Issue of April 15, pp. 7402–7405, 1992
Printed in U.S.A.

Isolation and Characterization of Exendin-4, an Exendin-3 Analogue, from *Heloderma suspectum* Venom

FURTHER EVIDENCE FOR AN EXENDIN RECEPTOR ON DISPERSED ACINI FROM GUINEA PIG PANCREAS*

(Received for publication, August 12, 1991)

John Eng‡§¶, Wayne A. Kleinman‡, Latika Singh‖, Gurcharn Singh‖, and Jean-Pierre Raufman‖

From the ‡Solomon A. Berson Research Laboratory, Veterans Affairs Medical Center, Bronx, New York 10468, the §Department of Medicine, Mount Sinai School of Medicine, New York, New York 10029, and the ‖Department of Medicine, Division of Digestive Diseases, State University of New York-Health Science Center, Brooklyn, New York 11203-2098

The recent identification in *Heloderma horridum* venom of exendin-3, a new member of the glucagon superfamily that acts as a pancreatic secretagogue, prompted a search for a similar peptide in *Heloderma suspectum* venom. An amino acid sequencing assay for peptides containing an amino-terminal histidine residue (His[1]) was used to isolate a 39-amino acid peptide, exendin-4, from *H. suspectum* venom. Exendin-4 differs from exendin-3 by two amino acid substitutions, Gly[2]-Glu[3] in place of Ser[2]-Asp[3], but is otherwise identical. The structural differences make exendin-4 distinct from exendin-3 in its bioactivity. In dispersed acini from guinea pig pancreas, natural and synthetic exendin-4 stimulate a monophasic increase in cAMP beginning at 100 pM that plateaus at 10 nM. The exendin-4-induced increase in cAMP is inhibited progressively by increasing concentrations of the exendin receptor antagonist, exendin-(9–39) amide. Unlike exendin-3, exendin-4 does not stimulate a second rise in acinar cAMP at concentrations >100 nM, does not stimulate amylase release, and does not inhibit the binding of radiolabeled vasoactive intestinal peptide to acini. This indicates that in dispersed pancreatic acini, exendin-4 interacts only with the recently described exendin receptor.

An assay for His[1] peptides was recently used to identify the presence of helospectin and a new, previously unrecognized His[1]-Phe[6] peptide in *Heloderma horridum* venom (1). This new peptide, designated exendin-3, is a pancreatic secretagogue. At concentrations greater than 100 nM, exendin-3 interacts with VIP[1] receptors on guinea pig pancreatic acini to stimulate an increase in cellular cAMP and amylase release (2). At lower concentrations (0.1–10 nM), however, exendin-3 interacts with a putative exendin receptor that causes an increase in acinar cAMP but not amylase release. This conclusion is based on the observation that increasing concentrations of a specific antagonist, exendin-3-(9–39) amide, progressively inhibit exendin-3-induced increases in cAMP (2). Because venom from a closely related lizard, *Heloderma suspectum*, has been shown to contain helodermin (3), a peptide closely related in structure to helospectin (4), a search was undertaken for a His[1] peptide in *H. suspectum* venom that might be analogous to exendin-3. We report the isolation from *H. suspectum* venom of such an analogue that has been named exendin-4. Exendin-4, unlike exendin-3, is not a pancreatic secretagogue. Instead, it interacts exclusively with the newly described exendin receptor (2) to increase pancreatic acinar cAMP.

MATERIALS AND METHODS

H. suspectum venom (lots HS19SZ and HS20SZ) was purchased from Miami Serpentarium Laboratories (Salt Lake City, UT). Diphenylcarbamyl chloride-treated trypsin was purchased from Sigma. Endoproteinase Asp-N was purchased from Boehringer Mannheim.

His[1] Assay and Amino Acid Sequencing—Amino-terminal amino acid analysis was performed by a single cycle of Edman degradation using an automated gas phase protein sequenator in combination with an on-line PTH-amino acid analyzer (Applied Biosystems, Foster City, CA). PTH-His was positioned to elute between PTH-Ala and PTH-dehydro-Ser. Purified peptides and peptide fragments were sequenced with the gas phase sequenator.

Isolation of His[1] Peptides from Heloderma Venom—Venom (25 mg) was dissolved in distilled water (10 mg/ml) and passed through a C_{18} Sep-Pak cartridge (Waters Associates, Milford, MA). The C_{18} cartridge was washed with 5 ml of water and eluted with 2 ml of 0.1% trifluoroacetic acid, 60% acetonitrile. Peptides in the eluate were separated by HPLC on an 8 mm × 10-cm μBondapak C_{18} Radial-Pak column (Waters Associates). The column was eluted with a linear gradient (20–60%) of acetonitrile in 0.13% heptafluorobutyric acid at a flow rate of 1 ml/min. One-minute fractions were collected, and aliquots were assayed for His[1] content.

Enzyme Cleavages—Purified exendin-4 (5–20 nmol) was digested with 0.2 μg of trypsin or with 0.2 μg of endoproteinase Asp-N. The peptide fragment exendin-(9–39) amide was prepared as described previously (2). Although this fragment was previously referred to as exendin-3-(9–39) amide (2), the name has been shortened to exendin-(9–39) amide to indicate that the carboxyl-terminal 31 amino acids of exendin-3 and exendin-4 are identical. Peptide fragments were purified by HPLC on a Nova C_{18} Radial-Pak column (Waters Associates).

Amino Acid Analysis—Peptides were dried and hydrolyzed with gas phase 6 M HCl at 150 °C for 60 min. Amino acids were analyzed with an automated amino acid derivatizer (Applied Biosystems) connected to an on-line phenylthiocarbamyl-derivative amino acid analyzer.

Mass Spectrometry—The mass of the COOH-terminal fragment generated by trypsin digestion of exendin-4 was determined by fast atom bombardment-mass spectrometry. Mass accuracy of greater than ±0.1 unit was achieved by peak matching to appropriate cesium chloride cluster ions. Fast atom bombardment-mass spectrometry was performed by the Laboratory for Macromolecular Analysis at the

* This paper was supported in part by the Department of Veterans Affairs. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

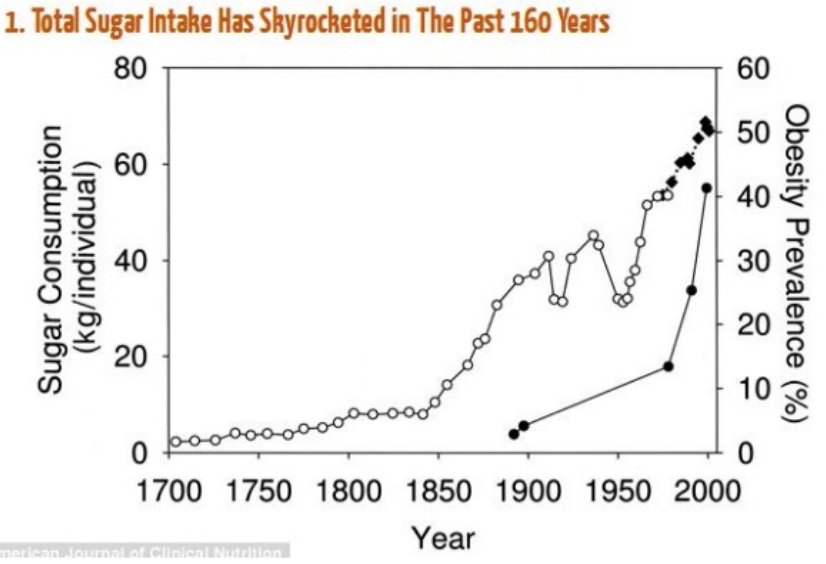
¶ To whom reprint requests and correspondence should be addressed: Solomon A. Berson Research Laboratory, Veterans Affairs Medical Center, Bronx, NY 10468. Tel.: 212-584-9000 (ext. 6069).

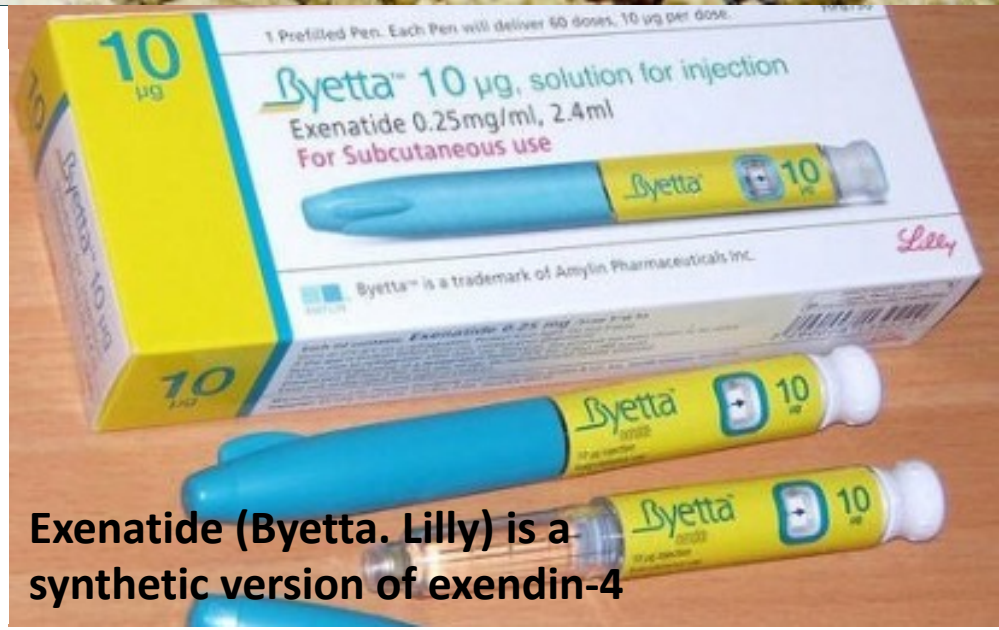
[1] The abbreviations used are: VIP, vasoactive intestinal peptide; PTH, phenylthiohydantoin; HPLC, high performance liquid chromatography.

Peptide drugs – when pharmaceutical sciences grab into the wild

- Gila monster: a species of venomous lizard; Mexico
- Eats only 4 times a year
- When fasting, it shuts down the pancreas, stopping insulin
- When its time to eat, it restarts pancreas with exendin-4 in its saliva -

  a GLP-1R agonist





Exenatide (Byetta. Lilly) is a synthetic version of exendin-4

Peptide drugs – GLP1 and incretins

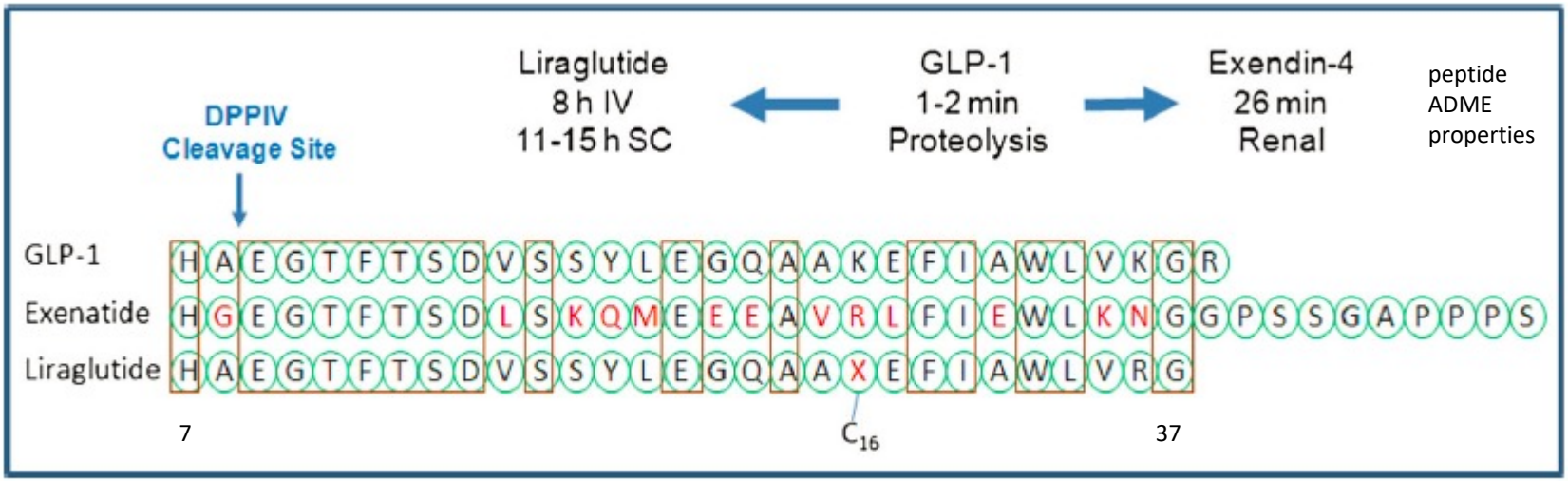
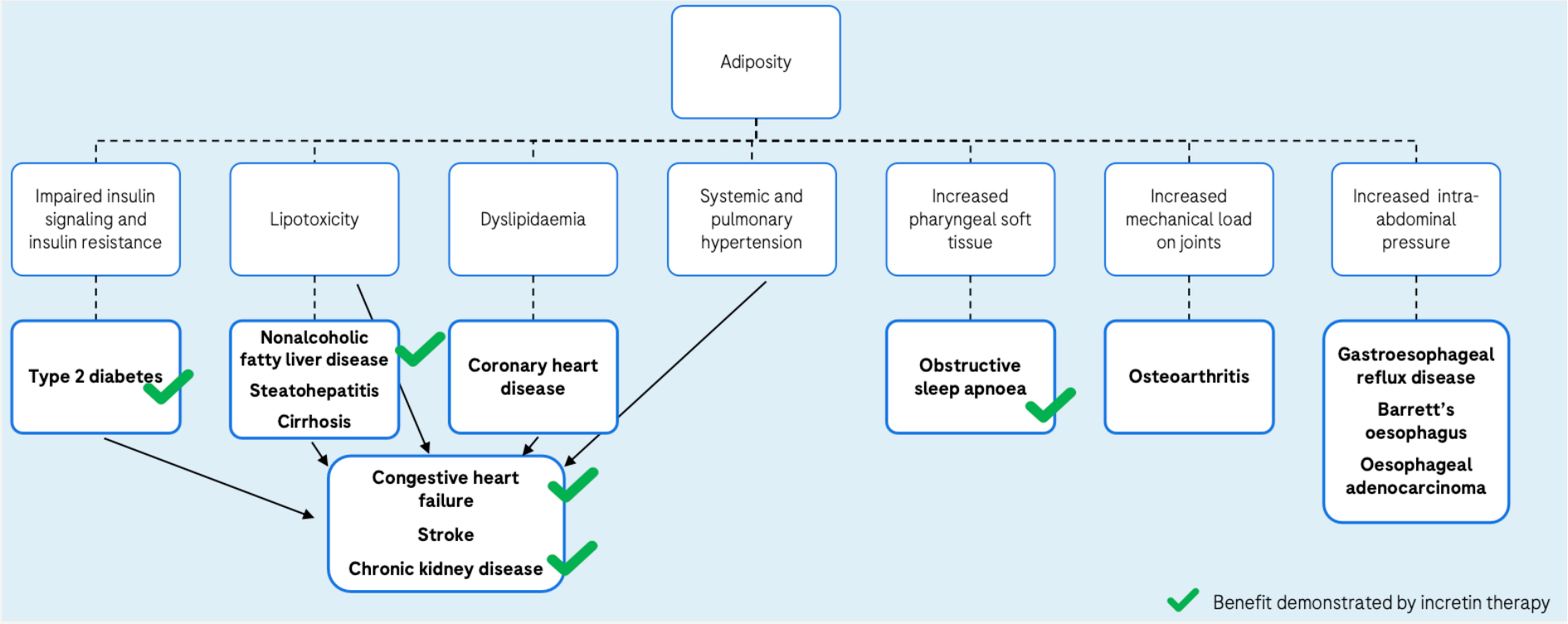


Fig. 2. Strategies to enhance peptide stability: GLP-1 peptide has a half-life of 1–2 min.

- **GLP1 receptor agonists (incretins) from gut L cells have entered the clinics in the treatment of diabetes type II, drawback: short half life**
- **Exendin-4 (synthetic Exenatide) has improved PK PD properties (lower clearance) allowing once a week dosing as compared to GLP1 (rapidly cleaved by peptidase DDPIV)**
- **Major challenges with the development of peptide drugs are stability and cost of goods (now about 2% of drug market)**

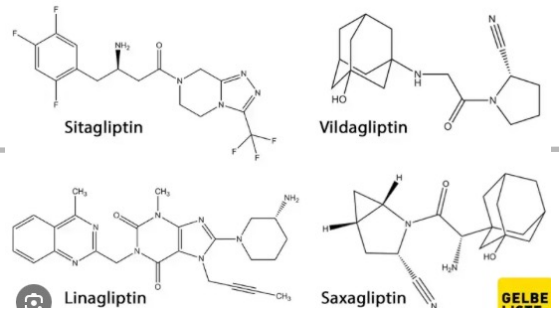
Obesity is associated with multiple complications
BMI>30 carry hazard ratio for overall mortality elevated more than 40%

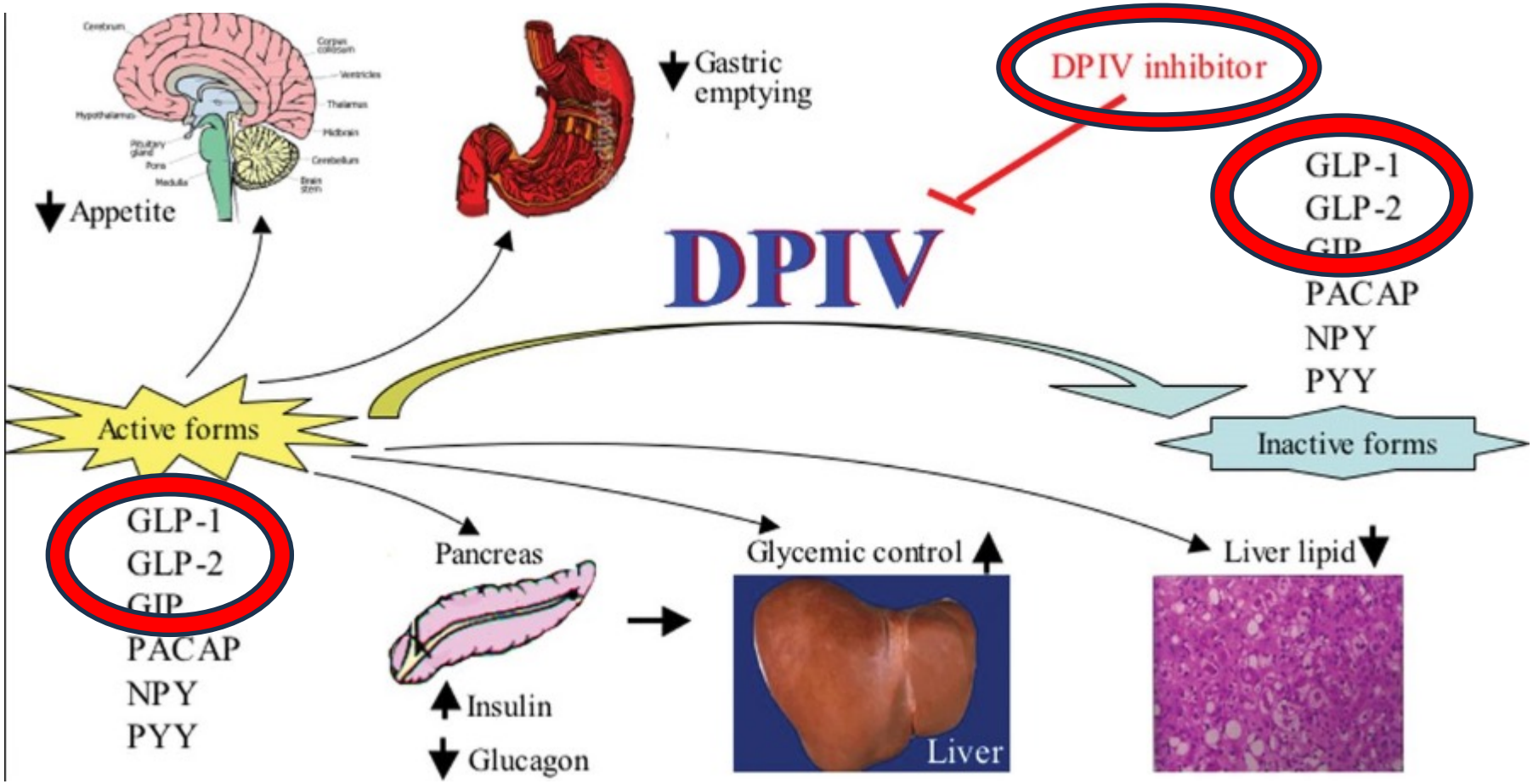


A critical threshold of weight loss is needed for disease modification (≥10% body weight loss)[2]

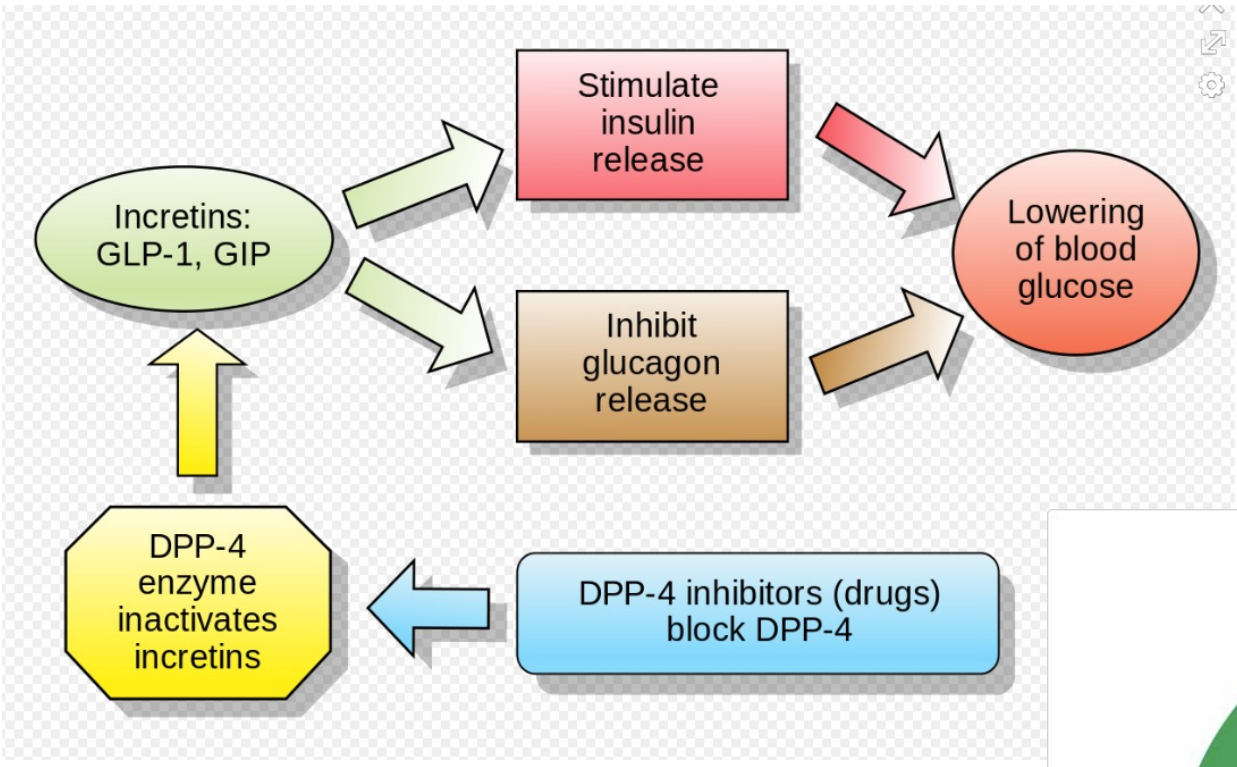
1. Müller et al. Anti-obesity drug discovery: advances and challenges. Nat Rev Drug Discov 21,2. Ryan et al, Weight Loss and Improvement in Comorbidity: Differences at 5%, 10%, 15%, and Over. Curr Obes Rep. 2017, Obesity Reviews, Volume: 22, Issue: 1, First published: 07 September 2020, DOI: (10.1111/obr.13112)

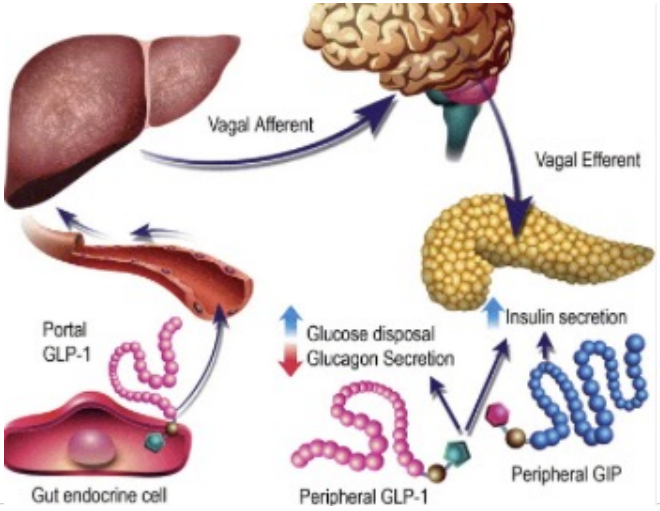
Peptide drugs – incretins – GLP1 GLP2 agonists in diabetes – vs DPP4 inhibitors - gliptins

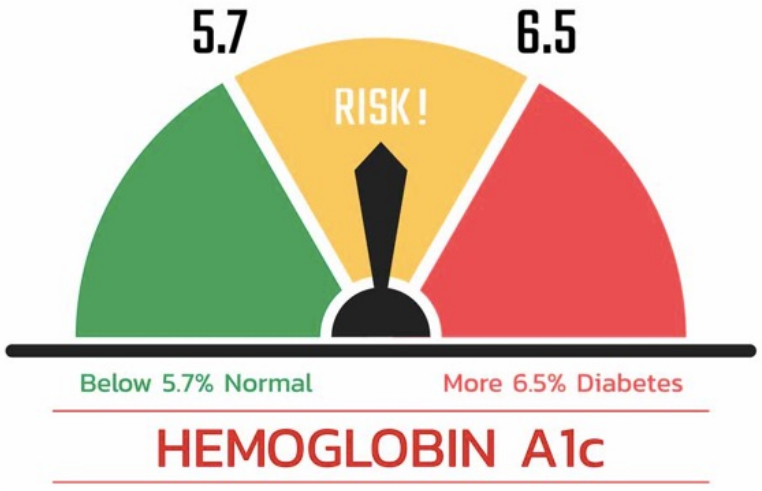




Peptide drugs – GLP1r incretins
lowering blood glucose, gastric emptying and body weight control







average glycemic level over 4-6 w !

GLP1 GIP1 receptor agonists (incretins) from gut cells have entered the clinics in the last decade in the management of diabetes type II and as a ”surprise” during late phase clinical trials in the obesity (BMI>30) first approved peptides drawback: short half life, hence recurrent injections (invalidating patient care)

Peptide drugs – GLP1 receptor and incretins

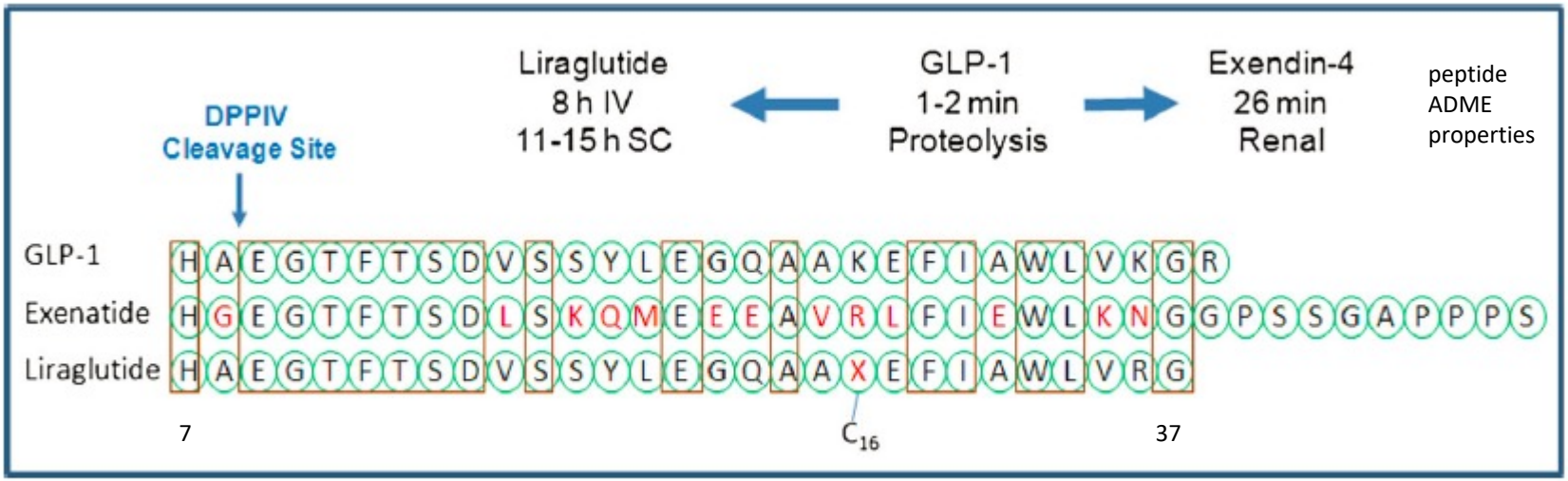


Fig. 2. Strategies to enhance peptide stability: GLP-1 peptide has a half-life of 1–2 min.

- **GLP1 receptor agonists (incretins) from gut L cells-ileum/colon- have entered the clinics in the treatment of diabetes type II, drawback: short half life**
- **Exendin-4 (synthetic Exenatide) has improved PK PD properties (lower clearance) allowing once a week dosing as compared to GLP1 (rapidly cleaved by peptidase DDPIV)**
- **Major challenges with the development of peptide drugs are stability and cost of goods (now about 2% of drug market)**

GLP1 peptides in drug research and development

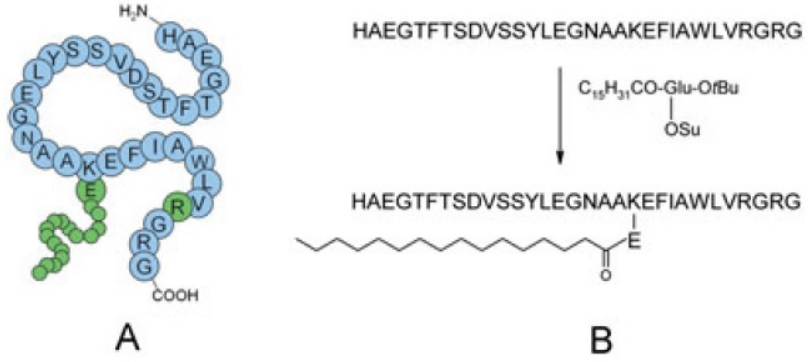
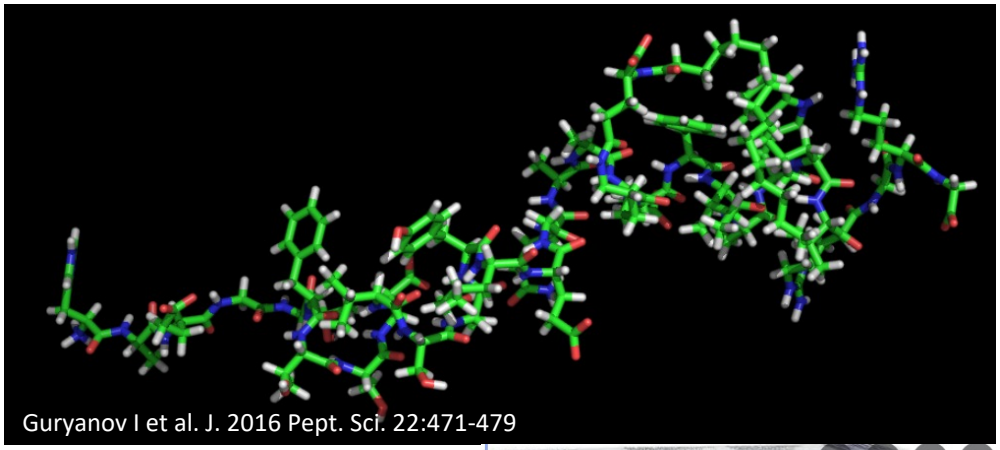
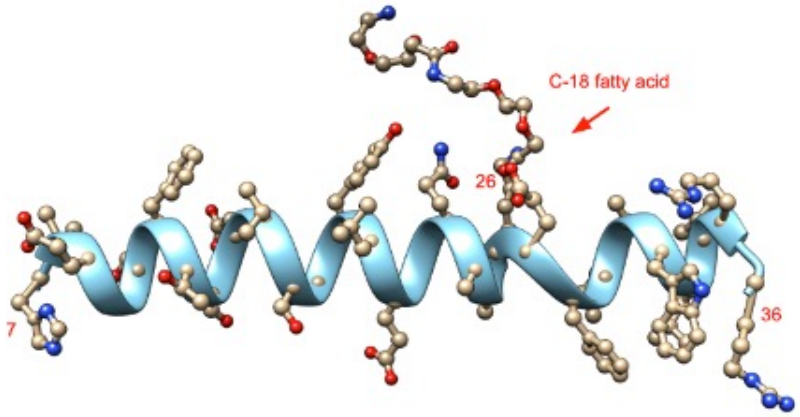


Figure 1. (A) The amino acid sequence of liraglutide. The changes with respect to glucagon-like peptide-1 (7-37) are shown in green; (B) A scheme of preparation of liraglutide by chemical modification of a recombinant peptide precursor (OSu, 1-oxysuccinimidoyl).



- Example **LIRAGLUTIDE** GLP1 (7-37) receptor agonist linked to 16C fatty acid residues (lipopeptide binds to albumin (not covalently) to stabilize the peptide)
- Example **DULAGLUTIDE** GLP1 receptor agonist linked to Fc fragment of antibody human IgG4
- Both medication for Type II diabetes, with improved administration (stabilized GLP1 peptide (compliance !) Liraglutide which mimics the action of glucagon-like peptide is also clinically approved as an obesity treatment.

INCRETINS : a salamander saliva poison peptide is turning a « game changer » in the management of metabolic diseases – T2D/pandemia



1. 2D and 3D structures of Semaglutide. a. The amino acid sequence of Semaglutide (PubChem) is numbered from 7 to 37. The sequen

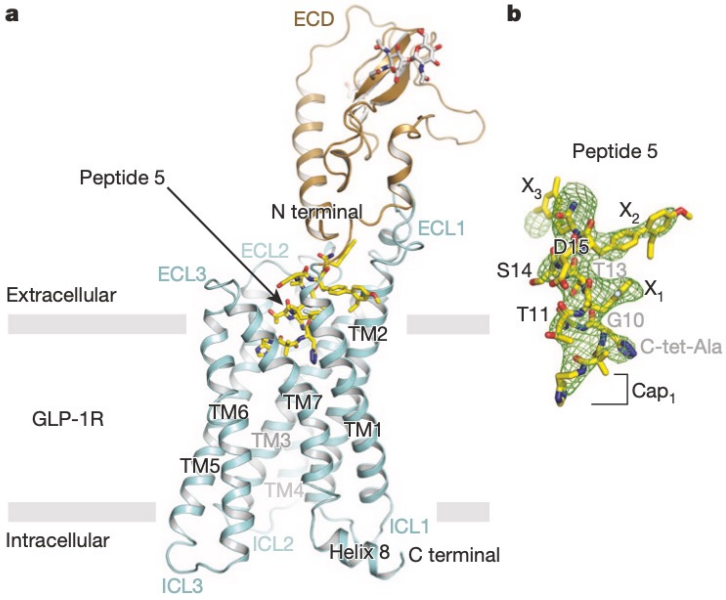
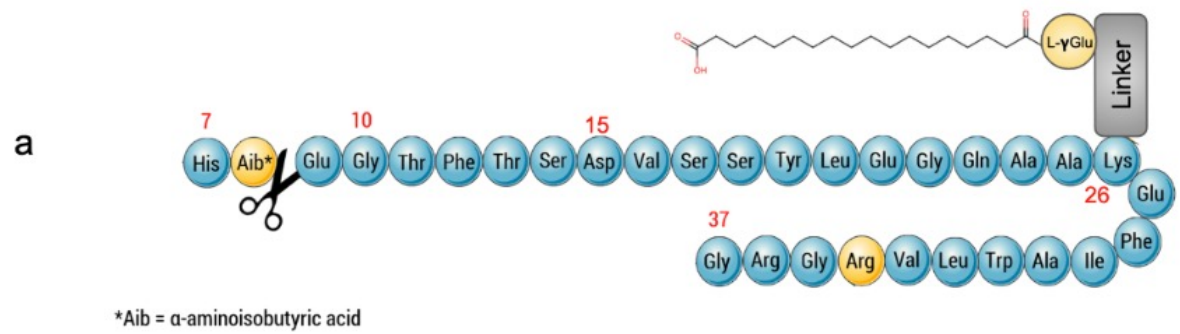


Figure 1 | The overall structure of GLP-1R in complex with peptide 5.

incretins : the wonder medicines ?!

Semaglutide ; synthetic peptide with improved PK PD

IUPAC Name	Semaglutide is a synthetic peptide (see the amino acid sequence below)
3D Structure of Semaglutide	Chain P in PDB ID 7ki0



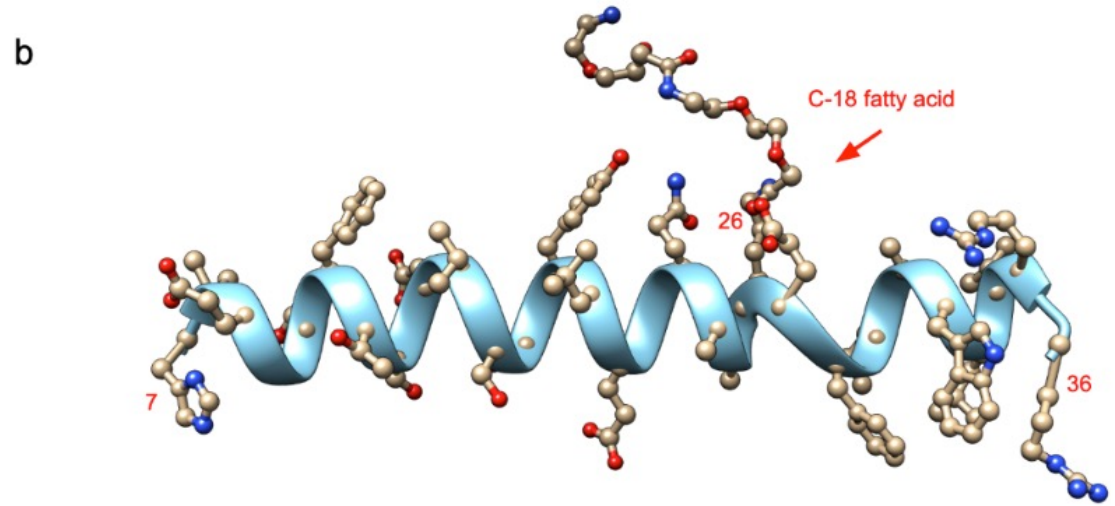
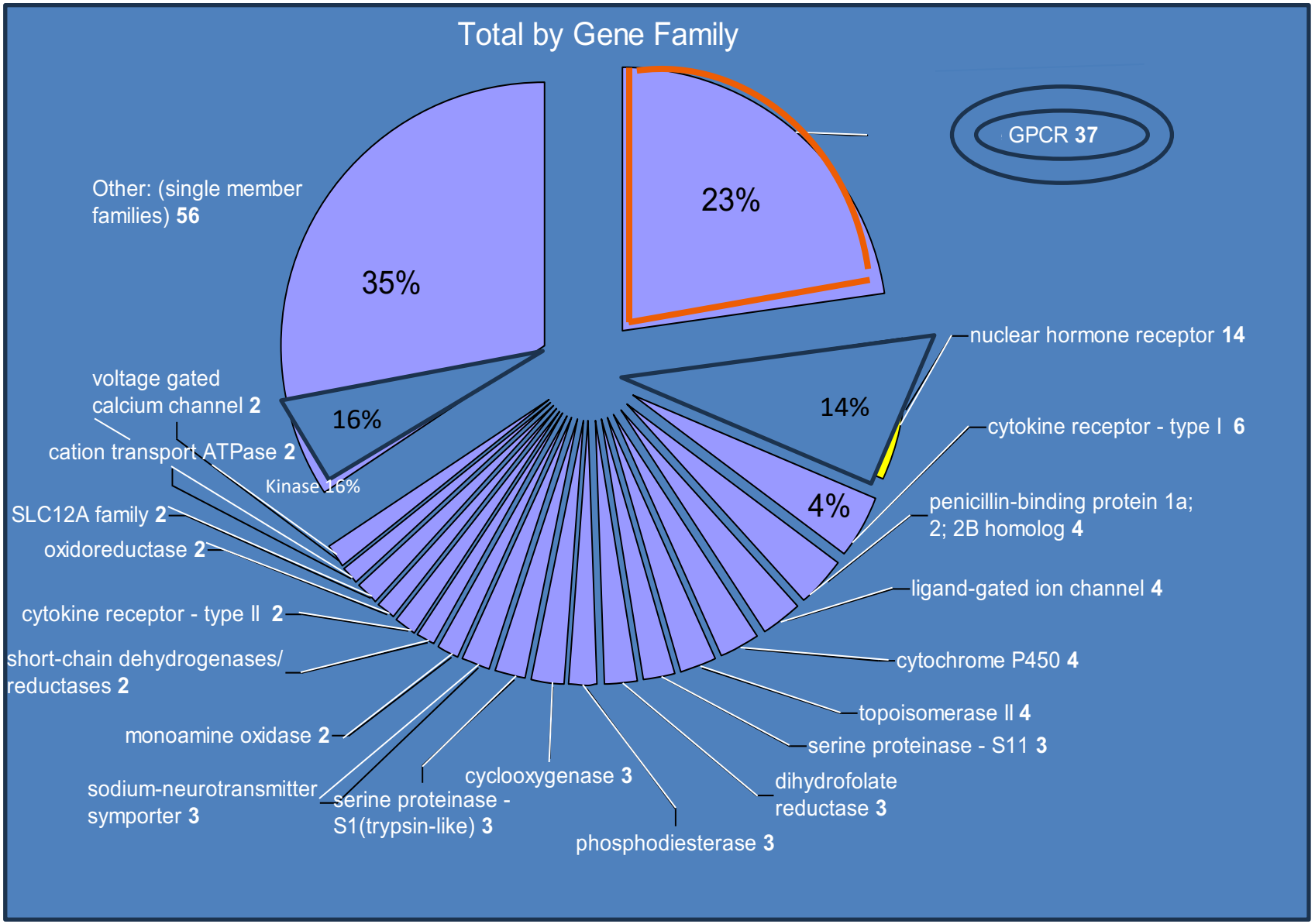


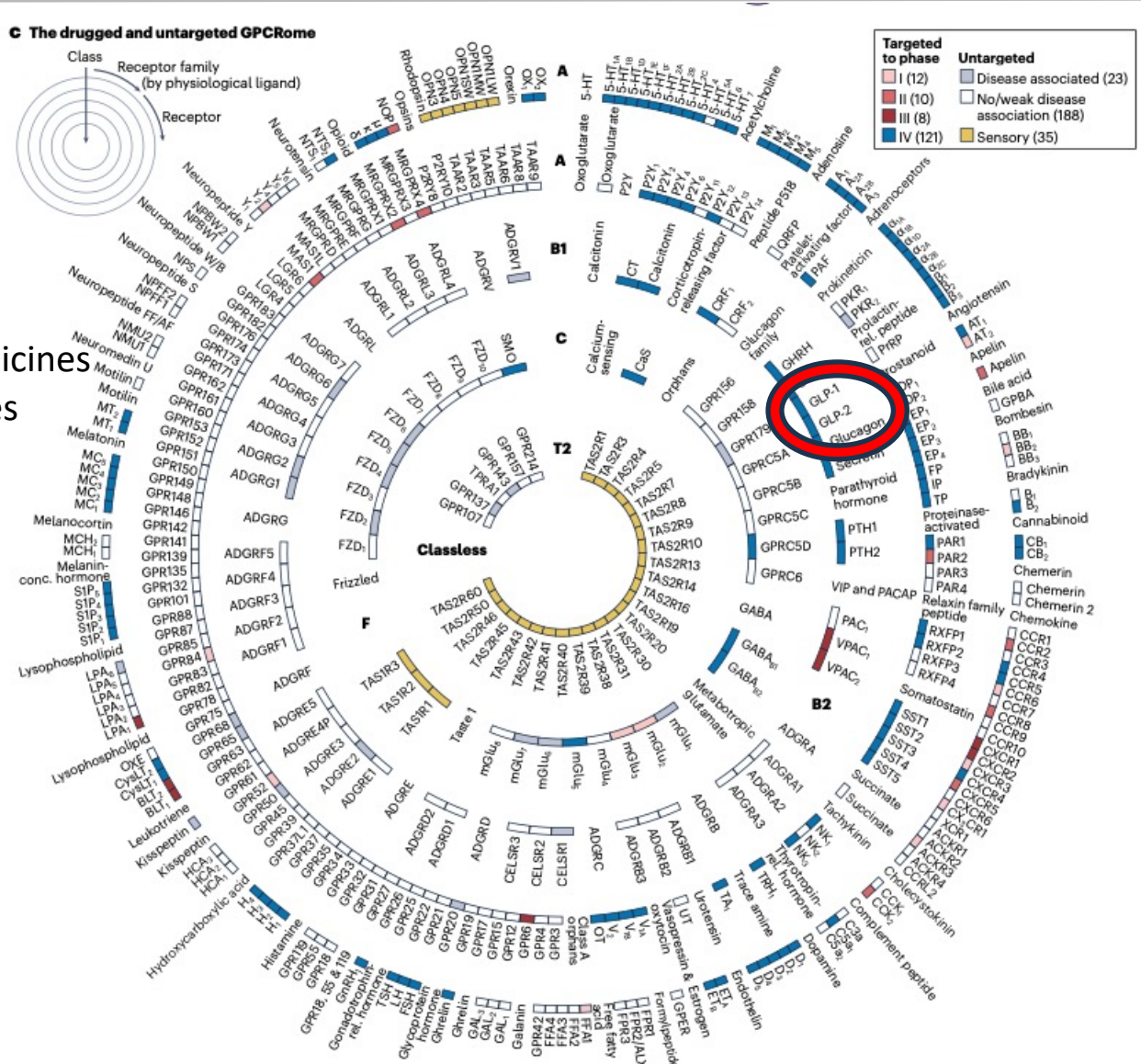
Figure 1. 2D and 3D structures of Semaglutide. a. The amino acid sequence of Semaglutide (PubChem) is numbered from 7 to 37. The sequence schematic shown is based on information presented in Knudsen and Lau, 2019. Amino acid modifications are highlighted in yellow and the DPP4 cleave site is indicated with the scissors. b. 3D structure of Semaglutide (PDB ID 7ki0, Zhang et al., 2021). Note: The C-18 fatty acyl chain shown here

GPCRs are the most precedented drug target family



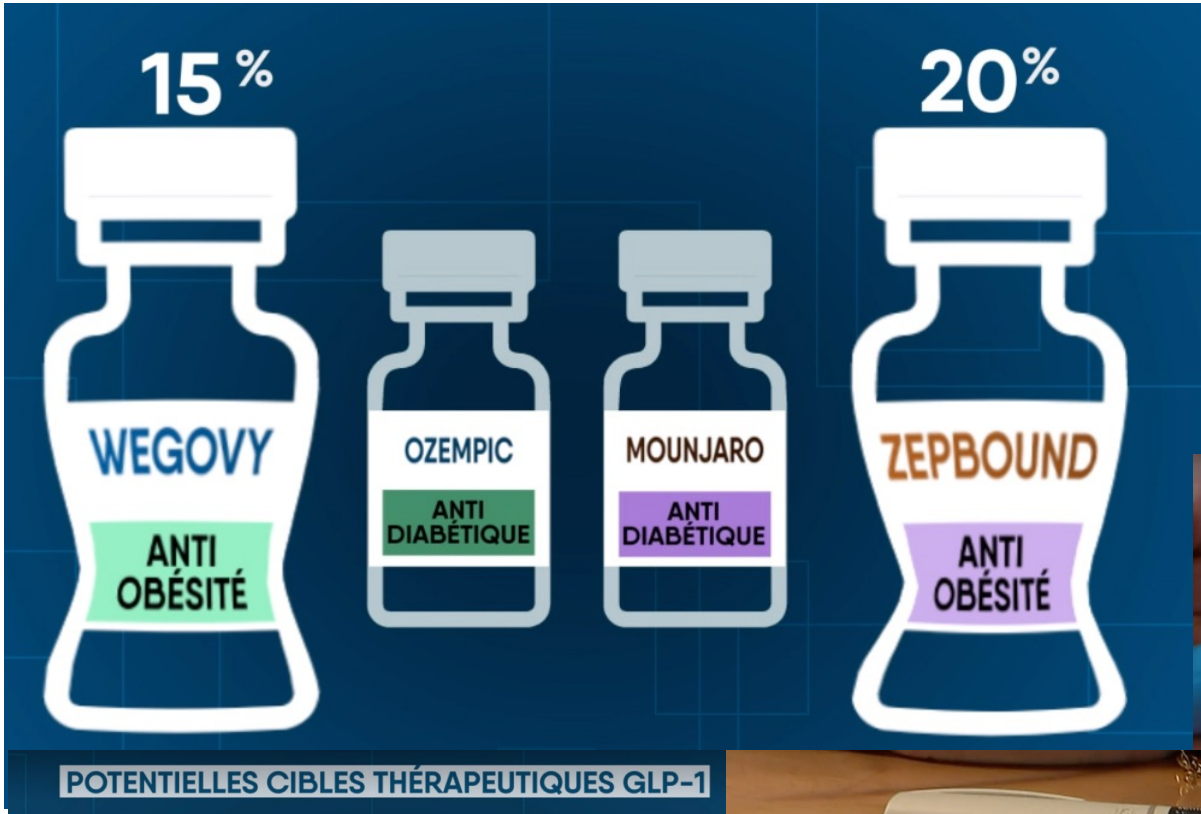
37% (n=516) of all approved medicines act on GPCRs : the largest drug target family by 2024

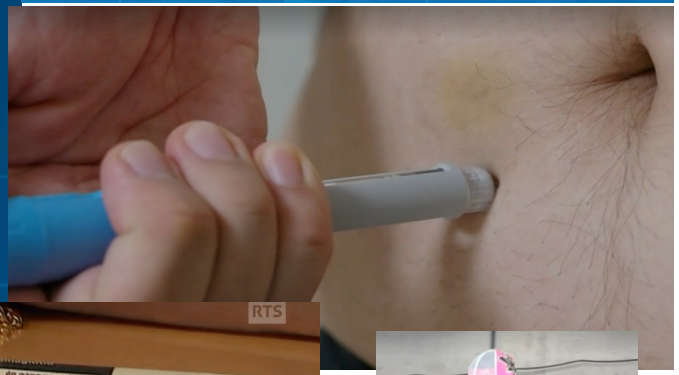
- 800 GPCRs (current estimate)
- 121 drugged GPCRs (2024)
- 516 GPCRs based approved medicines
- ie. 37% of all approved medicines

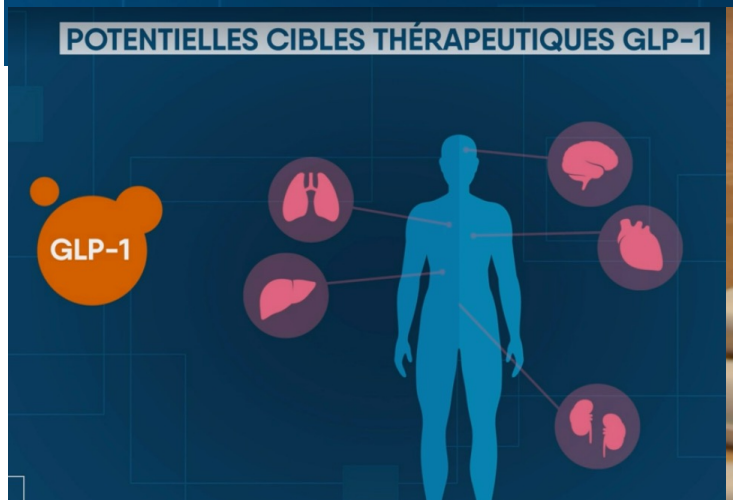


Sanchez Lorente J et al Nature Reviews drug discovery 2025
https://doi.org/10.1038/s41573-025-01139-y

GLP1-2 agonists – turning «unexpected» during the clinical trials an obesity medicine and perhaps more !







Tennis star effect on biologicals production – incretins shortage

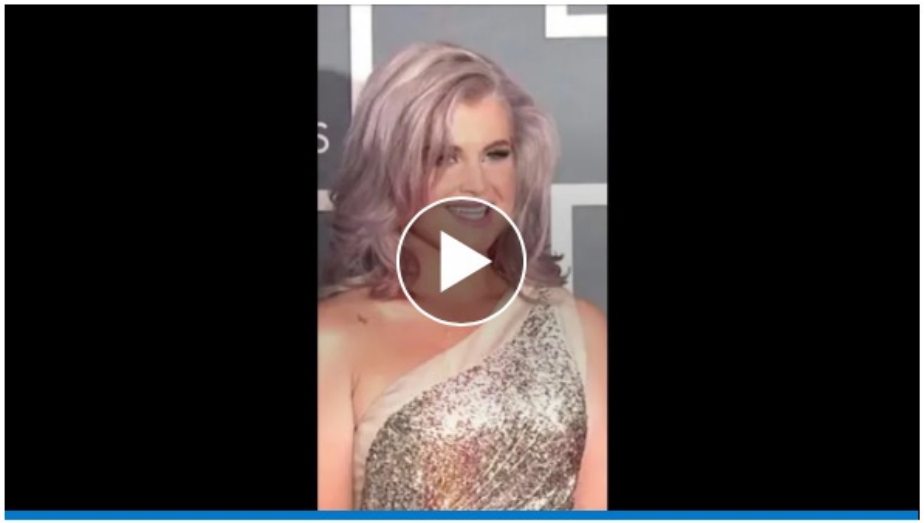
Die perfekte Botschafterin: Serena Williams wirbt für Abnehmspritzen.

- **Redirect/misuse of incretins have geopardized drug makers production lines of life threating medicines : T2D patients in danger !**
- **Incretins are designed for patients suffering from metabolic diseases such as diabetes and not for popstars and social media influencers promulgating pharmacological solutions in weight loss by convenience (instead of promoting diet and exercise !)**
- **Pharma/Biotech production shortage !!**

Popstars effect and biologicals - incretins

Kelly Osbourne says Ozempic use is 'amazing' after mom Sharon's negative side effects

JAY STAHL USA TODAY



Show Caption

Kelly Osbourne is making headlines again for her controversial comments.

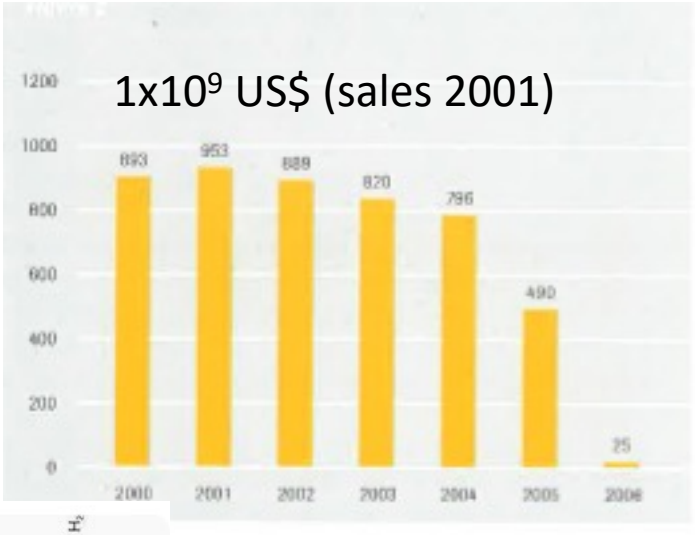
This time, the TV personality and daughter of Sharon and Ozzy Osbourne defended her use of Ozempic, a drug that is intended to treat those with Type 2 diabetes. On the Dolly Parton Pet Gala red carpet, Osbourne opened up about her opinions on Ozempic in an interview with E! News.

- **Redirect/misuse of incretins have geopardized drug makers production lines of life threating medicines**
- **Incretins are designed for patients suffering from metabolic diseases such as diabetes and not for popstars and social media influencers promulgating pharmacological solutions in weight loss by convenience (instead of promoting diet and exercise !)**

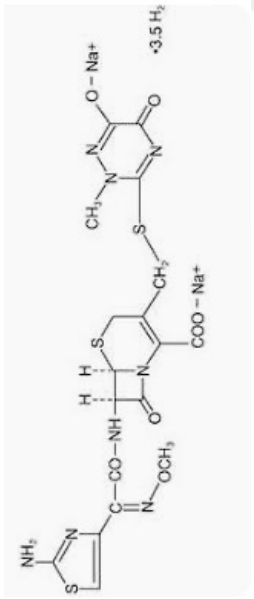
Overall sales of «blockbusters» : medicines reaching yearly ≥ 1 billion US$ revenue

2x10^9 US$ (sales 2024)

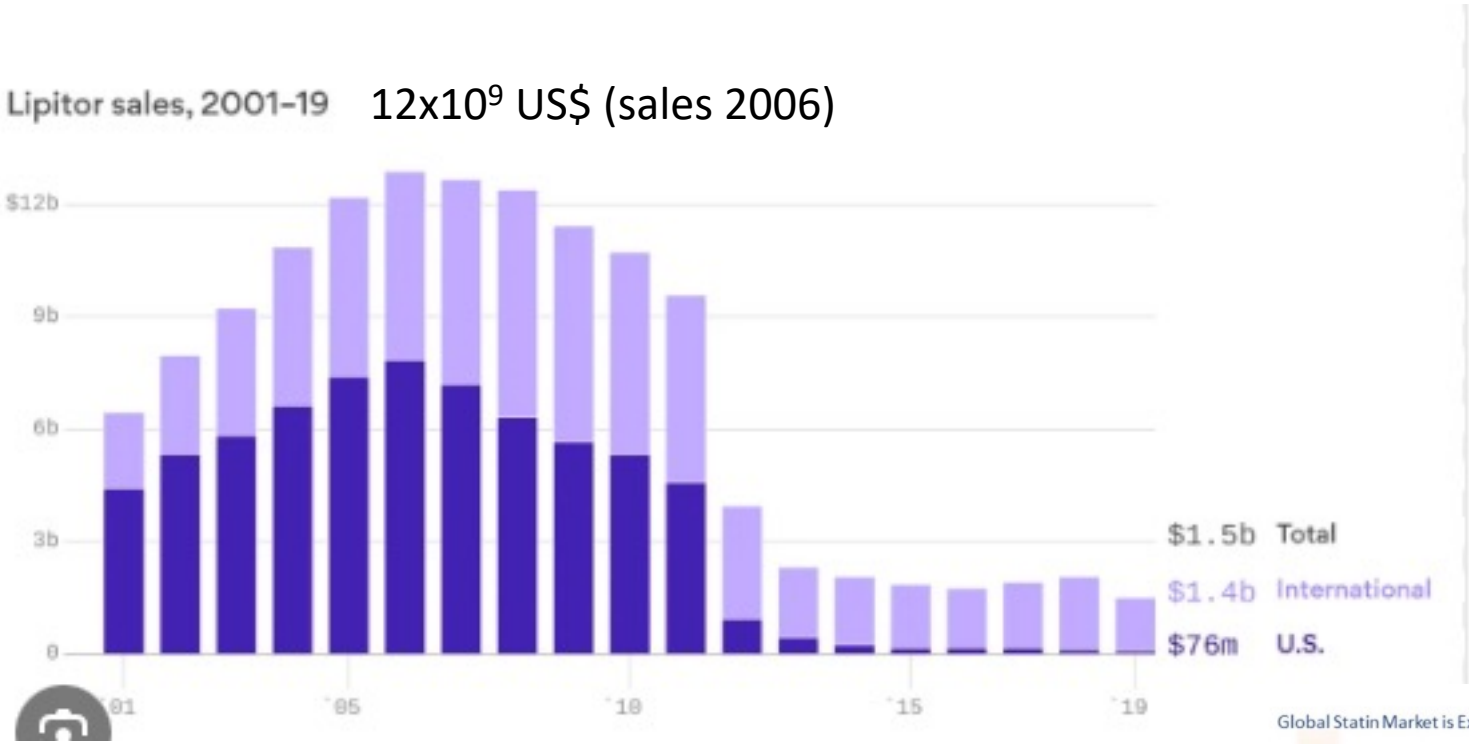
Yearly sales of Roche Rocephin from 2000 to 2006



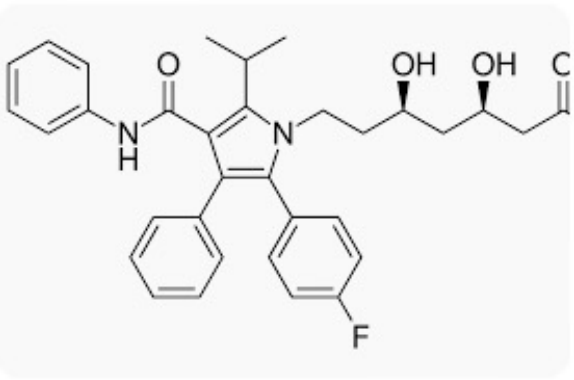
1x10^9 US$ (sales 2001)

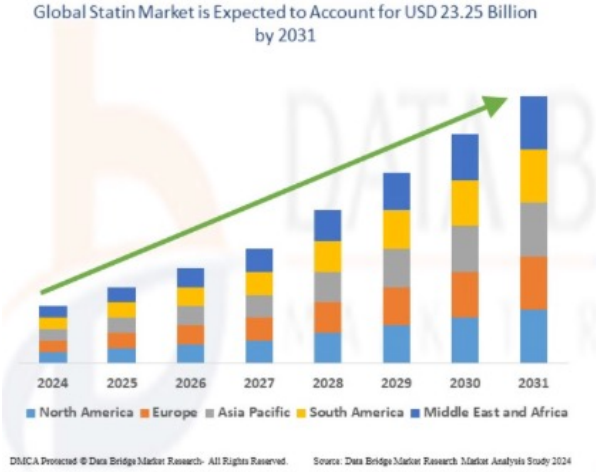


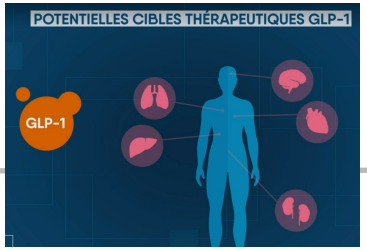
Overall sales of «blockbusters» : medicines reaching yearly ≥ 1 billion US$ revenue
statins still most ever sold medicine



12x10^9 US$ (sales 2006)



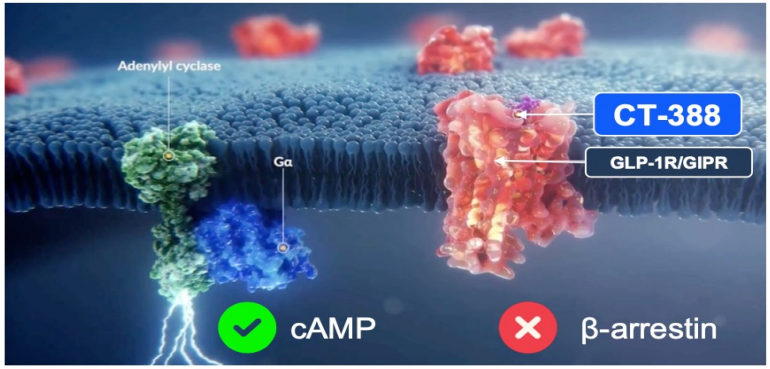


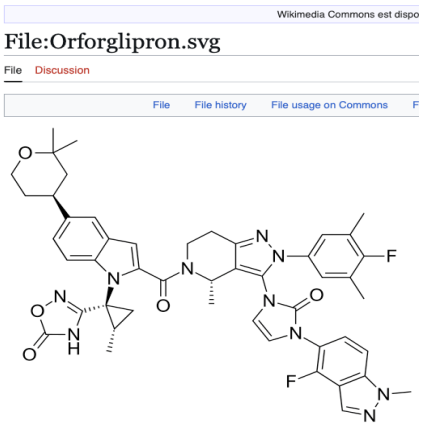


when new «reversed» therapeutic modalities arise ?!

SMW GLP1 agonists in clinical trials : new oral incretin mimetics work in progress !

- **Obesity**
  - Heterogenous, chronic, relapsing disease affecting millions worldwide[1,2]
  - Additional treatment options are needed
- **CT-388**
  - *Dual GLP-1/GIP receptor agonist* being developed for obesity, type 2 diabetes (T2D), and other weight-related comorbidities[3]
  - *Potent with minimal-to-no β-arrestin recruitment on both receptors*, which leads to reduced receptor internalization and potentially prolonged pharmacological activity[3]





SMW oral GLP1 agonists : away from biologics !

developing nonpeptidic small-molecule drugs targeting GLP-1R remains a challenge

771-P

Efficacy of CT-996, an Oral Small Molecule GLP-1 Receptor Agonist, in Human GLP-1 Receptor Knock-in Mice and Obese Cynomolgus Monkeys

Jian Luo[1], Ruben Rodriguez[1], Anne Hergarden[1], Ted Tracy[1], Davina Lam[1], Sumanta Garai[1], Daniel Marshall[1], Stig Hansen[2], Manu Chakravarthy[1]

[1]Carmot Therapeutics, a member of the Roche group; [2]Kimia Therapeutics.

Purpose

In the present studies we characterize CT-996, a potent, oral, small molecule, signal-biased GLP-1 receptor agonist, and evaluate its effect on glucose response, weight loss, and food intake in pre-clinical models.

Conclusions

- CT-996 is a small molecule GLP-1RA that exhibits biased cAMP signaling and reduced receptor internalization.
- Daily oral dosing of CT-996 enhanced glucose homeostasis and reduced weight and fat mass after 4-week treatment in obese cynomolgus monkeys.
- All doses of CT-996 were well-tolerated in cynomolgus monkey studies.
- These observations corroborate the continued development of CT-996 as a next-generation GLP-1RA. CT-996 is currently being evaluated in a Phase 1 clinical trial.

Acknowledgements

- Contribution to discovery: Sean Zhou, Ray Fucini, Xiaohui Du, and David Lloyd
- Presentation support: Damian Bialonczyk

Disclosures

- All authors are current or former employees of Carmot Therapeutics, a member of the Roche group.

Presented at the ADA 84th Scientific Sessions in Orlando, FL. June 21–24, 2024

Introduction

- Injectable GLP-1 receptor agonists (GLP-1RA) have demonstrated significant efficacy in glycemic control and weight management.
- While semaglutide is available in an oral peptide formulation, it requires specific timing and administration conditions.
- The increasing use of injectable incretin therapies underscores the urgent need for simpler, cost-effective solutions.
- Here, we describe CT-996, a potent, oral small molecule GLP-1RA, and present preclinical efficacy studies to support its development for daily oral use in humans.

Methods

In Vitro Characterization

- cAMP accumulation was measured in HEK293 cells expressing either human or cynomolgus monkey GLP-1R 30 minutes after stimulation. Maximal β-arrestin recruitment after stimulation was measured in HEK293 cells expressing human GLP-1R-LgBit and SmBiT-human-β-arrestin-2. Internalization was measured in HEK293 cells expressing HiBiT-tagged human GLP-1R 60 minutes after stimulation.
- Insulin secretion was measured in EndoC-bH5® cells, a human pancreatic β-cell line, 40 minutes after stimulation in the presence of 11 mM glucose. Curves depict non-linear regression analysis of data normalized to vehicle and a high dose of GLP-1. Insulin data is also normalized to total protein content. Data are mean ± SD of 2-3 replicates per concentration.

Mixed Meal Tolerance Test (MMTT) in Humanized GLP-1R Knock-in Mice

- Male humanized GLP-1R knock-in (hGLP-1R KI; Taconic) mice, aged 8-14 weeks, were fasted overnight. Vehicle and CT-996 were administered orally 30 minutes before the MMTT (Ensure, 10 ml/kg).

Glucose Stimulated Insulin Secretion (GSIS) in Obese Monkeys

- Male obese cynomolgus monkeys, > 8 years old (KBI), were fasted overnight.
- Vehicle and CT-996 were administered orally 2-3 hours prior to intravenous glucose tolerance test (IVGTT; 0.5 g/kg).

28-Day Daily Dose Study in Obese Monkeys

- Twenty-four (24) male cynomolgus monkeys, each weighing > 8 kg with BMI > 40 were included. Some of the animals had plasma glucose above 6 mmol/L.
- CT-996 was administered once daily.
- MMTT (Ensure, 5 ml/kg) was administered on Day 1, 3 hours post dose.
- Chow intake was recorded at each meal, and body weight was assessed weekly.
- Body composition was measured using dual-energy X-ray absorptiometry (DEXA) at baseline and Day 29.

Results

CT-996 is a potent and cAMP-biased GLP-1R agonist

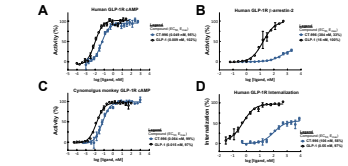


Figure 1. Dose response curves for human GLP-1R cAMP accumulation (A), Human GLP-1R β-arrestin recruitment (B), Cynomolgus monkey GLP-1R cAMP accumulation (C), and human GLP-1R internalization (D) in response to endogenous ligand GLP-1 (black circles) or CT-996 (blue circles).

CT-996 dose-dependently enhanced GSIS in human β-cells

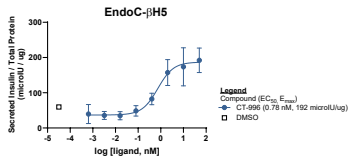


Figure 2. Dose response curves of insulin secretion after stimulation with CT-996 in the presence of 11 mM glucose. Abbreviations: nM is nanomolar, ug is microgram, and microIU is microinternational unit.

CT-996 improved glucose following MMTT in hGLP-1R KI mice

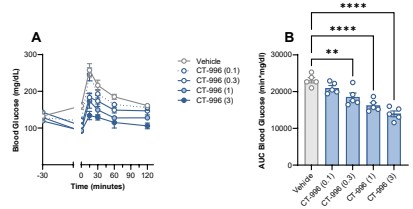


Figure 3. Mean (± SE) (A) glucose response to an MMTT and (B) the area under the glucose curves in hGLP-1R KI mice 30 minutes after vehicle and CT-996 administration. Statistical differences were evaluated using a one-way ANOVA followed by Bonferroni post hoc test. ** p < 0.01, **** p < 0.0001.

CT-996 dose-dependently enhanced GSIS in obese monkeys

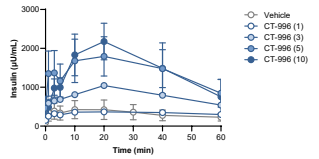


Figure 4. Effects of oral CT-996 on GSIS during IVGTT in obese cynomolgus monkeys. Plasma insulin concentrations are represented as mean (± SE) following intravenous glucose challenge (2-3 hours after vehicle and CT-996 administration).

CT-996 improved postprandial glucose following MMTT in obese monkeys

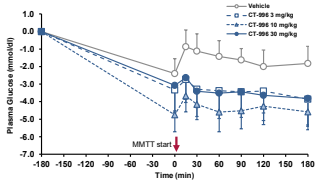


Figure 5. Effects of oral CT-996 on postprandial glucose in obese cynomolgus monkeys. Plasma glucose change is represented as mean (± SE) from baseline. N = 6/group. MMTT was administered on Day 1, 3 hours post CT-996 administration.

CT-996 progressively lowered body weight in obese monkeys

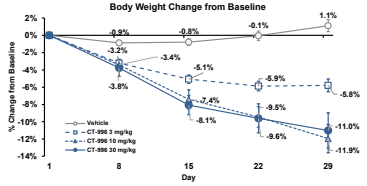


Figure 6. Effects of CT-996 on body weight in obese cynomolgus monkeys following once-daily oral administration. Weekly body weight percent change is represented as mean (± SE) from baseline. N = 6/group.

CT-996 preferentially reduced fat mass in obese monkeys

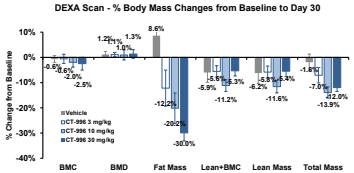


Figure 7. Effects of daily oral CT-996 on body composition in obese cynomolgus monkeys. Percent change from baseline [Mean (± SE)]. N = 6/group. Abbreviations: BMC is bone mineral content and BMD is bone mineral density.

CT-996 drastically suppressed chow intake in obese monkeys

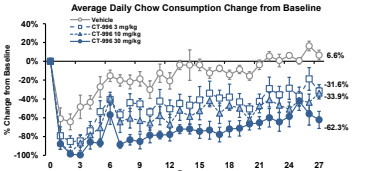
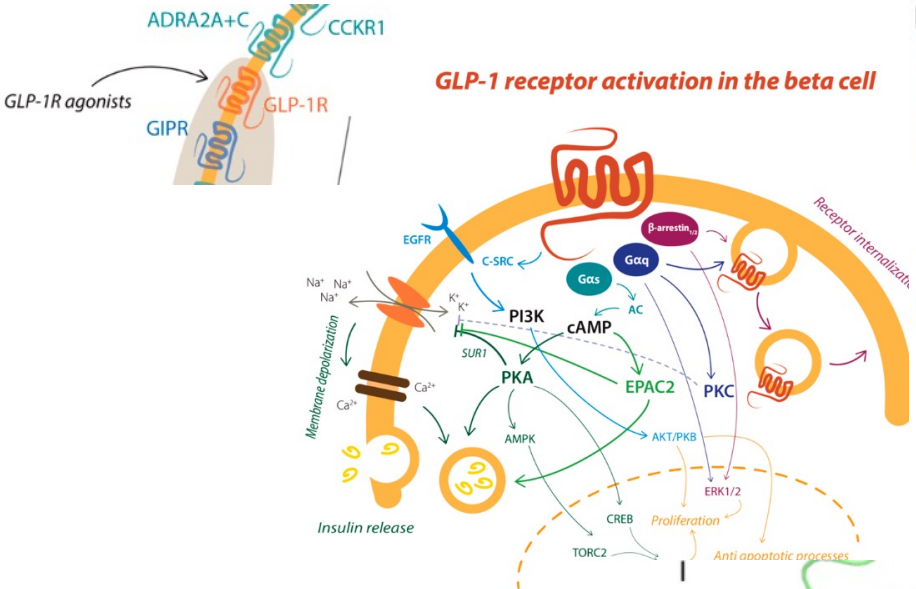
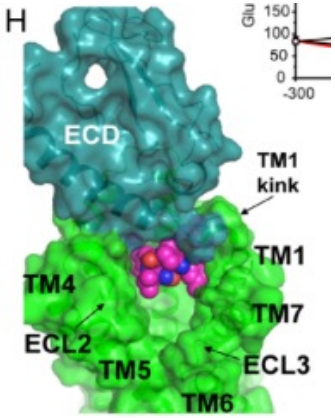
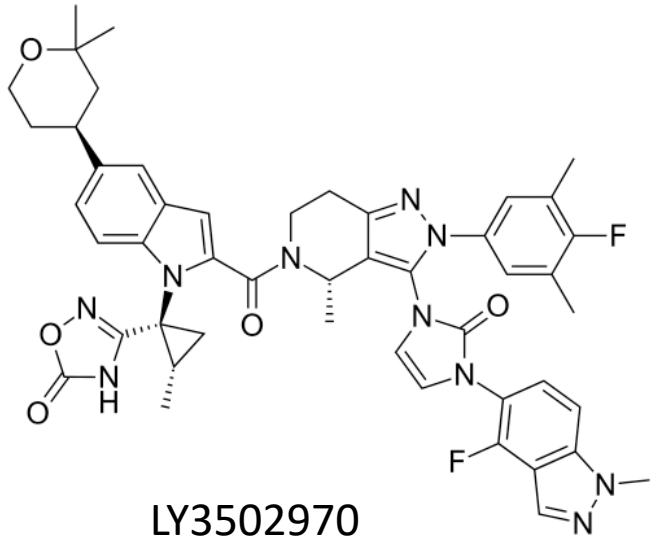


Figure 8. Effects of daily oral CT-996 on chow intake in obese cynomolgus monkeys. Daily chow consumption percent change is represented as mean (± SE) from baseline. N = 6/group.

GLP1R_incretins : from biologicals to small Mr compound







LY3502970
MW 882 Da

MW 4,2 kDa

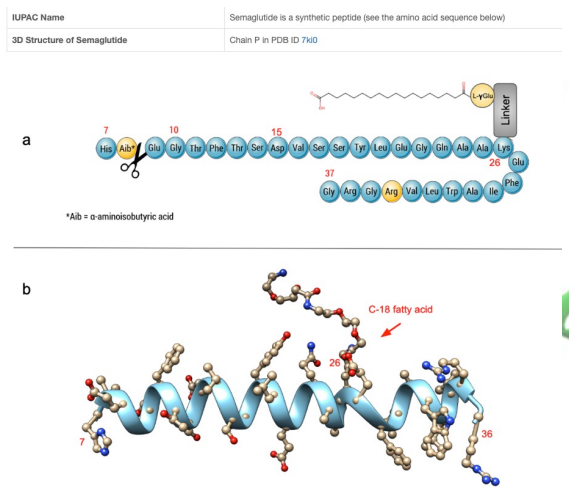
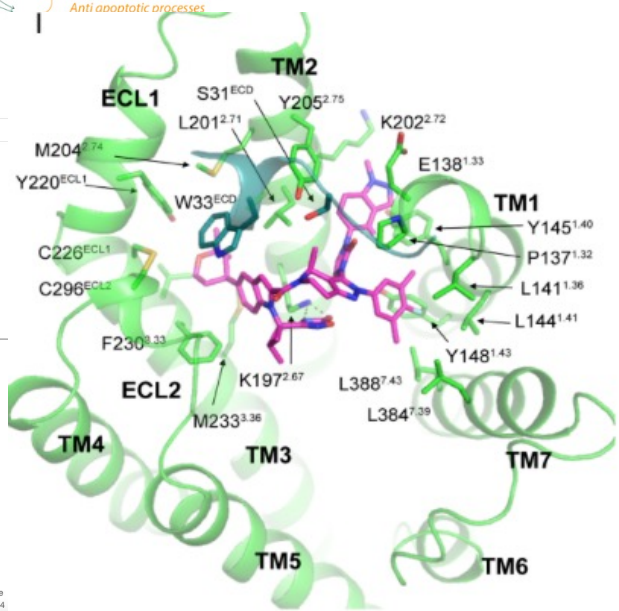
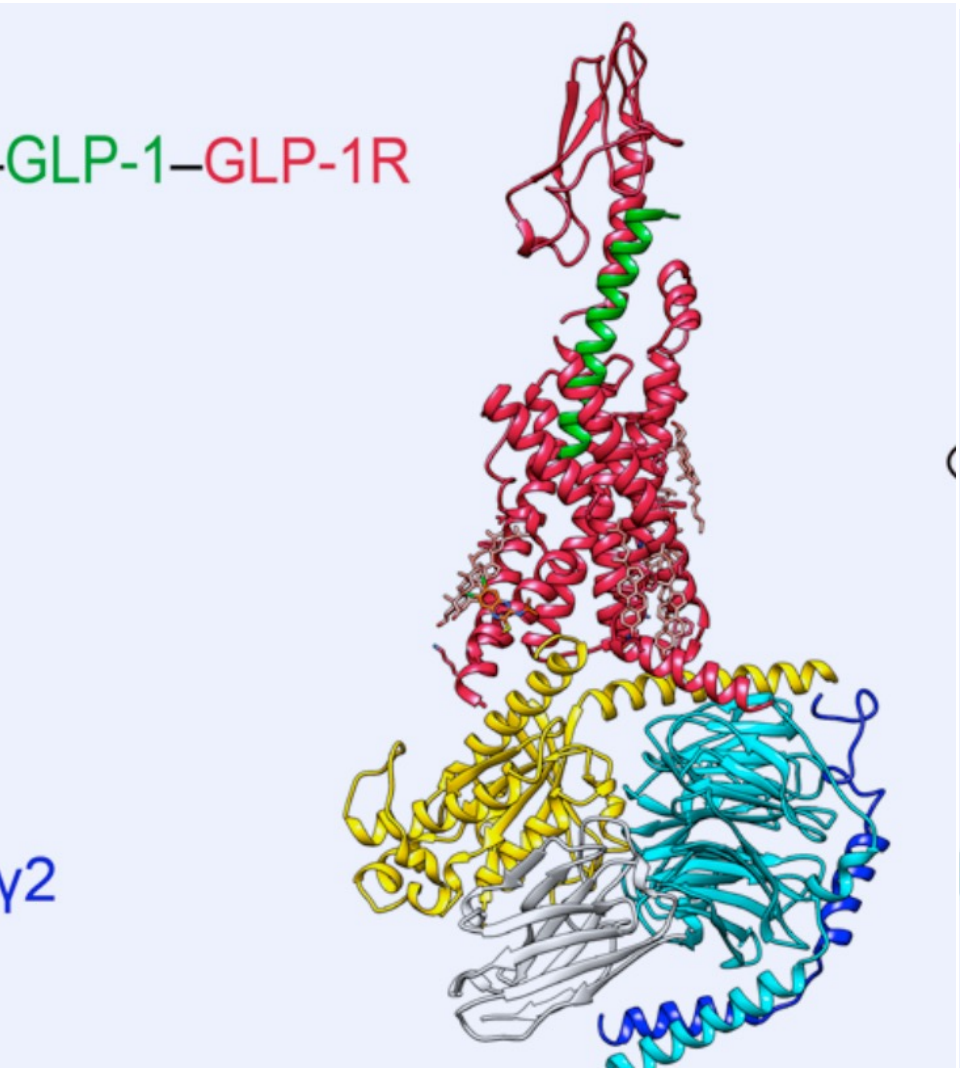


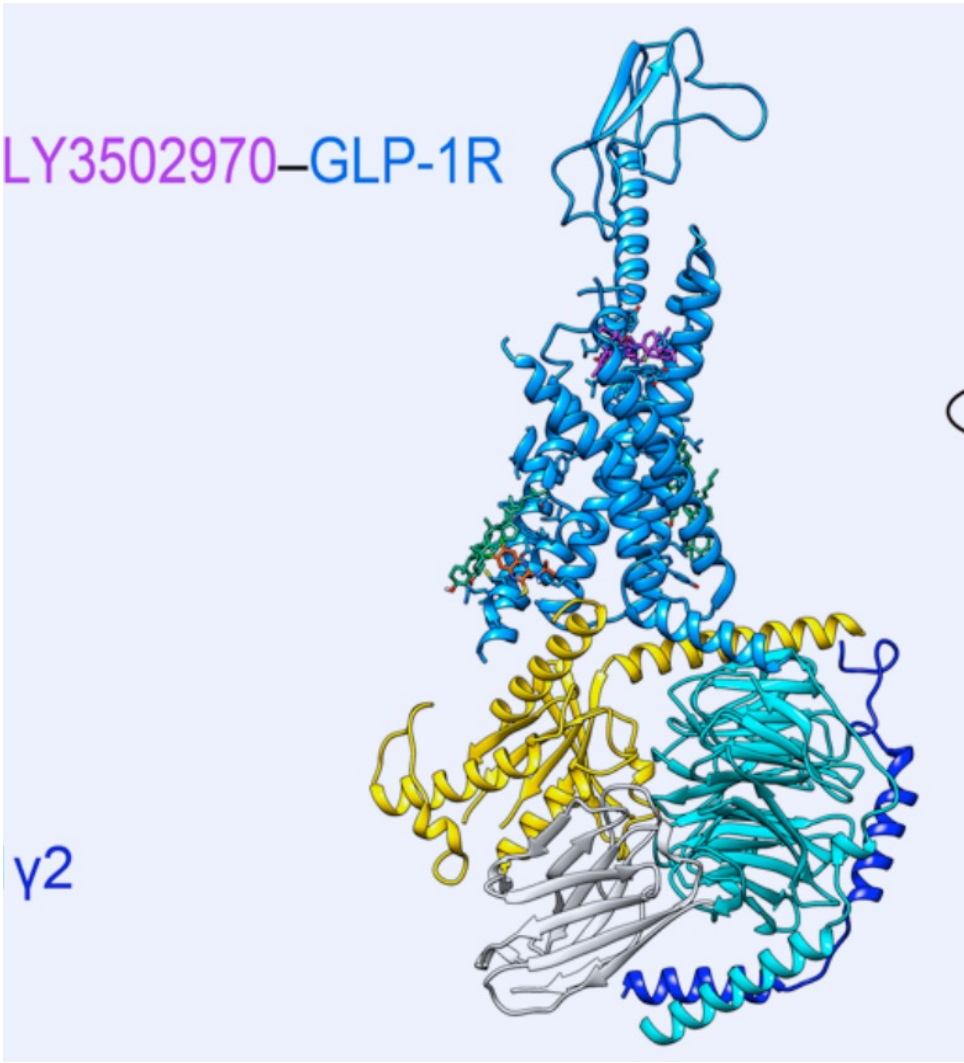
Figure 1. 2D and 3D structures of Semaglutide. a. The amino acid sequence of Semaglutide (PubChem) is numbered from 7 to 37. The sequence schematic shown is based on information presented in Knudsen and Lau, 2019. Amino acid modifications are highlighted in yellow and the DPP4 cleave site is indicated with the scissors. b. 3D structure of Semaglutide (PDB ID 7ki0, Zhang et al., 2021). Note: The C-18 fatty acyl chain shown i



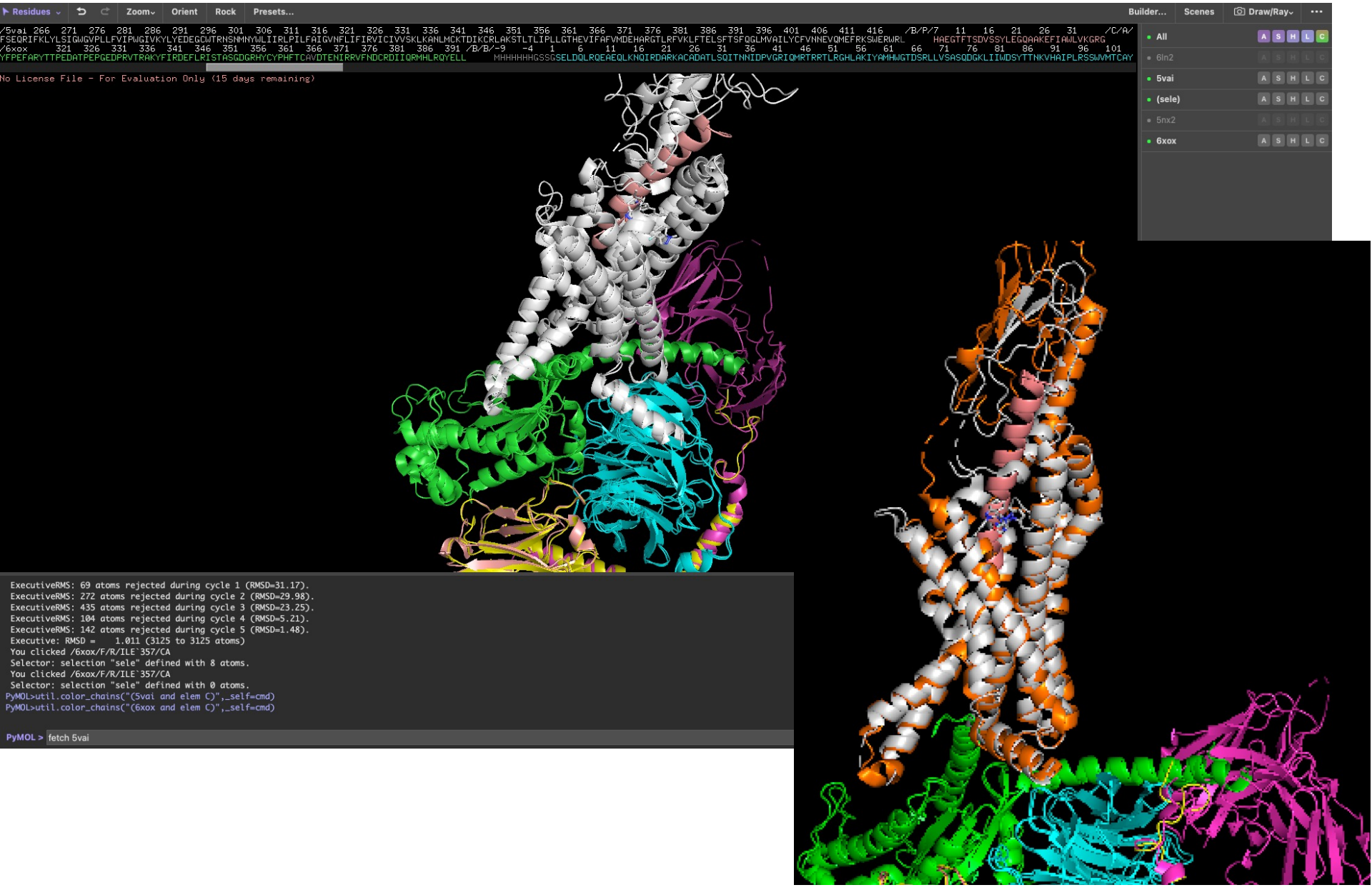
A high-resolution structure of LY3502970 in complex with active-state GLP-1R revealed a unique binding pocket in the upper helical bundle where the compound is bound by the extracellular domain (ECD), extracellular loop 2, and transmembrane helices 1, 2, 3, and 7.

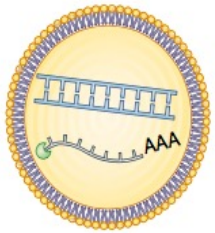
Cryo EM : GLP-1 peptide vs Orforglipron (LY3502970)





Session 7 - when new therapeutic modalities arise
Plug and Play with PyMOL !





Nucleic acid based medicines: a new therapeutic modality

ASOs
(antisense oligonucleotides)
RNAs
(**miRs, siRNAs etc**)

STARTING POINT : THE "UNDRUGGABLE GENOME" EXPLOITED BY DNA DRUGS !

The human genome project 1990-2003

1996: The human genome project may reveal «***3000-10'000 new drug targets***» *(J Drews)*

2002: Release of Human Genome

out of 20'500 proteins encoded in the human genome
- ~400 proteins bind to "drug-like molecules"
- ~200 proteins are targets of marketed small molecule drugs

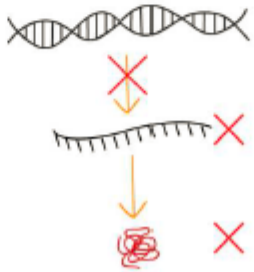
The druggable genome
Andrew L. Hopkins and Colin R. Groom
NATURE REVIEWS | DRUG DISCOVERY
VOLUME 1 | SEPTEMBER 2002

Have we identified all viable drug targets already ?

No!

The undruggable genome is a huge space:

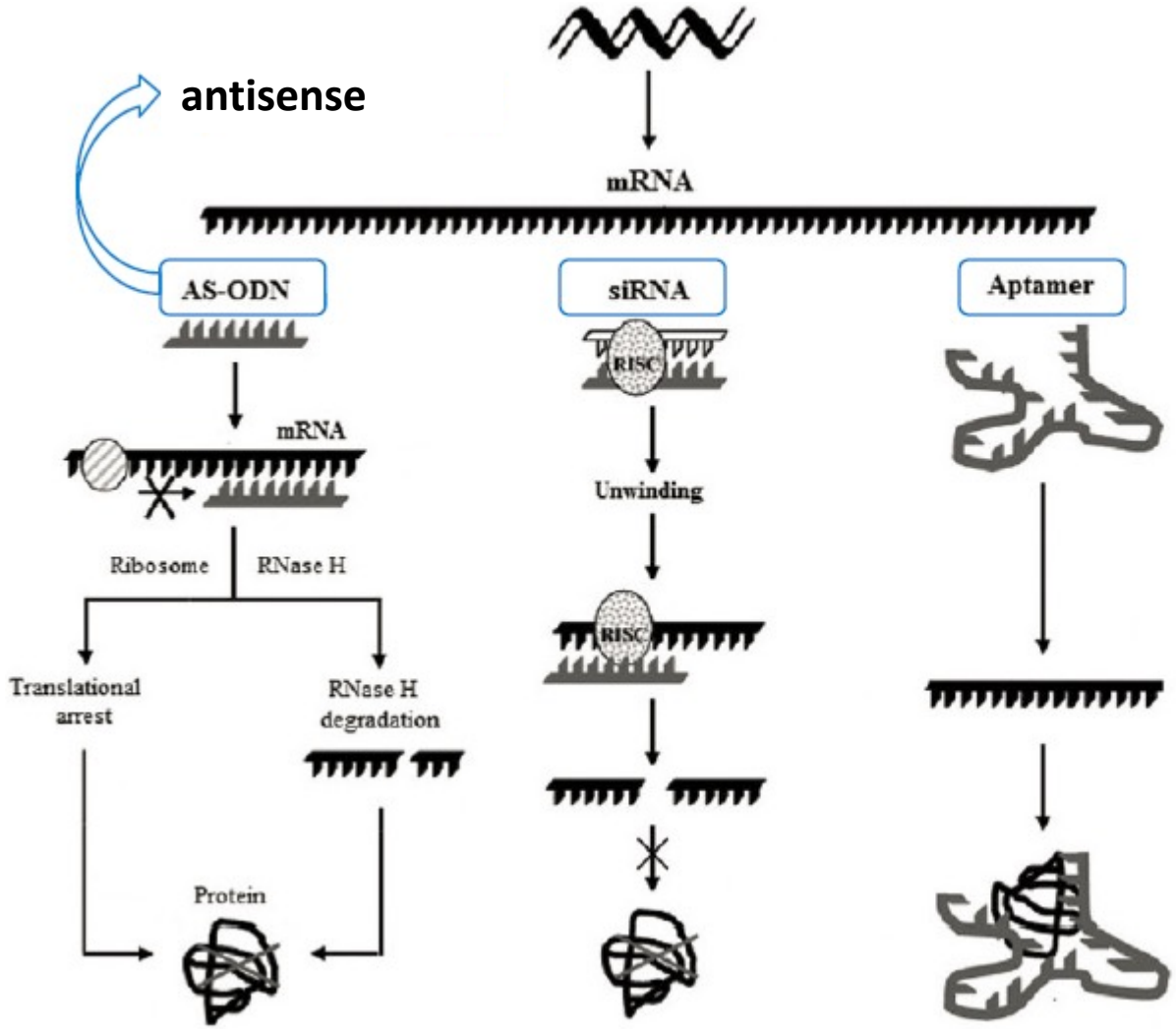
Human genome: 3 Billion Bases
- **76% of DNA is transcribed into mRNA**
  - **3% into Protein**
  - **73% is 'non-coding' RNA**



Nucleic Acid based Therapeutics aim to exploit druggable & undruggable genome

DNA RNA therapeutics

"undruggable" genome

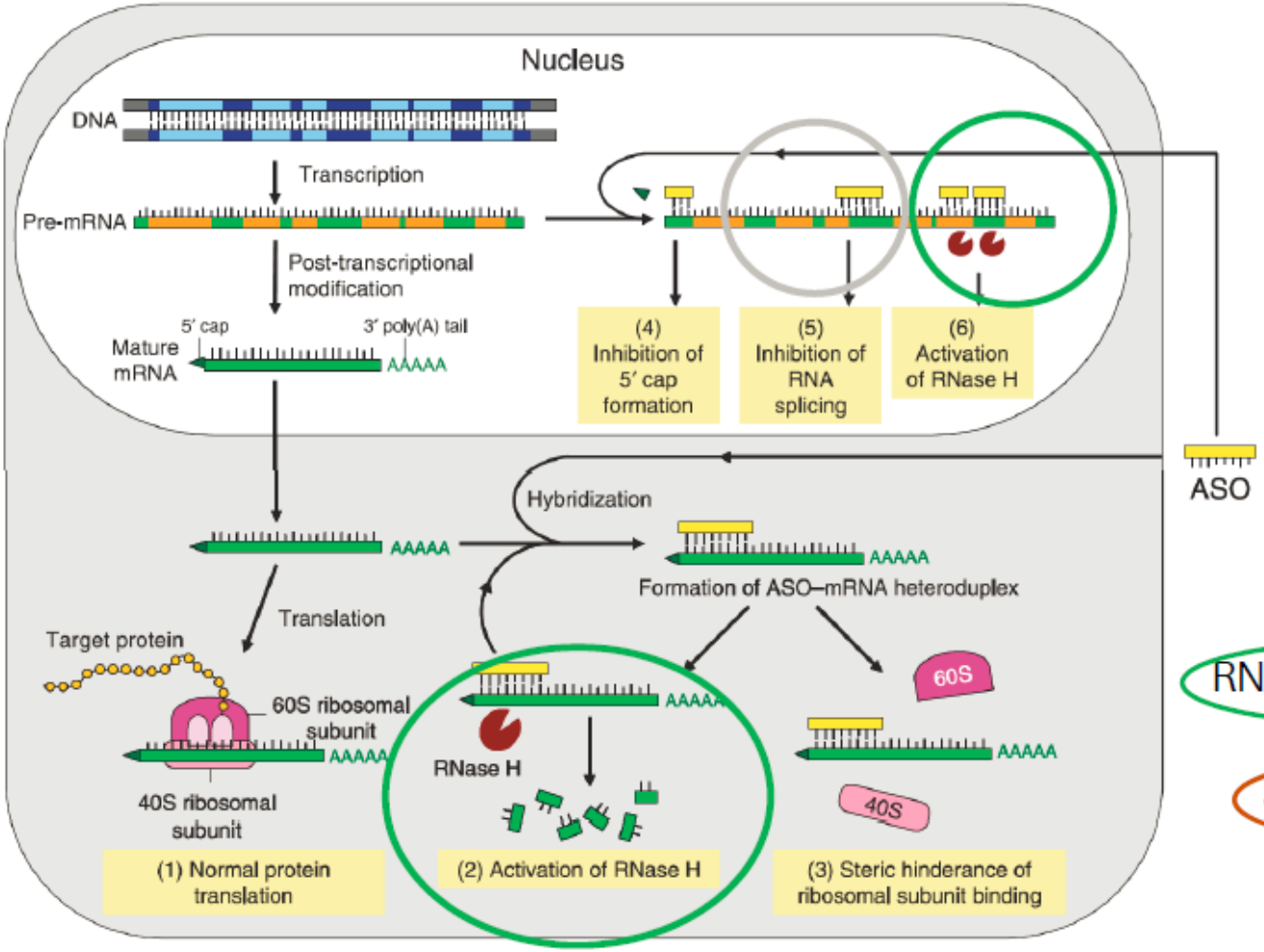


Basic concept:
Instead of binding to a mature protein and thereby alter its function

- **Synthesis** of a protein is inhibited by blocking / degrading mRNA
- **Noncoding/regulatory** regions are targeted
- A **Protein's function** is blocked via mechanism other than targeting ligand binding

Antisense oligonucleotides (single stranded)

Modulation of mRNA functionality by specific hybridization of ASO



Chan et al, Clin Exp Pharmacol Physiol (2006) 33, 533–540

CELL ENTRY : HOW ?

- **Endocytosis:** Non-specific or receptor-mediated uptake of ASO into endosomes (1)
- Endosomes mature into lysosomes (2)
- Majority of ASO accumulate in lysosomes (2):

  'non-productive' pathway
- Small amount of ASO escapes into cytosol and nucleus (3) and is pharmacologically active:

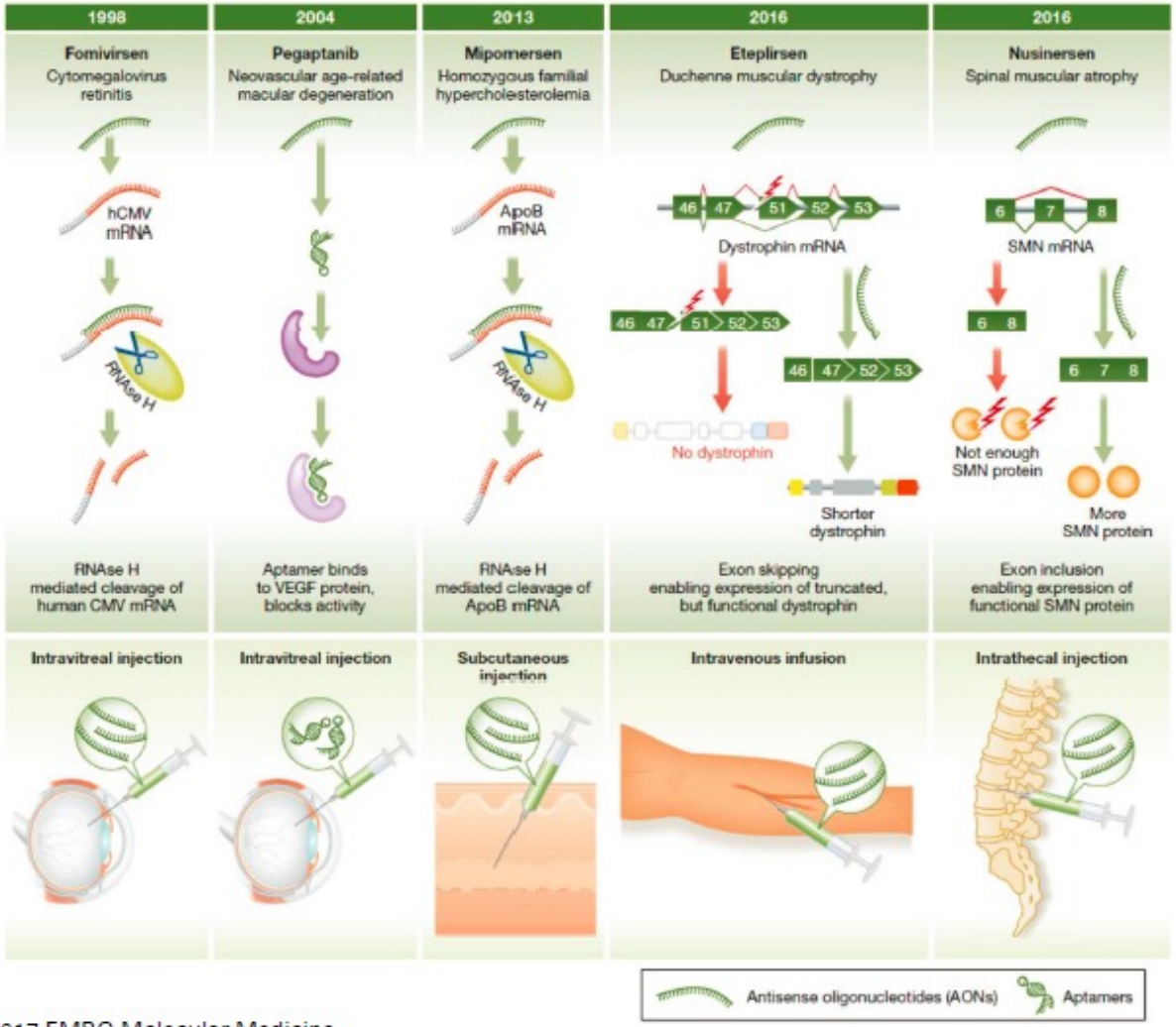
  'productive' pathway
- Exact mechanisms of escape poorly understood

RNAseH degradation

Splice switching

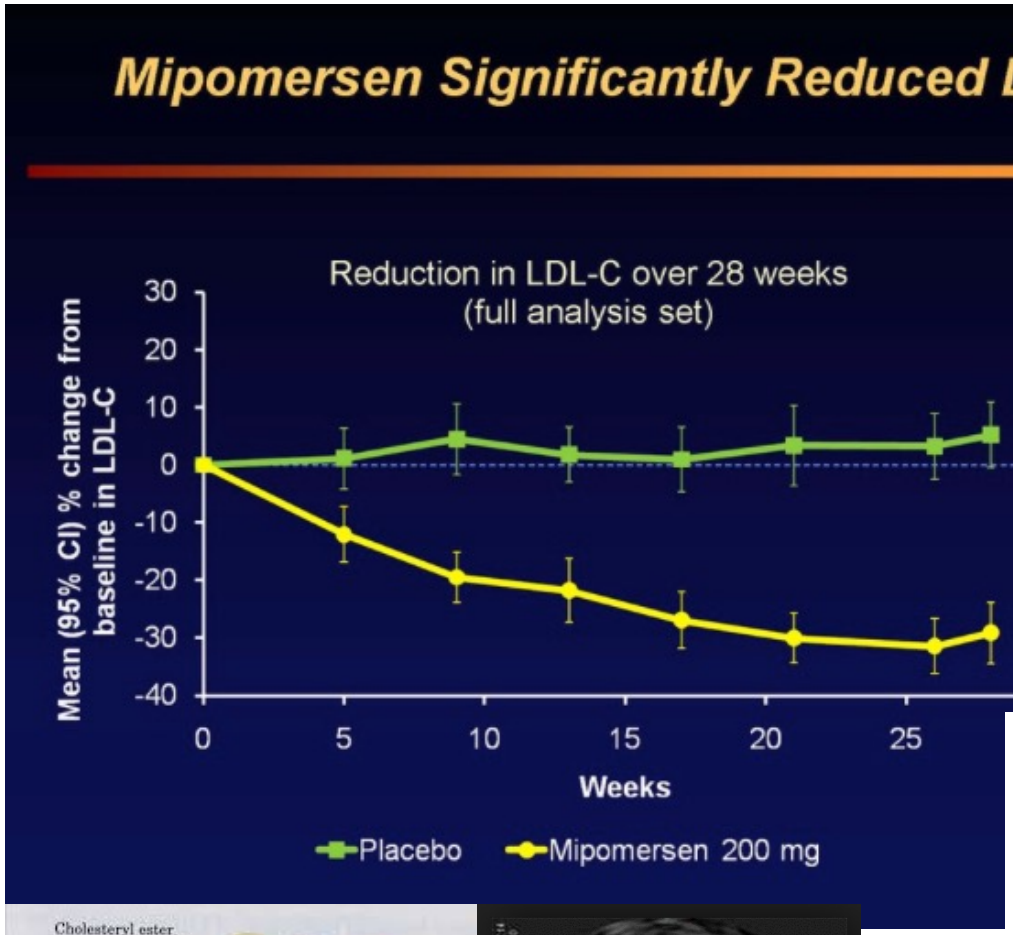
Antisense DNA oligonucleotides in the clinical setting

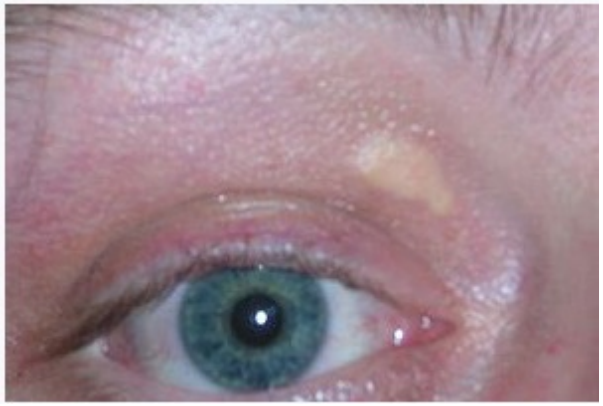
FDA Approved drugs:



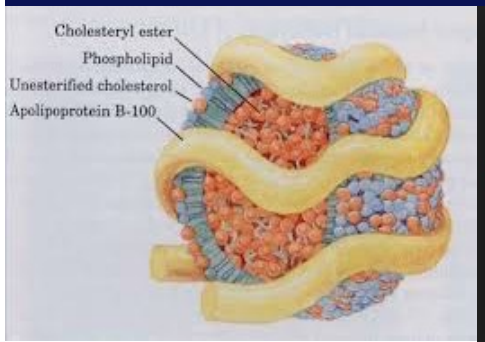
C. Godfrey et al. 2017 EMBO Molecular Medicine

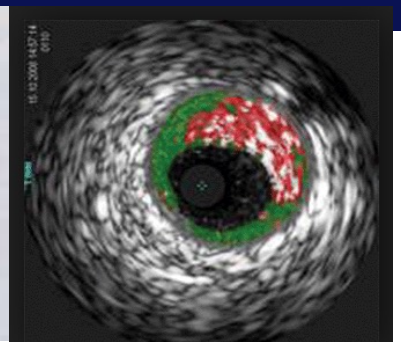
Mipomersen ASO in eg. fam. hypercholesterolemia





FH: cholesterol deposit on eyelid; 80% carry LDLr loss-of-function mutations





Genzyme Inc

Antisense DNA oligonucleotides : route of administration
main issue : poor biodistribution (target organ exposure) and toxicity

SEARCHING FOR THE OPTIMAL SEQUENCE IN SILICO AND TESTING IN VITRO

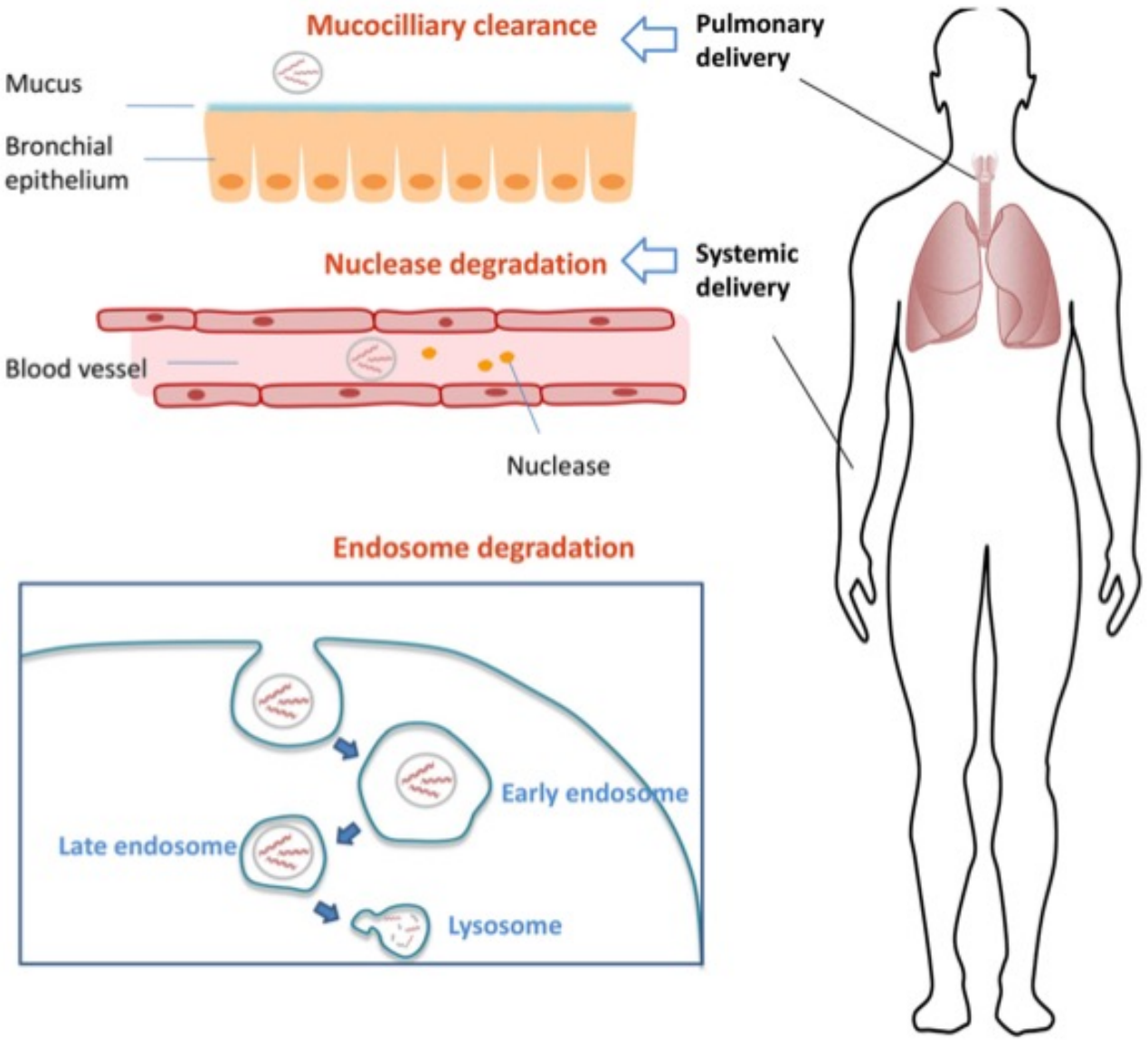
- Classical delivery is by injection:
- **Intravenous (iv)** or **subcutaneous (sc)** for systemic delivery
- **Intramuscular (IM), intratumoral (ITM), intravitreal (IVI)** injection of ASOs have been used to achieve more tissue-specific delivery.
- ASOs do not readily cross the blood brain barrier when administered peripherally
- GalNAc conjugation is the most mature effort so far: a method to reduce dose by a factor 20 or more for hepatocyte specific targets

Antisense DNA oligonucleotides : route of administration
main issue : poor biodistribution (target organ exposure) and toxicity

ASO are given iv, sc, for systemic exposure

ASO do not readily pass the blood brain barrier

Organ exposure include intramuscular and oral administration

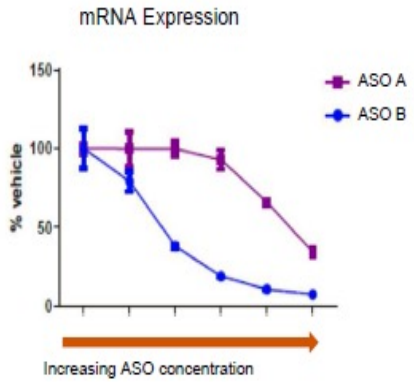


Antisense DNA oligonucleotides

SEARCHING FOR THE OPTIMAL SEQUENCE IN SILICO AND TESTING IN VITRO
RNA FOLDS INTO 3D STRUCTURE WHICH DETERMINES ITS ACCESSIBILITY !

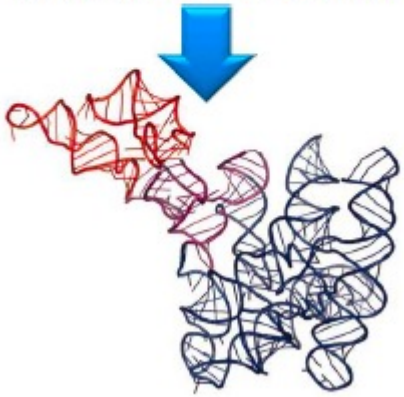
- Synthesize ASOs along the mRNA sequences

- Test activity in vitro in a cell based assay:
- Cell line expressing the intended mRNA
- Determine IC50



Huge differences in activity can be obtained!

Predicted 2D structure of a mRNA



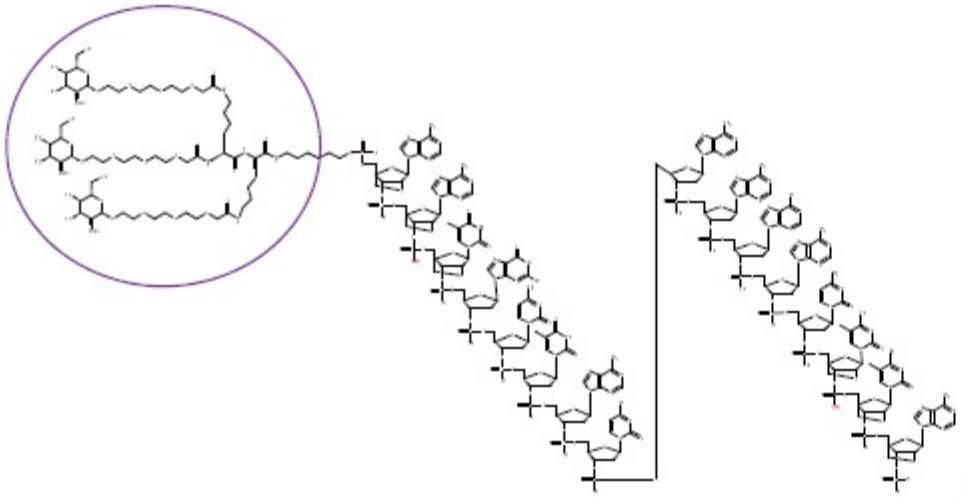
Crystal structure of a bacterial RNA

Antisense DNA oligonucleotides

SEARCHING FOR THE OPTIMAL SEQUENCE IN SILICO AND TESTING IN VITRO

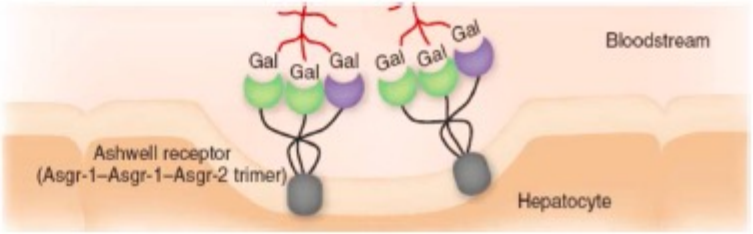
- GalNAc conjugation is the most mature effort so far: a method to reduce dose by a factor 20 or more for hepatocyte specific targets

GalNac cluster



N-acetyl-D-Galactosylamine (GalNAc)

- GalNAc conjugate:
  - Asialoglycoprotein receptor is used **to target hepatocytes**
  - Trimeric GalNAc cluster is recognized by the receptor and internalized together with the cargo (ASO)

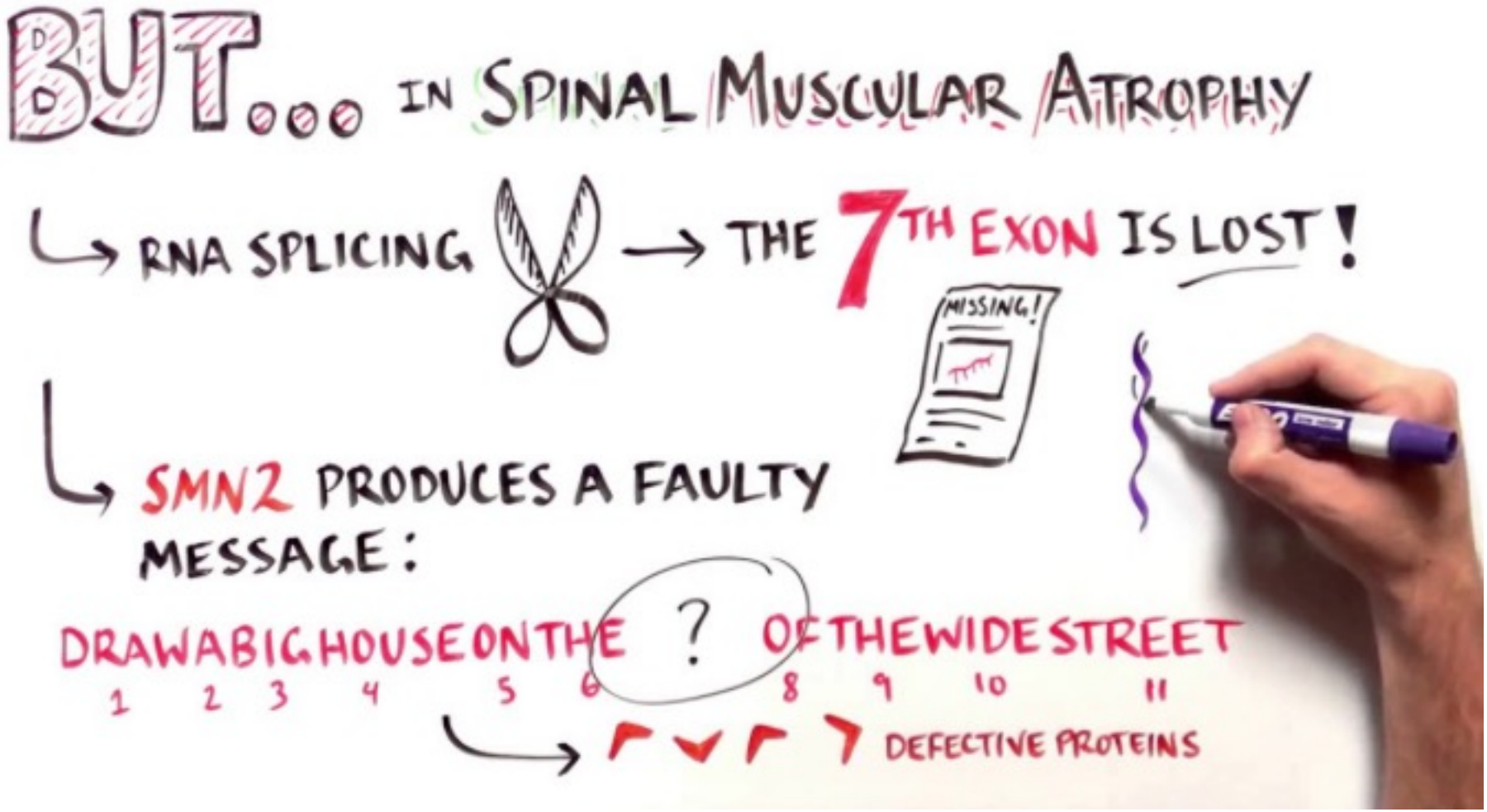


Antisense DNA oligonucleotides : spinal muscular atrophy : the nusinersen trials

Antisense DNA oligonucleotides : spinal muscular atrophy

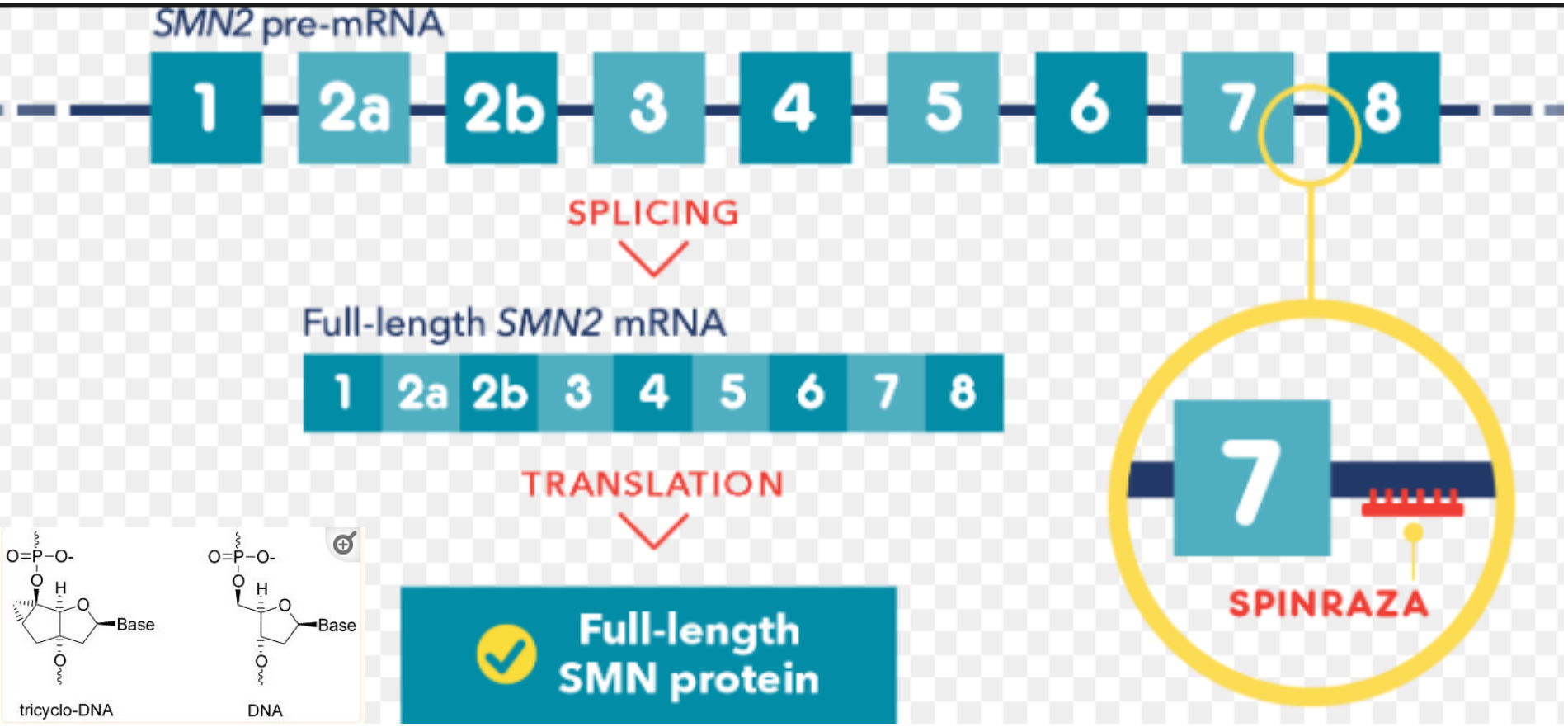
- Spinal muscular atrophy (SMA) is a degenerative rare disease of the motor nuclei in the spinal cord and lower brain stem
- SMA presents in childhood with muscle weakness and most severely fatal respiratory failure (10-20'000 kids in US)
- Mutations of the SMN locus on 5q13 (surviral of motor neuronal gene 1 and 2) are the cause of SMA rare disease
- In humans the locus contains splice site mutations at both the telomeric SMN1 gene and the centromeric homologous SMN2 gene
- Prevalence is 1.2 over 100 000 individuals (1/6000 babies)
- Carrier rates for the autosomal recessive is 1 in 40 for genetic counselling purposes (one copy of the altered gene)
- Spinal muscular atrophy (SMA) type I (Hoffmann Disease) was found in 4:1,600 (1:400) infants of the egyptian Karaite community (endemistic propagation)

SEARCHING FOR THE OPTIMAL SEQUENCE IN SILICO AND TESTING IN VITRO



SMA : alternate RNA splicing defects by exon7 skipping of SMN2 pre mRNA

SEARCHING FOR THE OPTIMAL SEQUENCE IN SILICO AND TESTING IN VITRO



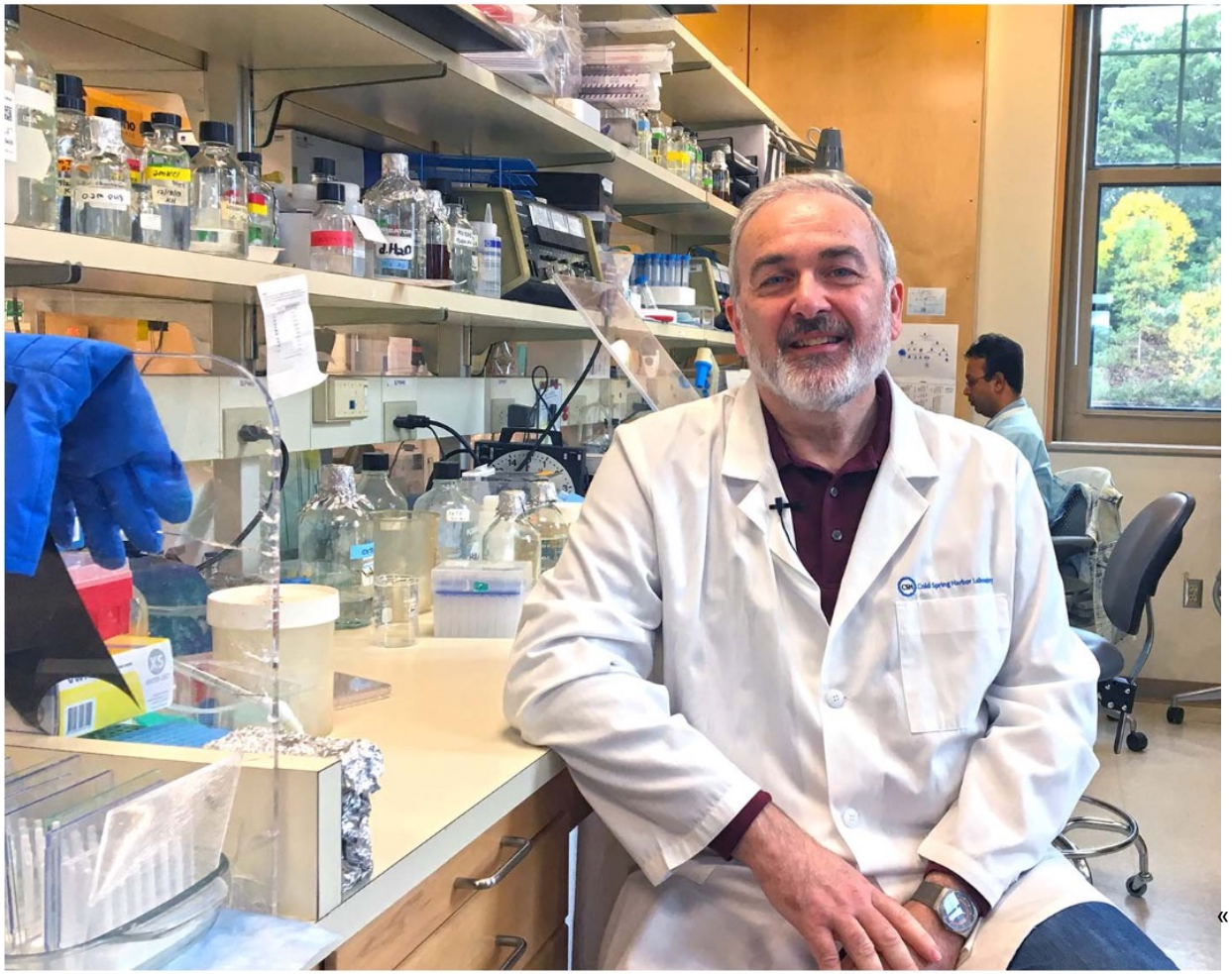
Intronic silencing sequence regulate splicing of SMN2 exon 7

Tricyclo DNA molecules to improve on stability of AOS (RNAseH resistance) and BBB exposure

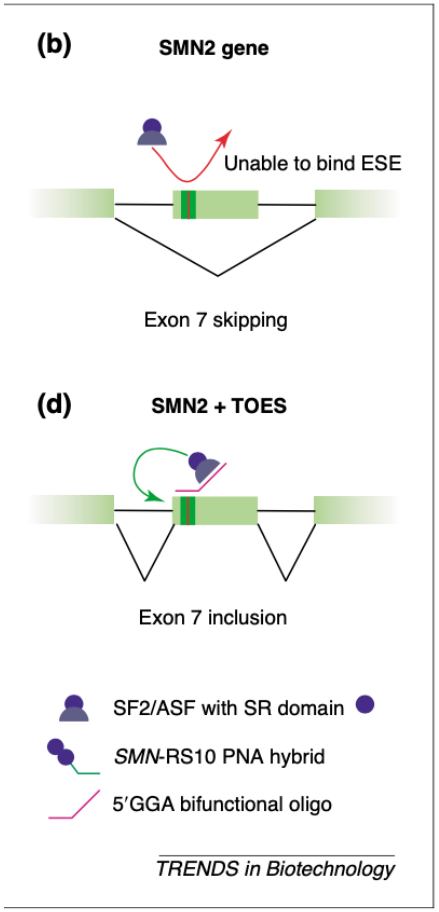
SMA : pre mRNA splicing research leads to therapeutics

Cold Spring Harbor Laboratory ABOUT US EVENTS NEWSSTAND PARTNER WITH US CAREERS GIVING

RESEARCH BANBURY CENTER CSHL PRESS DNA LEARNING CENTER MEETINGS & COURSES PH.D. PROGRAM



CSHL Professor Adrian Krainer, winner of the 2019 Breakthrough Prize in Life Science, in his laboratory.



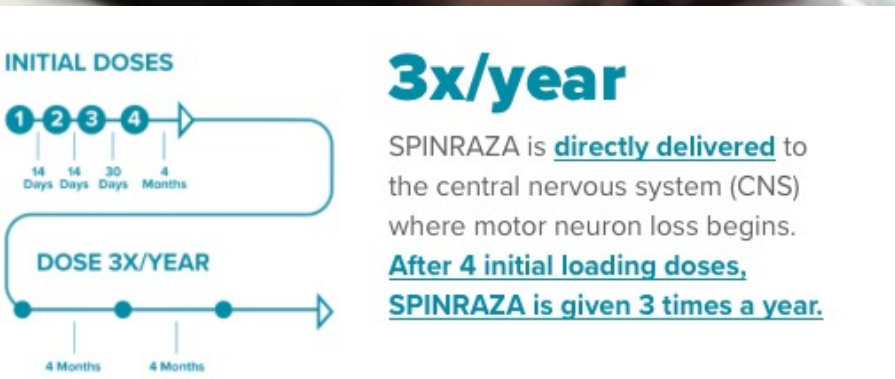
« Cold Spring Harbour lab 2017 medicine « 2017

Cartegni,L.Krainer,A.(2002) Nat. Genet. 30, 377–384. Disruption of an SF2/ASF-dependent exonic splicing enhancer in SMN2 causes spinal muscular atrophy in the absence of SMN1.

Spinraza: spinal muscular atrophy innovative medicine
12mg AOS dosis as intrathecal injections (spine puncture)

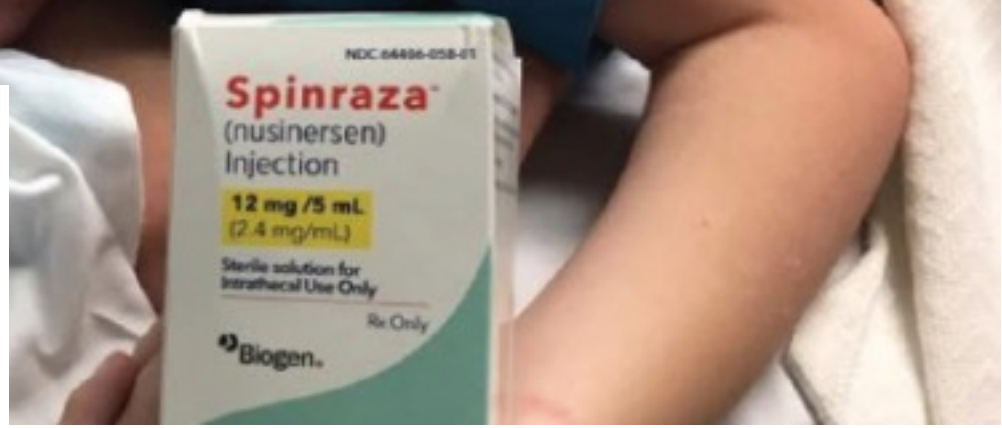
The most common adverse reactions that occurred in at least 20% of SPINRAZA-treated patients and occurred at least 5% more frequently than in control patients were:

- lower respiratory infection and constipation in patients with infantile-onset SMA
- pyrexia, headache, vomiting, and back pain in patients with later- onset SMA



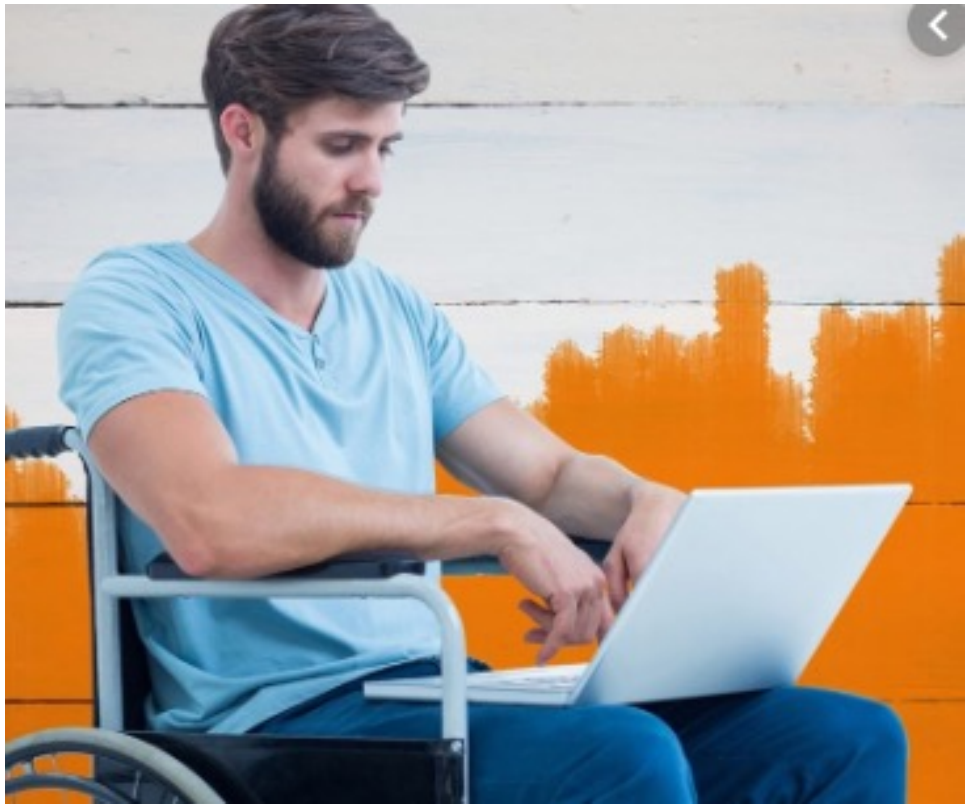
3x/year

SPINRAZA is directly delivered to the central nervous system (CNS) where motor neuron loss begins. After 4 initial loading doses, SPINRAZA is given 3 times a year.



Spinraza: correcting RNA splicing defects by targeting splice factor at genomic site

SMA : spinal muscular atrophy splicing ailment



Biomedical scientists with Biogen Inc receive the pretigious « Prix Galien » for innovative medicine

Gene therapy : spinal muscular atrophy innovative medicine

$2.1m Novartis gene therapy to become world's most expensive drug

US approves the one-time treatment for deadly spinal muscular atrophy in infants

▲ Swiss drugmaker Novartis has gained US approval for its $2.1m spinal muscular atrophy gene therapy Zolgensma.

Zolgensma: spinal muscular atrophy innovative gene therapy medicine

BIOTECH AND PHARMA

FDA approves Novartis' $2.1 million gene therapy — making it the world's most expensive drug

PUBLISHED FRI, MAY 24 2019 • 1:03 PM EDT | UPDATED FRI, MAY 24 2019 • 3:11 PM EDT

Berkeley Lovelace Jr. @BERKELEYJR
Angelica LaVito @ANGELICALAVITO

SHARE

KEY POINTS

- The FDA approved Novartis' Zolgensma, a one-time treatment for spinal musc atrophy.
- Zolgensma, a gene therapy, will cost $2.1 million.
- Novartis had previously said it could price the treatment between $1.5 million and $5 million.

Adeno-associated viral dependent SMN expression gene therapy indicated for the treatment of pediatric patient less than 2 years of age with spinal muscular atrophy (SMA) with bi-allelic mutations in the survival motor neuron 1 (*SMN1*) gene.

Marketing

Zolgensma safety scare hits Novartis again as FDA halts spinal injection trial

by Angus Liu I Oct 30, 2019 10:42am

According to Novartis, its gene therapy unit AveXis alerted regulators about dorsal root ganglia mononuclear cell inflammation, sometimes accompanied by neuronal cell body degeneration or loss, that cropped up in a preclinical study. It's a new finding in Zolgensma animal testing.

The condition can be associated with sensory changes, the Swiss drugmaker said, adding that it has not cropped up in humans so far.

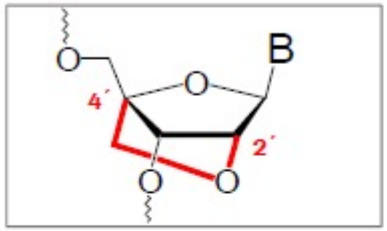
In a Wednesday note to clients, SVB Leerink analyst Mani Foroohar suspected the clinical halt is "likely reflective of FDA taking a more stringent attitude towards [Zolgensma] review" after a recent data scandal.

The company said it's working with the FDA to "identify any additional actions necessary to resume dosing" in the spinal-injection trial, dubbed Strong, and will continue to monitor safety events in patients.

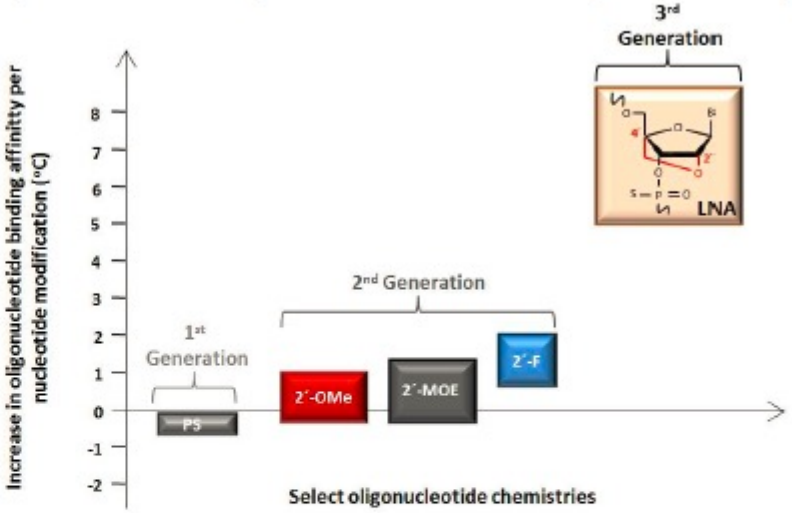
Known as intrathecal delivery, the direct injection into the spine is one avenue Novartis is pursuing to expand Zolgensma's patient base. The gene therapy is currently only approved for SMA patients younger than two, but the Strong trial is testing it in older SMA type 2 patients up to five years of age.

Antisense DNA oligonucleotides (single stranded)

LNA – locked nucleic acid



..provides unprecedented target affinity



.. resulting in oligos with:

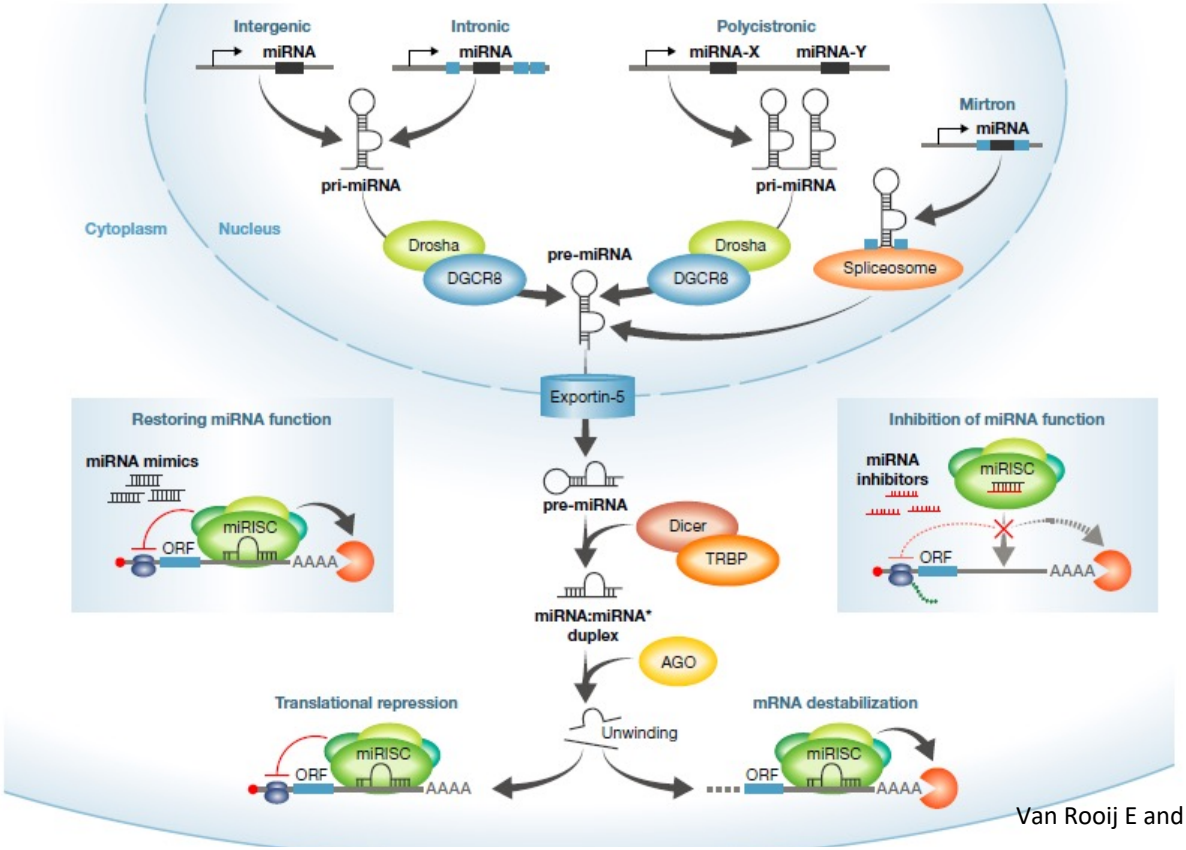
- Substantially improved potency and specificity
- Multiple *modes-of-action*
- Activity in multiple tissues
- Potential for good tolerability profiles
- Lower cost of goods

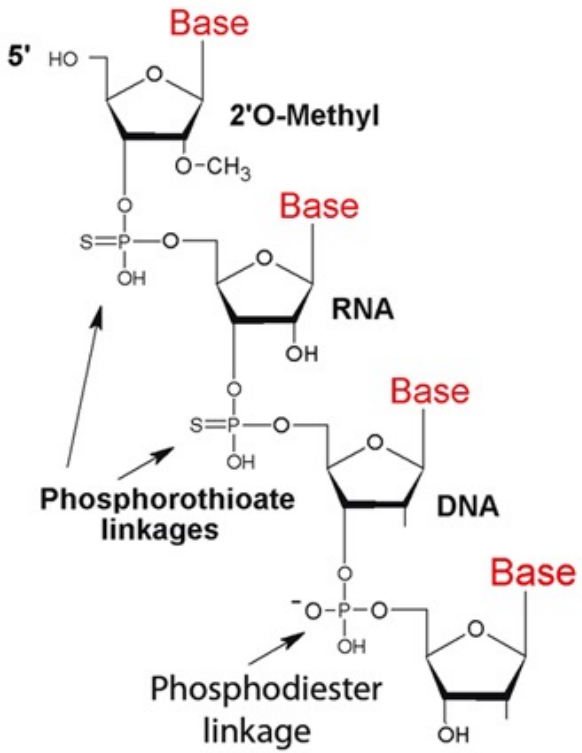
.. enables smaller and diverse designs

LNA gapmers
mRNA degradation
→ reduction of disease causing proteins

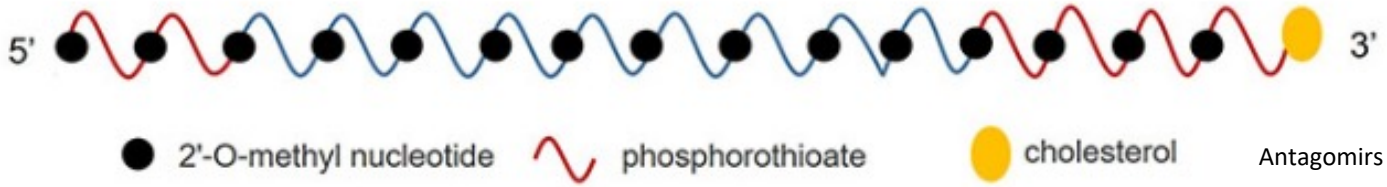
LNA mixmers /antimiRs
Splice modulation of pre-mRNAs
→ creation of disease ameliorating protein
Steric inhibition of miRNA
→ upregulation of protein pathways

Development of microRNA therapeutics (agomirs – antagomirs) is coming of age
>30 % of human genes regulated by miRs !

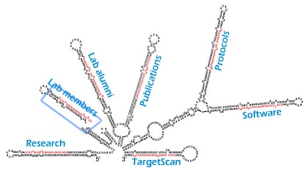




Van Rooij E and Kauppinen S. 2014 EMBO Mol Med J 6:851-864

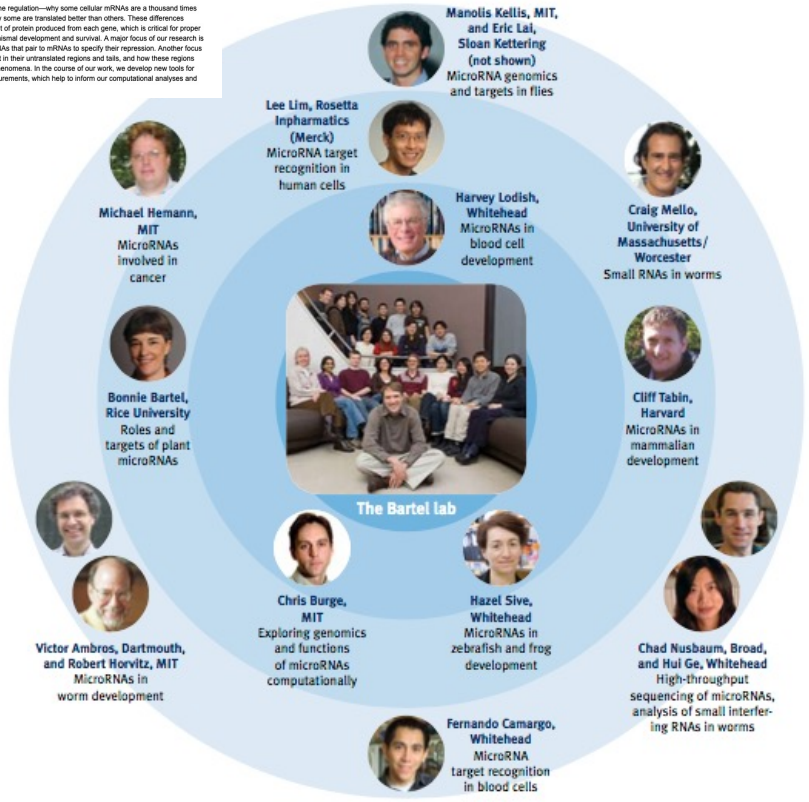


Antagomirs sequences



Antagomirs - RNA therapeutics in drug development

We study post-transcriptional gene regulation—why some cellular mRNAs are a thousand times more stable than others, and why some are translated better than others. These differences dramatically influence the amount of protein produced from each gene, which is critical for proper cellular function, as well as organismal development and survival. A major focus of our research is microRNAs, which are ~22-nt RNAs that pair to mRNAs to specify their repression. Another focus is mRNAs, with particular interest in their untranslated regions and tails, and how these regions recruit and mediate regulatory phenomena. In the course of our work, we develop new tools for high-throughput molecular measurements, which help to inform our computational analyses and in-depth mechanistic studies.



The RNA connection

A snapshot of joint projects by David Bartel's lab highlights the crucial role of collaborations

More than a third of the human genome is partially regulated by microRNAs—tiny snippets of RNA that can disable a gene's ability to create proteins.

So it's no surprise that the lab of Whitehead Member David Bartel, the first to report this surprisingly widespread role for microRNAs, has found many colleagues happy to collaborate. At the same time, "as our lab looks at the particular targets of particular microRNAs, then we become interested in what's going on in other labs that specialize in those targets," Bartel says.

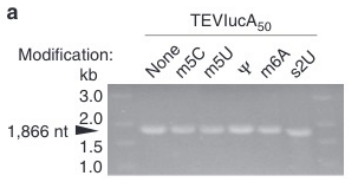
Here's a glimpse at some current connections for the 20-person lab—and it *is* just a glimpse. It shows only the principal investigators, not the postdocs and students who do all the bench work, let alone the ongoing streams of informal discussions.

original article

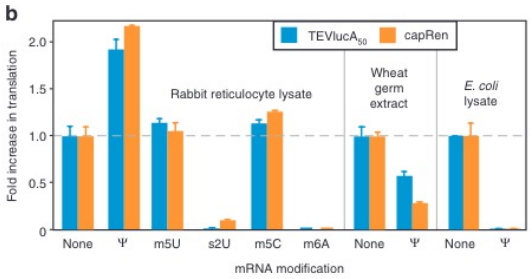
Incorporation of Pseudouridine Into mRNA Yields Superior Nonimmunogenic Vector With Increased Translational Capacity and Biological Stability

Katalin Karikó[1], Hiromi Muramatsu[1], Frank A Welsh[1], János Ludwig[2], Hiroki Kato[3], Shizuo Akira[3] and Drew Weissman[4]

[1]Department of Neurosurgery, University of Pennsylvania, Philadelphia, Pennsylvania, USA; [2]Laboratory of RNA Molecular Biology, The Rockefeller University, New York, New York, USA; [3]Department of Host Defense, Research Institute for Microbial Diseases, Osaka University, Osaka, Japan; [4]Department of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, USA

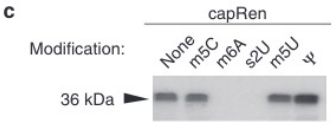


BIONTECH



Katalin Karikó

Biographie



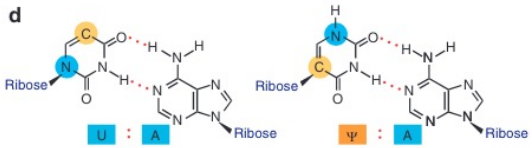


Figure 1 In vitro transcription and translation of nucleoside-modified mRNAs. (a) Aliquots of *in vitro*–transcribed TEVIucA$_{50}$ containing no

Antagomirs (anti miRs) therapeutics in drug development

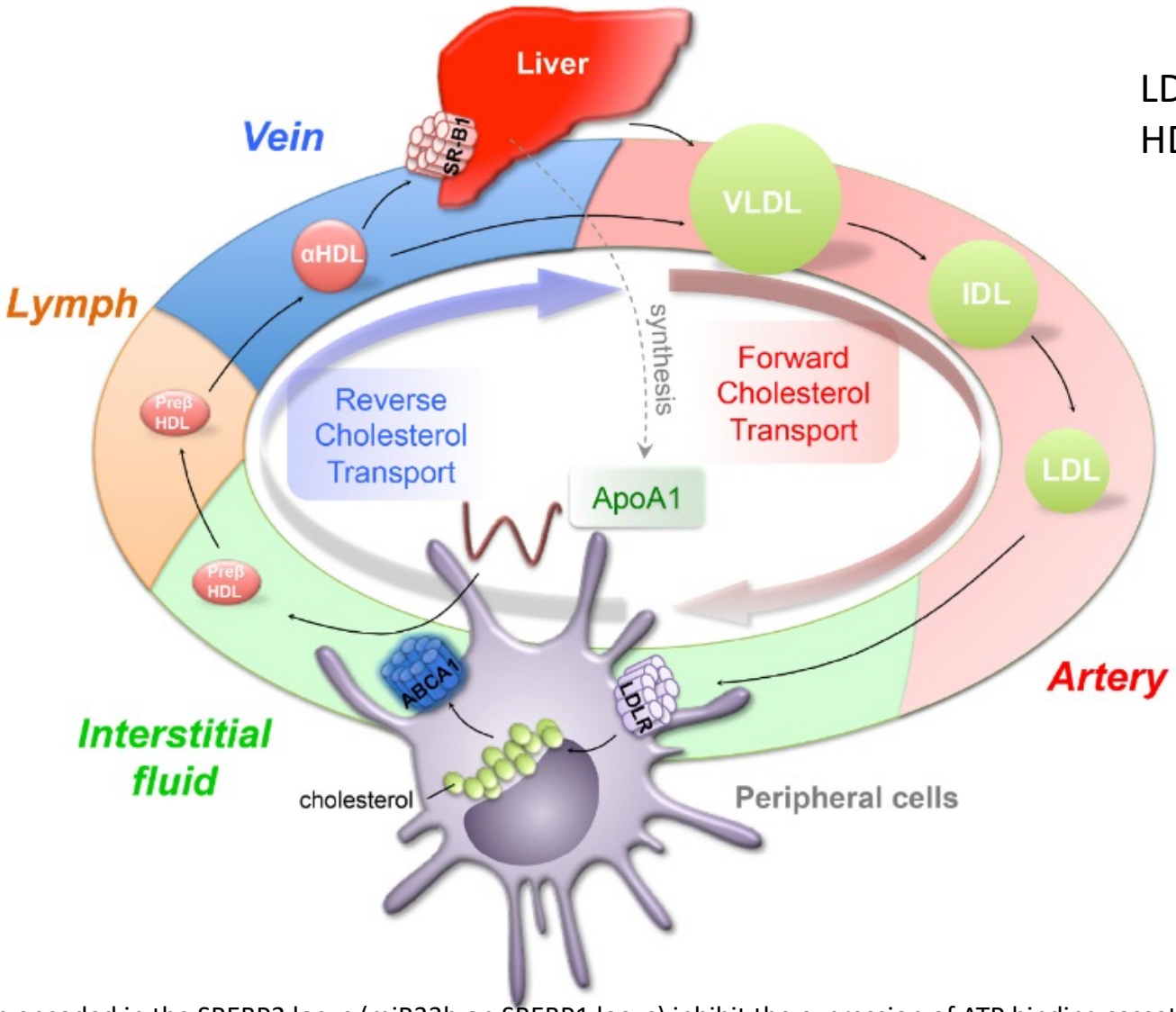
Table 1. MicroRNA-based therapeutics in development

Company	miRNA target	Mode of action	Indication	Status
Santaris Pharma	miR-122	antimiR	HCV	Clinical Phase II
Mirna Therapeutics	miR-34	mimic	Unresectable primary liver cancer	Clinical Phase I
	let-7	mimic	Cancer	Preclinical
Regulus Therapeutics	miR-122	antimiR	HCV	Clinical Phase I
	miR-221	antimiR	Hepatocellular carcinoma	Preclinical
	miR-10b	antimiR	Glioblastoma	Preclinical
	miR-21	antimiR	Hepatocellular carcinoma	Preclinical
	miR-21	antimiR	Kidney fibrosis	Preclinical
	miR-33	antimiR	Atherosclerosis	Preclinical
miRagen Therapeutics	miR-208	antimiR	Heart failure	Preclinical
	miR-15/195	antimiR	Post-MI remodeling	Preclinical
	miR-145	antimiR	Vascular disease	Preclinical
	miR-451	antimiR	Myeloproliferative disease	Preclinical
	miR-29	mimic	Fibrosis	Preclinical
	miR-208	antimiR	Cardiometabolic disease	Preclinical
	miR-92	antimiR	Peripheral artery disease	Preclinical

Antagomir (anti-miRNAs) are antisense oligonucleotides that silence endogenous microRNA from their gene target binding capabilities

eg. miR33a intron encoded in the SREBP2 locus (miR33a on SREBP1 locus) inhibit the expression of ATP binding cassette transporter ABCA1, a cholesterol efflux pump essential in RCT (reverse cholesterol transport)

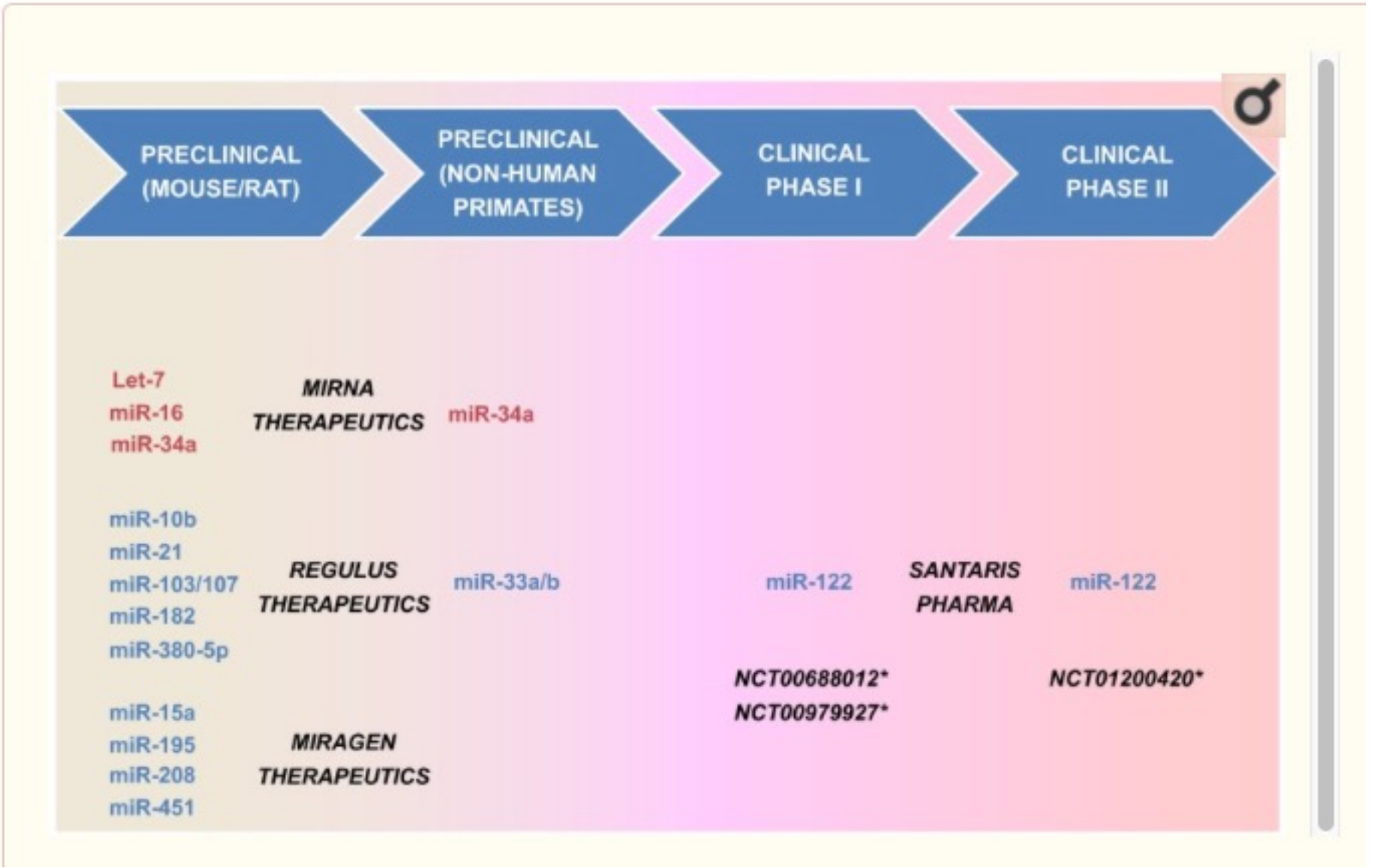
Antagomirs (anti miR33a) therapeutics in CVD drug development



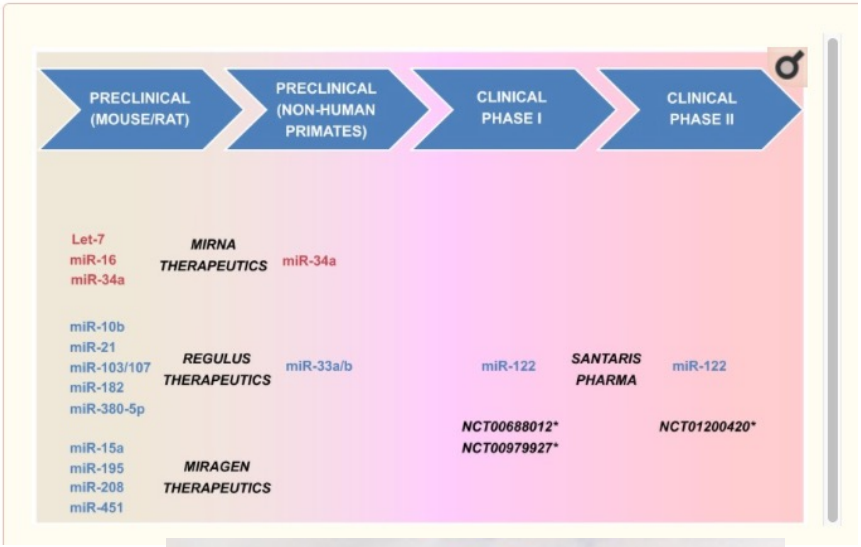
LDL = mg/dl
HDL = mg/dl

eg. miR33a intron encoded in the SREBP2 locus (miR33b on SREBP1 locus) inhibit the expression of ATP binding cassette transporter ABCA1, a cholesterol efflux pump essential in RCT (reverse cholesterol transport) (SREBP = sterol response binding protein)

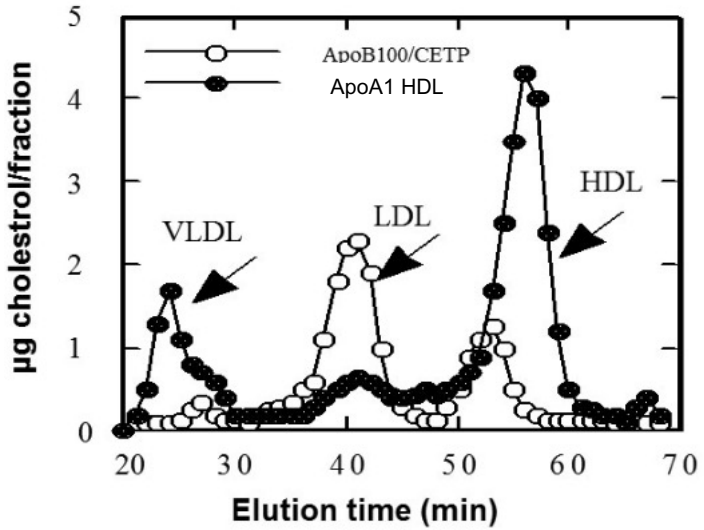
Development of microRNA therapeutics is coming of age



Development of miR33a antagomirs therapeutics in CVD



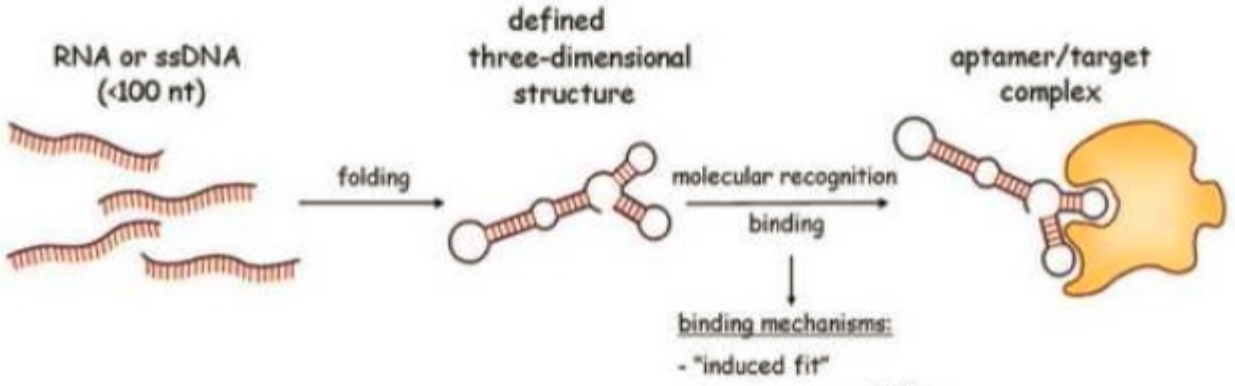
SUBSTANTIAL PRECLINICAL WORK HAS BEEN COMPLETED INCLUDING A NON- HUMAN PRIMATE STUDY OF INHIBITING miR-33A/B FOR THE TREATMENT OF ATHEROSCLEROSIS. BY TREATING AFRICAN GREEN MONKEYS SUB CUT WITH ANTI miR-33 , A DECREASE IN VLDL AND LDL AND AN INCREASE OF HDL WAS OBSERVED. REDUCED REPRESSION OF ABCA1 GENE OBSERVED (MOA VERYFIED !!)



Rayner KJ et al 2012 Nature 478 : 404-407

DNA RNA therapeutics : aptamers

- Oligonucleotides (DNA or RNA, 15-60 Bp) having high affinity and specificity in identifying target molecules (typically proteins)
- can be selected to bind any given target by chemical recognitions different from Watson–Crick base pairing.
- Interaction to target based on 3-Dimensional folding of the single stranded oligonucleotide (intramolecular hybridization) into particular shape.



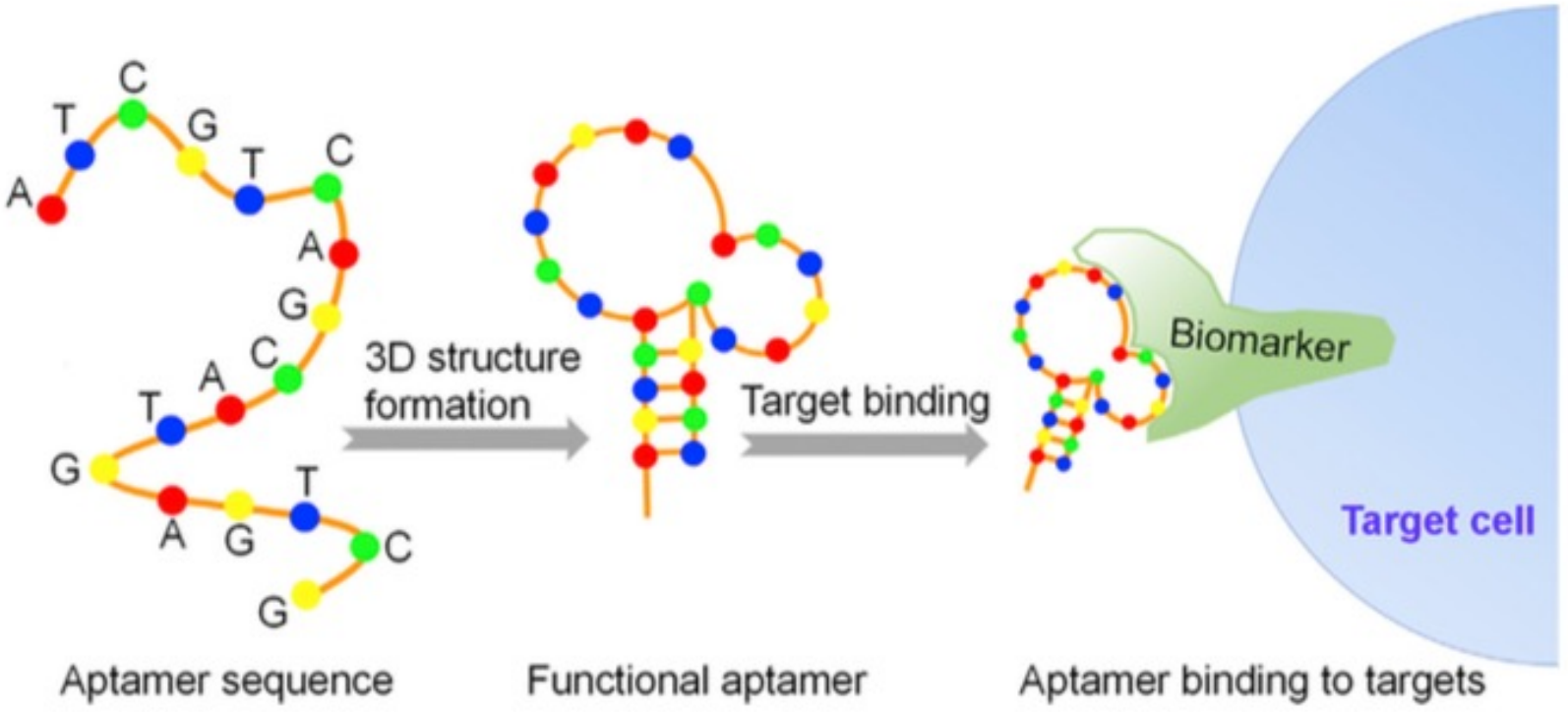
SELEX (systematic evolution of ligands by exponential enrichment) allows over several rounds of selection from a pool of sequences against the therapeutic target high affinity and specificity cdps to the target : similar affinities as ABs with low immunogenitcity however

DNA RNA therapeutics : aptamers

www.moleculartherapy.org

Review

Yang S et al.(2018) Molecular Therapy Nucleic Acids 13:164-175. Oligonucleotide aptamer-mediated precision therapy of hematological malignancies.



DNA RNA therapeutics : aptamers

Table 2 | **Aptamers in the clinic**

Name (company)	Composition	Target	Indication	Current phase
Pegaptanib sodium/Macugen (Pfizer/Eyetech)	2′-O-methyl purine/2′-fluoro pyrimidine with two 2′-ribo purines conjugated to 40 kDa PEG, 3′ inverted dT	Vascular endothelial growth factor	Age-related macular degeneration	Approved in the US and the EU
AS1411/ AGRO001 (Antisoma)	G-rich DNA	Nucleolin	Acute myeloid leukaemia	Phase II
REG1/RB006 plus RB007 (Regado Biosciences)	2′-ribo purine/2′-fluoro pyrimidine (RB006)/40 kDa PEG plus 2′-O-methyl antidote (RB007)	Coagulation factor IXa	Percutaneous coronary intervention	Phase II
ARC1779 (Archemix)	DNA and 2′-O-methyl with a single phosphorothioate linkage conjugated to 20 kDa PEG, 3′ inverted dT	A1 domain of von Willebrand factor	Thrombotic microangiopathies and carotid artery disease	Phase II
NU172 (ARCA biopharma)	Unmodified DNA aptamer	Thrombin	Cardiopulmonary bypass to maintain steady state of anticoagulation	Phase II
ARC1905 (Ophthotech)	2′-ribo purine/2′-fluoro pyrimidine conjugated to 40 kDa PEG, 3′ inverted dT	Complement component 5	Age-related macular degeneration*	Phase I
E10030 (Ophthotech)	DNA and 2′-O-methyl 5′-conjugated to 40 kDa PEG, 3′ inverted dT	Platelet-derived growth factor	Age-related macular degeneration*	Phase I
NOX-A12 (NOXXON Pharma)	L-RNA with 3′-PEG	CXCL12	Multiple myeloma and non-Hodgkin's lymphoma‡	Phase I
NOX-E36 (NOXXON Pharma)	L-RNA with 3′-PEG	CCL2	Type 2 diabetes, diabetic nephropathy	Phase I

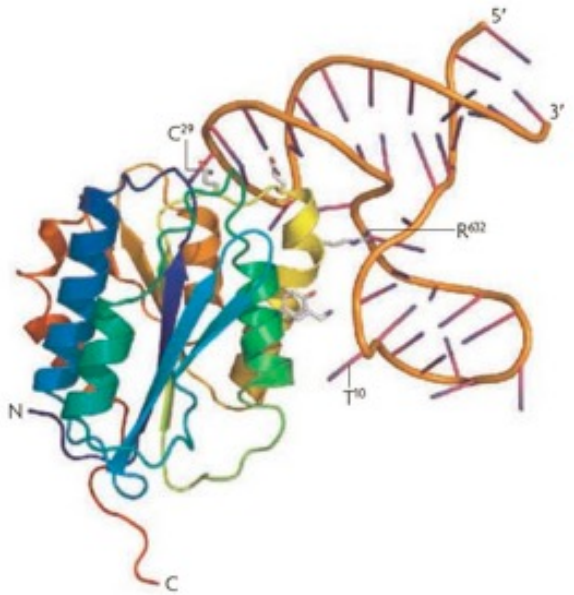


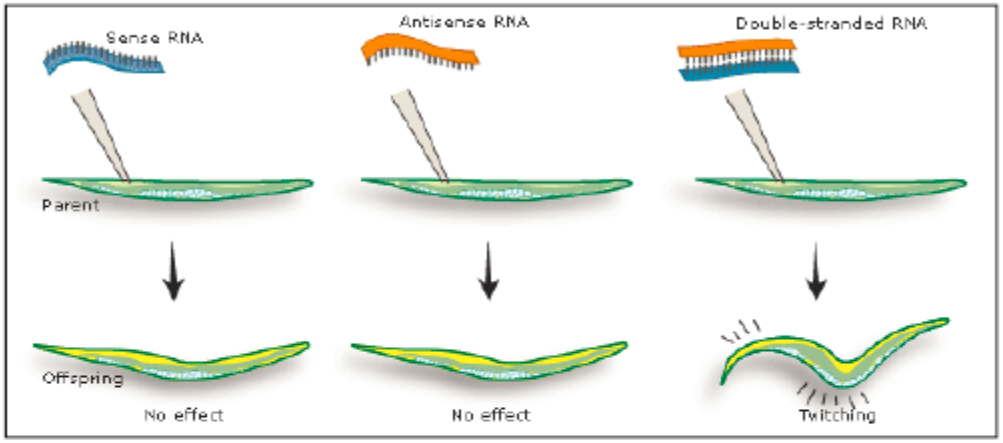
Figure 3 | **Crystal structure of the all-DNA parent of ARC1779 bound to the A1 domain of von Willebrand factor.***

*part of the coagulation cascade, important for thrombus formation

Nucleic acid based therapeutics : RNA interference

RNA INTERFERENCE : A RECENT MOLECULAR EXPLANATION ON AN OLD OBSERVATION

1998 •Andrew Fire and Craig Mello publish groundbreaking work done in *C. elegans.*

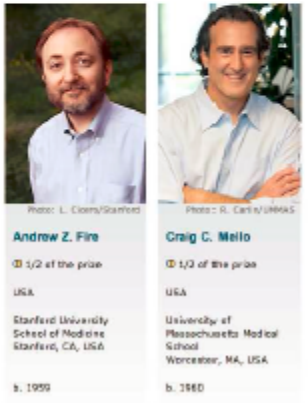


unc-22 encodes a myofilament protein. Decrease in unc-22 activity is known to produce severe twitching movements

1. silencing triggered efficiently by injected dsRNA, but weakly or not by single-stranded RNAs.
2. silencing specific for mRNA homologous to the dsRNA (other mRNAs were unaffected)
3. dsRNA had to correspond to the mature mRNA sequence; neither intron nor promoter sequences triggered a response → a posttranscriptional, presumably cytoplasmic mechanism
4. the targeted mRNA disappeared suggesting that it was degraded
5. only a few dsRNA molecules per cell were sufficient to accomplish full silencing.

The Nobel Prize in Physiology or Medicine 2006

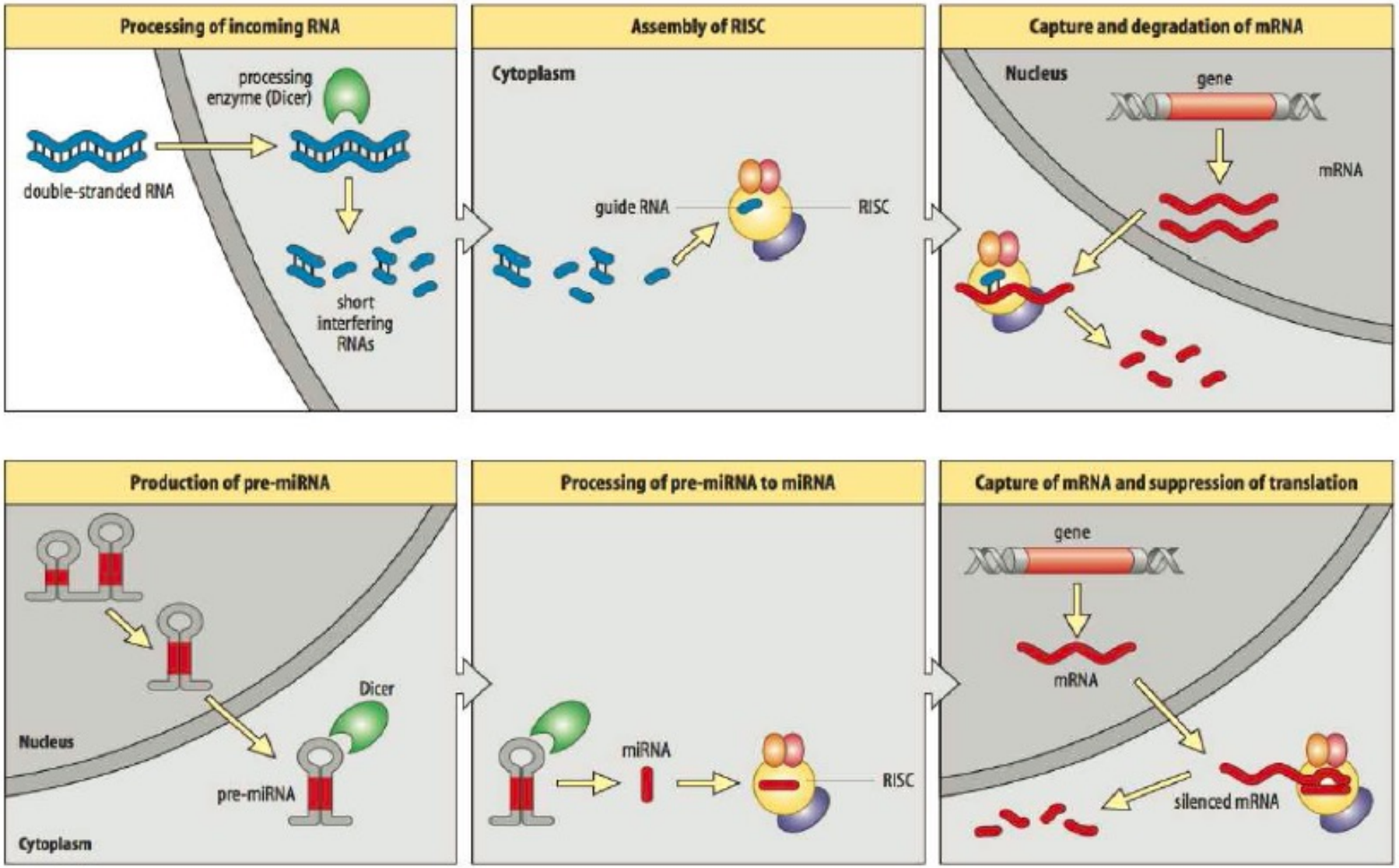
"for their discovery of RNA interference - gene silencing by double-stranded RNA"



2001 •Thomas Tuschl demonstrates potent and specific RNAi silencing in mammalian cells

RNA interference: small RNAs as therapeutics ?

siRNAs and miRNAs hit respectively transcription and translation !

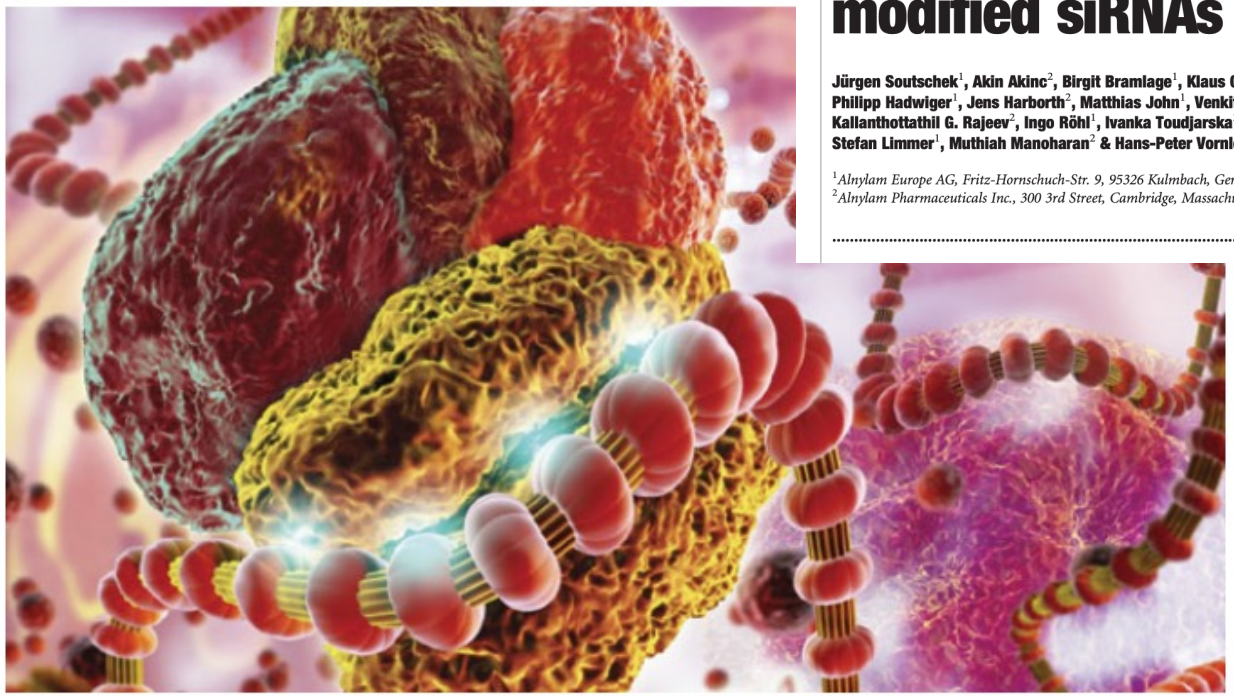


RNA interference: small RNAs as therapeutics eg in liver : nanoparticle, cholesterol moiety, systemic delivery improved ?!

The Second Coming of RNAi

Now showing clinical progress against liver diseases, the gene-silencing technique begins to fulfill some of its promises.

By Eric Bender | September 1, 2014



THE ART OF SILENCING: Small interfering RNA molecules are incorporated into an RNA-induced silencing complex where they bind and degrade target messenger RNAs (yellow with red rings). Taking advantage of this natural RNA interference (RNAi) pathway, researchers are developing therapeutics for liver-based diseases, viral infections, cancer, and more.

articles

Therapeutic silencing of an endogenous gene by systemic administration of modified siRNAs

Jürgen Soutschek[1], Akin Akinc[2], Birgit Bramlage[1], Klaus Charisse[2], Rainer Constien[1], Mary Donoghue[2], Sayda Elbashir[2], Anke Geick[1], Philipp Hadwiger[1], Jens Harborth[2], Matthias John[1], Venkitasamy Kesavan[2], Gary Lavine[2], Rajendra K. Pandey[2], Timothy Racie[2], Kallanthottathil G. Rajeev[2], Ingo Röhl[1], Ivanka Toudjarska[2], Gang Wang[2], Silvio Wuschko[1], David Bumcrot[2], Victor Koteliansky[2], Stefan Limmer[1], Muthiah Manoharan[2] & Hans-Peter Vornlocher[1]

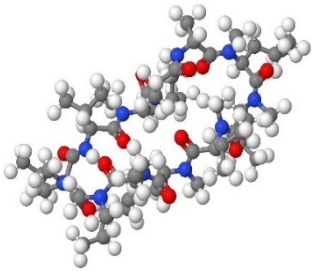
[1]*Alnylam Europe AG, Fritz-Hornschuch-Str. 9, 95326 Kulmbach, Germany*
[2]*Alnylam Pharmaceuticals Inc., 300 3rd Street, Cambridge, Massachusetts 02142, USA*

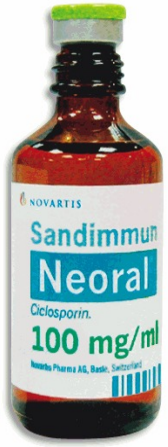
Cyclic Peptides in drug research_pioneering therapeutic modalities

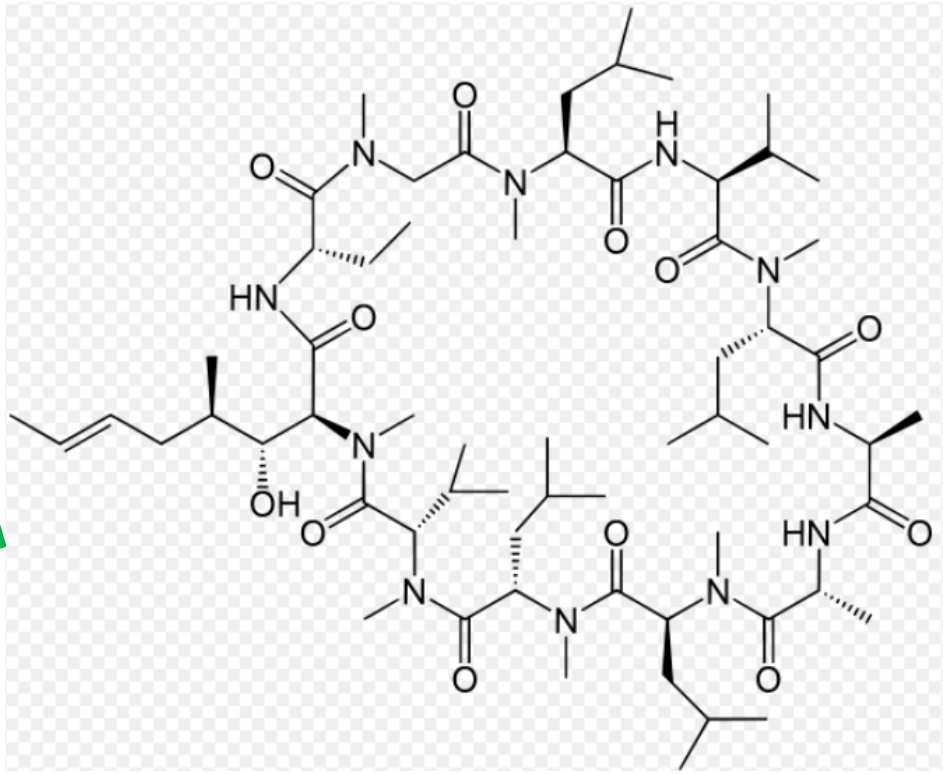
- **Only 2% of the marketed medicines**
- **200 peptide drugs are currently in clinical development**
- **Advantage: high selectivity, low amount need for optimal exposure, agonists**
- **Disadvantage, formulation issue, (oral no option) stability, cost of goods**

Cyclosporin A

Immunesupressor,block calcineurin transplantation medicine, antifungal natural product







WORKSHOP : ALLOIMMUNITY_SANDIMUN

- Cyclosporin**, pioneering peptide drug**, originally fungistatic (!) with oral administration (stable cyclic peptide (exceptional) turned out immunosupressor (Sandimun) derived from a natural fungi compound library screen by JF Borel et al in Sandoz Basel.

Guryanov I et al. J. 2016 Pept. Sci. 22:471-479

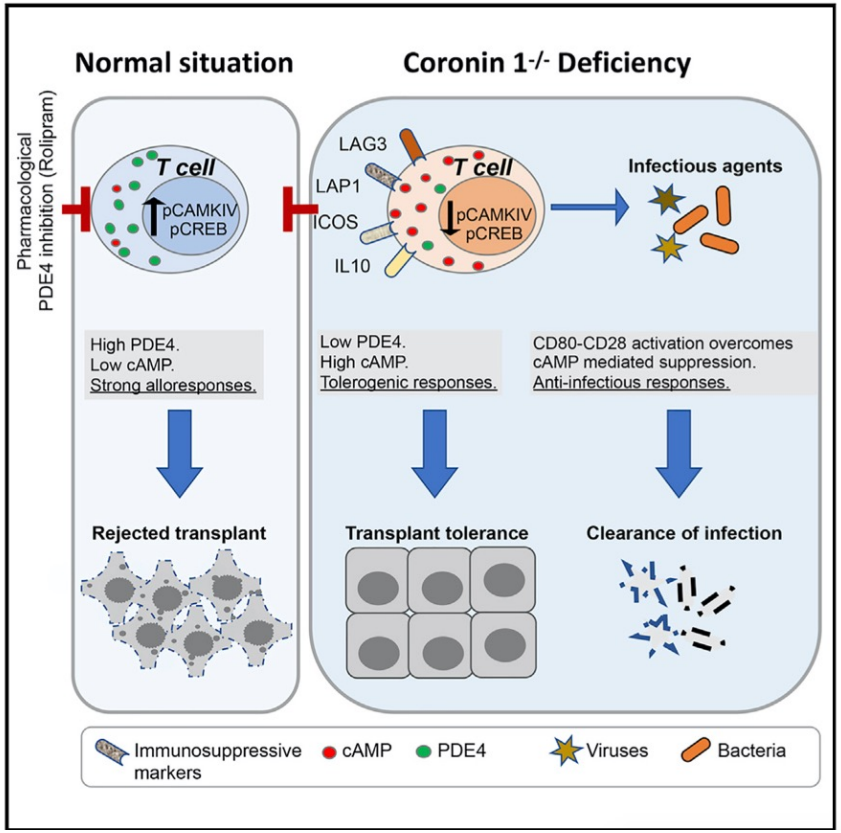
Immunosupression in clicnics-opportuinties for coronin1 inhibitors

Article

Immunity

Disruption of Coronin 1 Signaling in T Cells Promotes Allograft Tolerance while Maintaining Anti-Pathogen Immunity

Graphical Abstract



Authors

Rajesh Jayachandran, Aleksandra Gumienny, Beatrice Bolinger, ..., Nina Khanna, Simona W. Rossi, Jean Pieters

Correspondence

rajesh.jayachandran@unibas.ch (R.J.), jean.pieters@unibas.ch (J.P.)

In Brief

Avoiding infection as a consequence of immunosuppression following organ transplantation has been elusive. Here, Jayachandran et al. demonstrate that targeting the immunoregulatory protein coronin 1 in mice results in allograft-specific tolerance in the absence of immunosuppression-associated co-morbidities. Coronin 1-deficiency increased cAMP concentrations to suppress allo-specific T cell responses

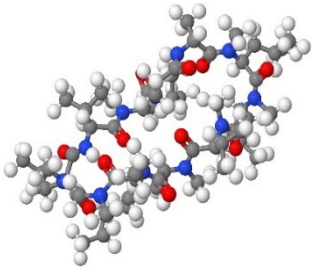
Cyclic Peptides in drug research_pioneering therapeutic modalitie

Calcineurin is a phosphatase in the signal cascade towards NFAT T cell transcriptional regulation

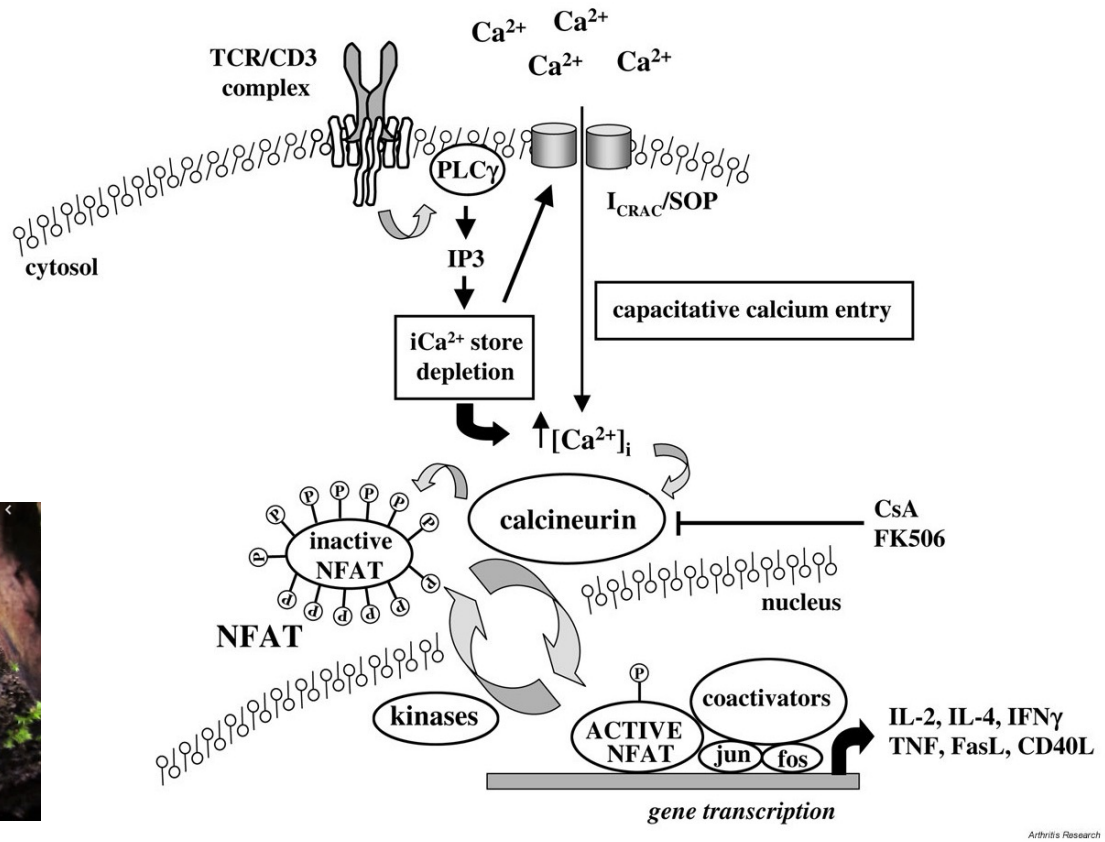
Cyclosporin A

Immunesupressor,block calcineurin transplantation medicine, antifungal natural product

Trichoderma polysporum



From: Studies of T-cell activation in chronic inflammation



- Cyclosporin**, pioneering peptide drug**, originally fungistatic (!) with oral administration (stable cyclic peptide (exceptional) turned out immunosupressor (Sandimun) derived from a natural fungi compound library screen by JF Borel et al in Sandoz Basel in 1970's Guryanov I et al. J. 2016 Pept. Sci. 22:471-479

Drugging the undruggable targets – direct pharmacological intervention by protein:protein interaction disruption

- The genomic revolution provided drug discovery an ever since increasing number of therapeutic targets
- Mutated genes and pathologic protein products have emerged as precision medicine anti cancer drug target
- **In particular the relevance of protein:protein interactions highlights a number of new challenges for medicinal chemists with often no suitable/ targetable binding pocket for the design of a classical small molecule chemistry is available**

- **Stapling peptide based drug modalities** are getting developed to bridge the gap between **BIOLOGICALS**/protein therapeutics (MABs) modalities and **CHEMICALS** small MW cpds from undruggable potential therapeutic targets

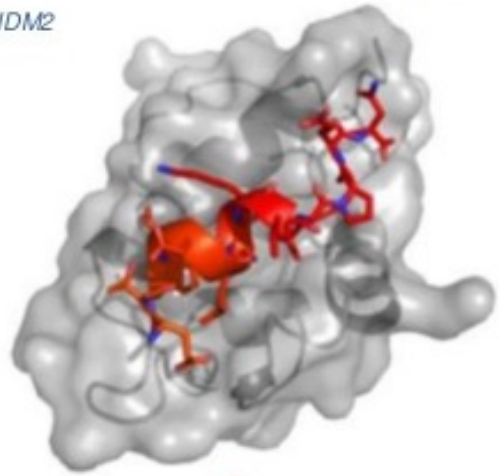
Moellering RE et al. 2002 Nature 462:182-188

„ Drugging the undruggable therapeutic targets“

Fragment Linking – Protein-Protein Interactions

Why protein-protein interactions as targets?

p53-HDM2

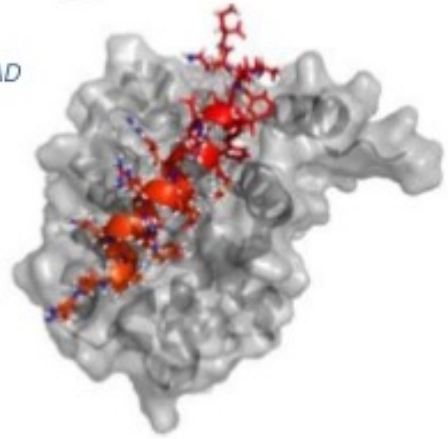


*Protein-Protein interactions (PPI's) are found throughout biological systems. Typically these are defined as **difficult targets** as success rates in targeting these has been low especially using HTS approaches.*

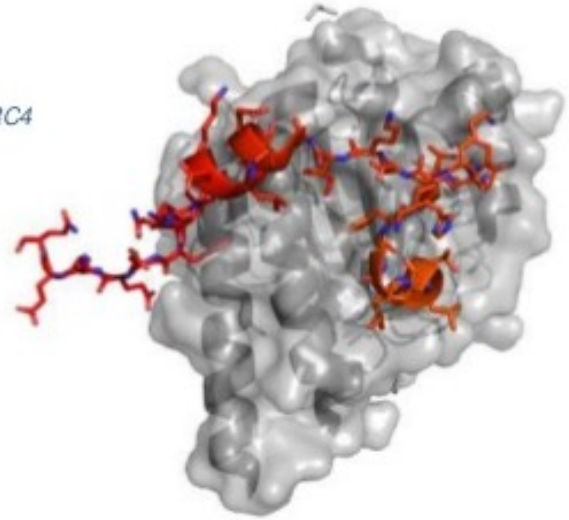
Unlike conventional targets they do not have distinct binding pockets however they have what is known as 'hot-spots' typically on the surface of the protein

FBDD has been used successfully against a number of these targets however none to date have been approved as drugs although in a number of cases there are compounds in Phase I/II development.

Bcl-BAD



RAD51-BRC4

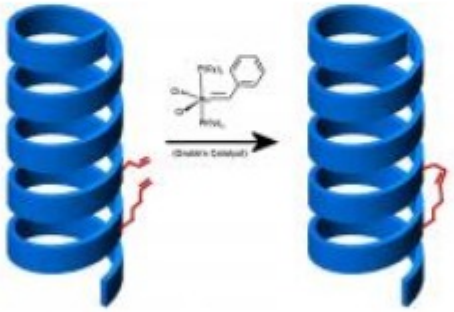


Drugging undruggable targets – peptide stapling

Alkenyl Amino Acids for Stapled Peptides

Stapled peptides are promising intracellular drug targets. They may have increased target affinity, cell penetrating ability and increased proteolyic resistance in comparison to non-stapled analogs (GL Verdine, GJ Hilinski *Methods Enzymol*. **2012**, *503*, 3-33).

Stapled peptides are formed by incorporating special amino acids with olefinic side chains at the i, i+4 positions (one-loop staple) or at i, i+7 positions (two-loop staple). The hydrocarbon bridge is formed by a ring-closing metathesis reaction catalyzed by benzylidenebis (tricyclohexyl-phosphine)-dichlororuthenium (Grubb's catalyst).



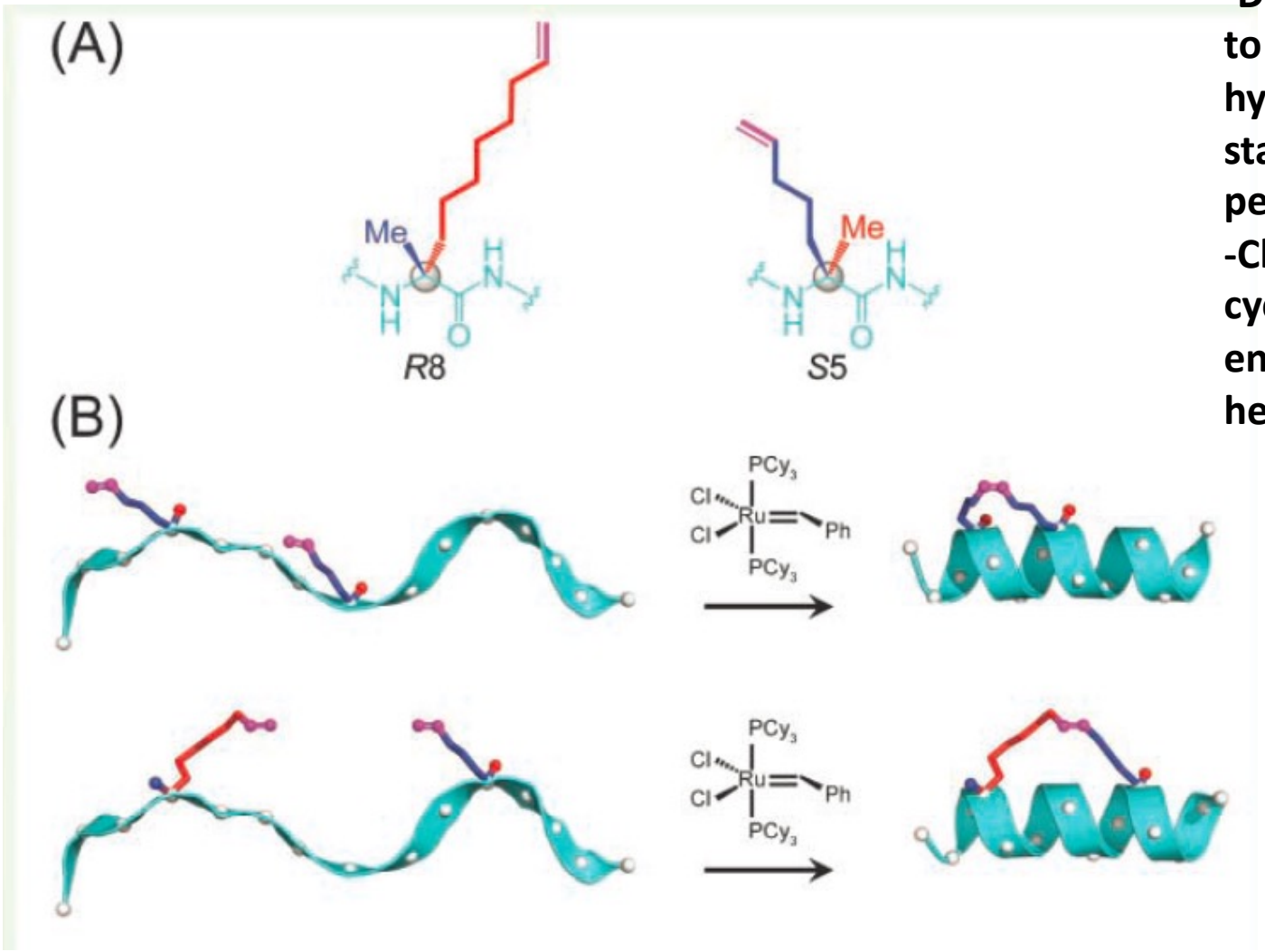
AAPPTec supplies a wide selection of high purity Fmoc-alkenyl alanines used to prepare stapled peptides through a ring-closing metathesis reaction. **AAPPTec's products are affordably priced and are also available in bulk quantities for additional savings. Please send an e-mail to sales@aapptec.com for a quotation on larger quantities or use the On-Line Quote Request.**

DESIGNER AA USED TO INTRODUCE ALL-HYDROCARBON STAPLES INTO PEPTIDES

CLOSING THE MACRO CYCLIC RING ENFORCES ALPHA HELICAL STRUCTURE

Verdine G et al. 2007 Clin Cancer Res 13:7264-7220

Drugging undruggable targets – peptide stapling



-Designer AA used to introduce all-hydrocarbon staples into peptides
-Closing the macro cyclic ring enforces alpha helical structure

Verdine G et al. 2007 Clin Cancer Res 13:7264-7220

Drugging undruggable targets – direct NOTCH signaling inhibition by protein:protein interaction disruption

Direct inhibition of the NOTCH transcription factor complex

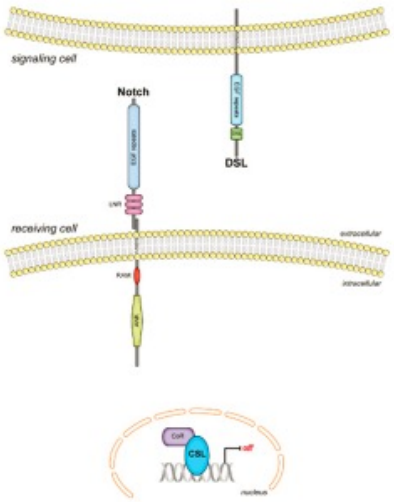
Raymond E. Moellering[1,2,3], Melanie Cornejo[4], Tina N. Davis[6], Cristina Del Bianco[5], Jon C. Aster[5], Stephen C. Blacklow[5], Andrew L. Kung[6], D. Gary Gilliland[4,7], Gregory L. Verdine[1,3] & James E. Bradner[2,3,8]

Direct inhibition of transcription factor complexes remains a central challenge in the discipline of ligand discovery. In general, these proteins lack surface involutions suitable for high-affinity binding by small molecules. Here we report the design of synthetic, cell-permeable, stabilized α-helical peptides that target a critical protein–protein interface in the NOTCH transactivation complex. We demonstrate that direct, high-affinity binding of the hydrocarbon-stapled peptide SAHM1 prevents assembly of the active transcriptional complex. Inappropriate NOTCH activation is directly implicated in the pathogenesis of several disease states, including T-cell acute lymphoblastic leukaemia (T-ALL). The treatment of leukaemic cells with SAHM1 results in genome-wide suppression of NOTCH-activated genes. Direct antagonism of the NOTCH transcriptional program causes potent, NOTCH-specific anti-proliferative effects in cultured cells and in a mouse model of NOTCH1-driven T-ALL.

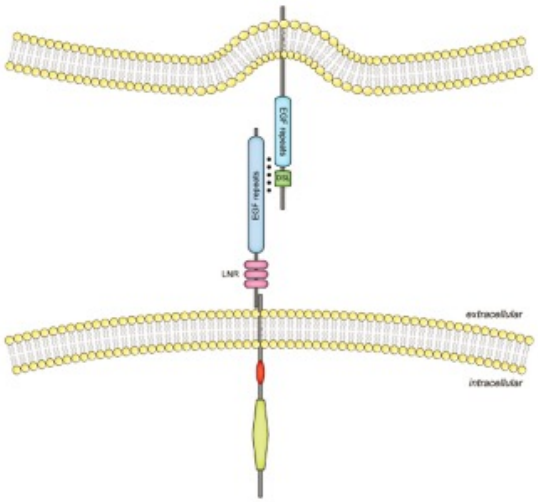
Transcription factors are the final effectors of the cellular signaling cascade

Moellering RE et al. 2002 Nature 462:182-188

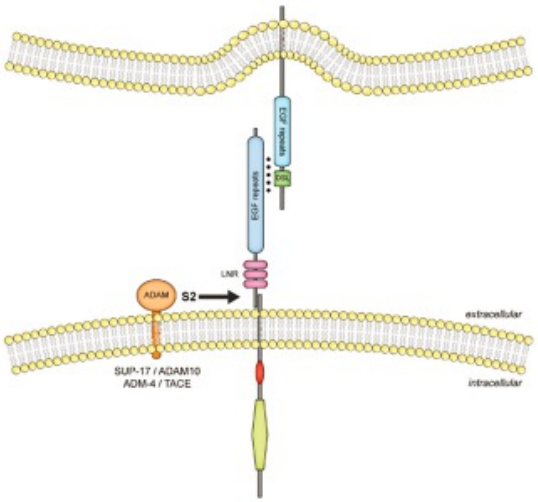
NOTCH cellular signal pathway : cell to cell communication



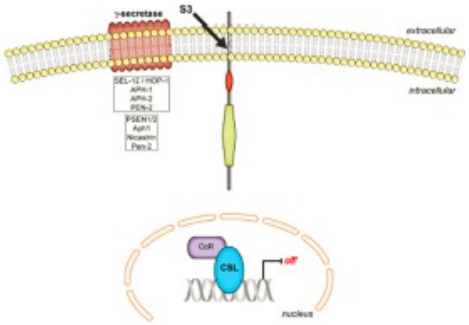
1. In the absence of Notch signal transduction CSL represses target genes.



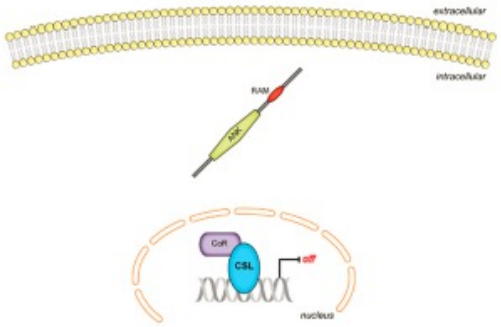
2. Endocytosis in the signaling cell is required for ligand activity.



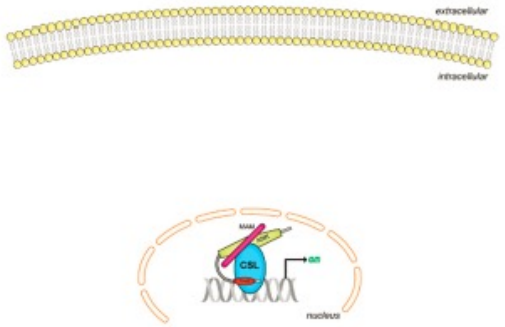
3. Ligand binding triggers S2 cleavage and ectodomain shedding.



4. Ectodomain shedding triggers S3 cleavage.



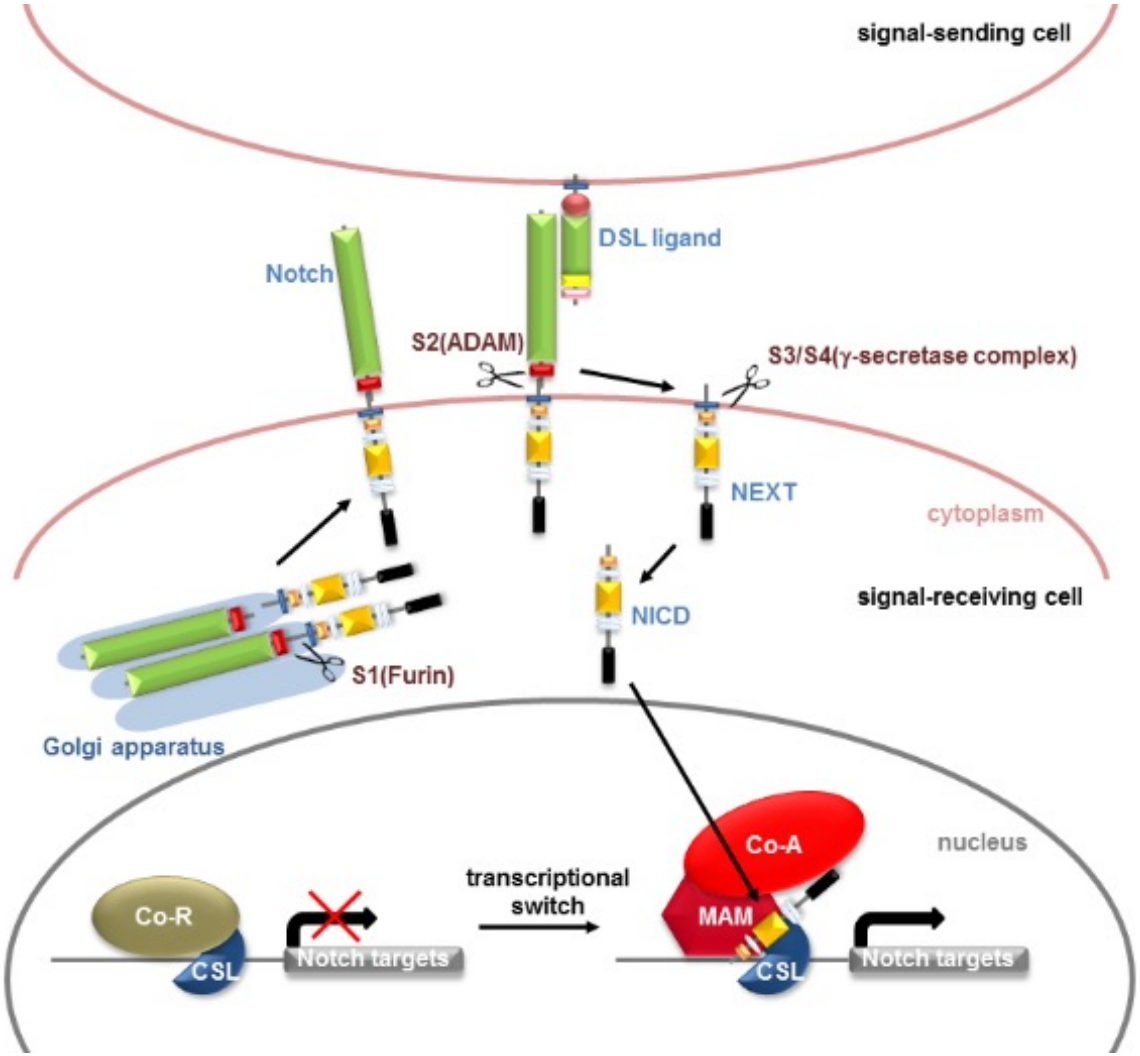
5. S3 cleavage releases the intracellular domain for nuclear translocation.



6. The CSL-NICD-MAM ternary complex activates target gene transcription.

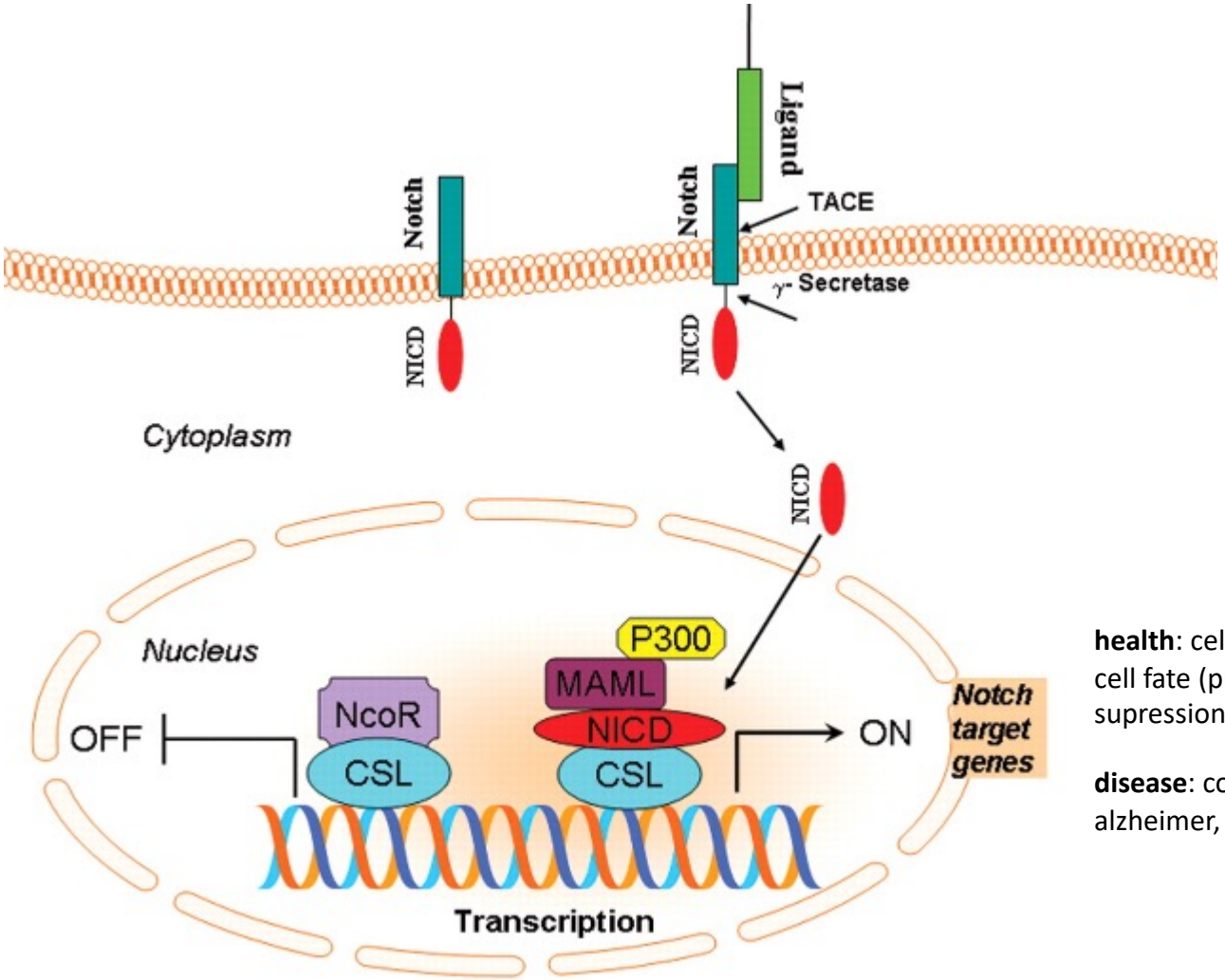
Greenwald I et al 2012 Wormbook.com

NOTCH signal and the transcriptional ON /OFF readout pathway



Greenwald I et al 2012 Wormbook.com

NOTCH signal and the transcriptional ON /OFF readout pathway



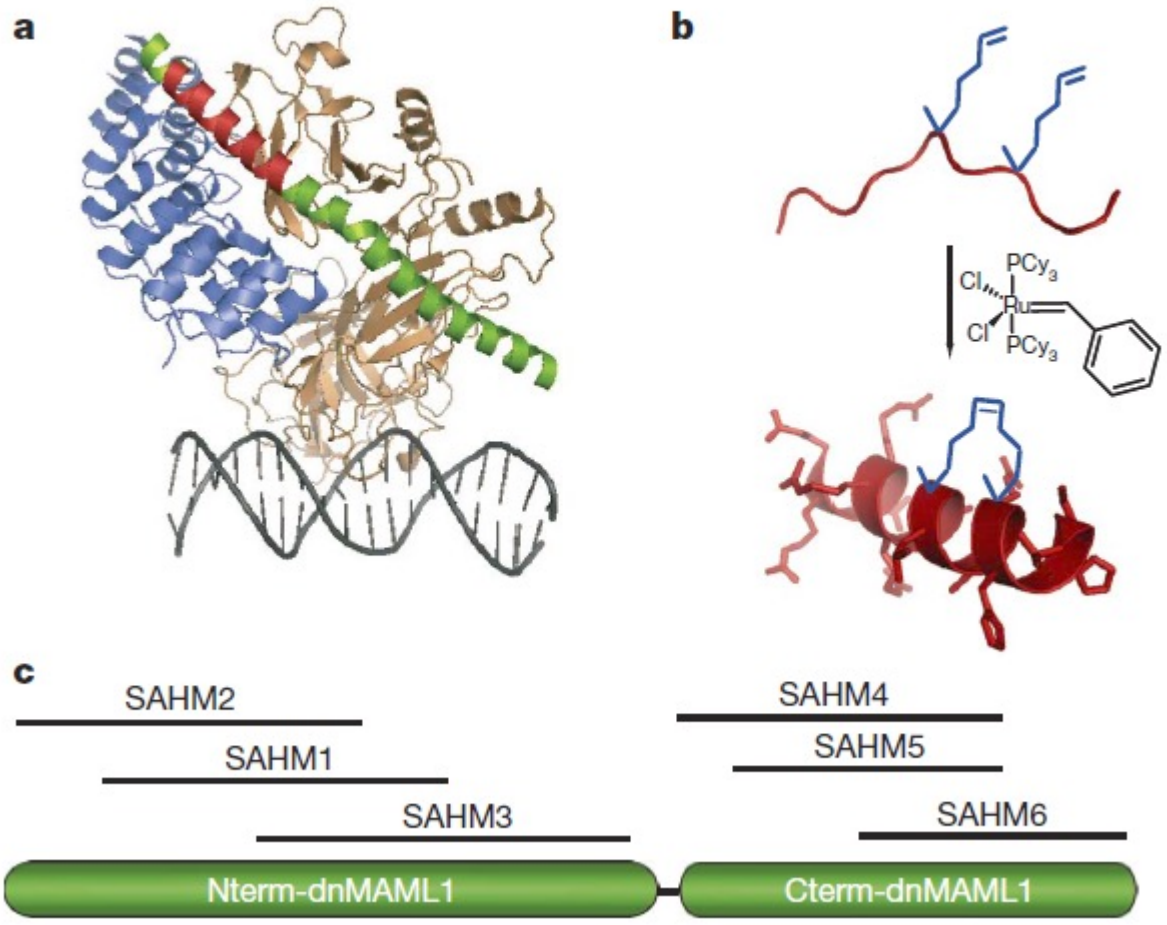
health: cell-cell com, cell fate (promotion supression of proliferation

disease: colon cancer, alzheimer, T-ALL leukaemia

Greenwald I et al 2012 Wormbook.com

Drugging undruggable targets – direct NOTCH signal inhibition by protein:protein interaction disruption at the target genes

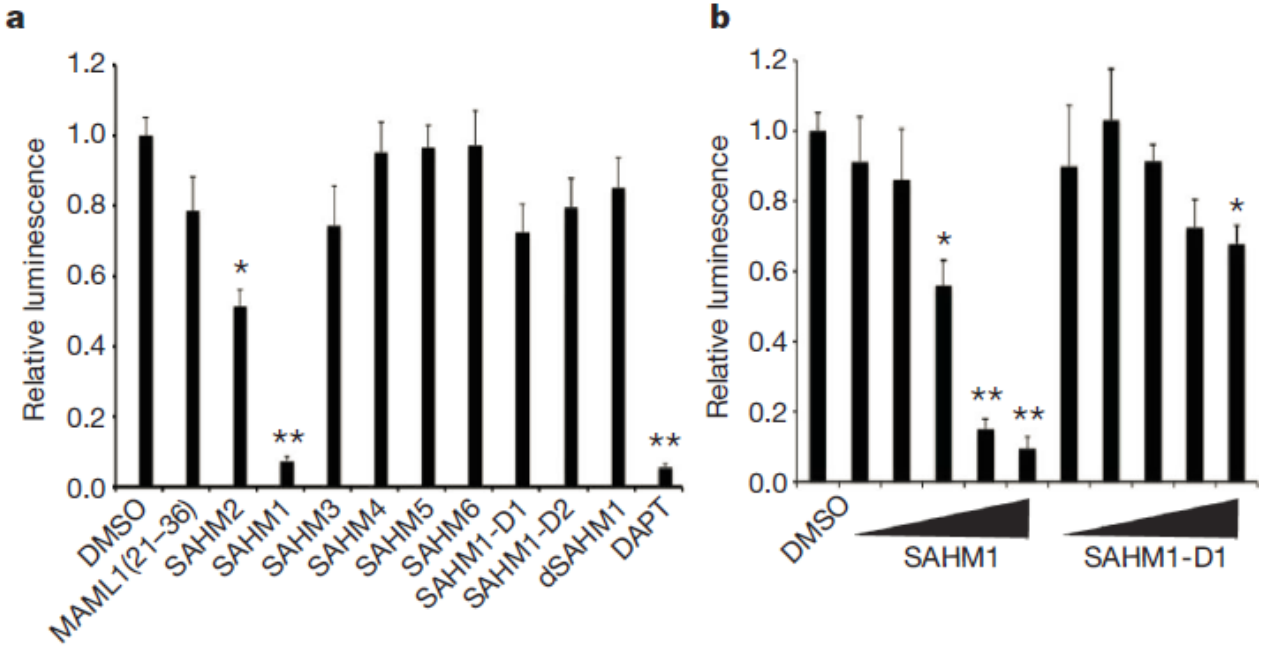
NATURE|Vol 462|12 November 2009



Compound	Sequence	Helicity
MAML1(21-36)	βA1a-ERLRRRIELCRRHHST	23%
SAHM1-UN	βA1a-ERLRRRI*LCR*HHST	40%
SAHM1	βA1a-ERLRRRI*LCR*HHST	94%
SAHM2	βA1a-SAVMERL*RRI*LCRRHH	73%
SAHM3	βA1a-RIELCRRHH*TCE*RYEAV	59%
SAHM4	βA1a-ERLELER*HTF*LHQR	67%
SAHM5	βA1a-ELER*HTF*LHQR	72%
SAHM6	βA1a-QHTFALH*RCI*AKAKR	56%
SAHM1-D1	βA1a-RELRREI*LCR*HHST	83%
SAHM1-D2	βA1a-RELRRRI*LCE*HHST	78%
dSAHM1	βA1a-DRLRRRM*NYR*RQTD	55%

Moellering RE et al. 2002 Nature 462:182-188

Drugging undruggable targets – direct NOTCH final effector inhibition by protein:protein interaction blockade using „peptide stapling“



Moellering RE et al. 2002 Nature 462:182-188

THANK YOU.....

DO YOU HAVE ANY QUESTIONS ?