N E W L A Y O U T

1,000,000,000 *CHF investment*

7,000,874 *hours of work*

6,587 *experiments*

423 *researchers*

1 *medicine*

With Prof Susan M Gasser
and Prof Olivier Michielin

THE MAKING OF AN INNOVATIVE MEDICINE

Introductory workshops on translational biomedical research and drug discovery and development

BIO-698 resumes Thursday September 21. 2023
4:15 PM @ AAC 108

EPFL

Sciences de la Vie -SV

Prof Roger G. Clerc

The Making Of An Innovative Medicine – course schedule

Thursday's @ 4-6 PM except 14.12/21.12.23 @2-6 PM

Session 1: **Scope of the course _ general organization _ case study**
21.09.23 *Embracing a career at the heart of biomedical research !?*
AAC108

Session 2: **Historical perspective: the modern pharmacy**
28.09.23 *Advent of modern medicines - placebo controlled drug development*
AAC108

Session 3: **Introduction to translational research: crossing the bridge**
05.10.23 *A chasm has opened wide between biomedical research and patients in need*
AAC014

Session 4: **Therapeutic target identification I & II**
12-19.10.23 *"me too" vs a wealth of innovative targets _ small MW cpds vs biologicals*
AA014 AAC108 *Early front loading of biomarker identification for cohort stratification*

Session 5: **Structure based drug design _medicinal chemistry_low/high throughtput**
26.10.23 **screening assays_ multiple parallel parameters optimization MDO**
AAC108 *Setting up screening assays, the robotics, the million cpds librairies*

Session 6: **Therapeutic modalities peptides and biologicals: today's -**
02.11.23 **tomorrow's pharmacy NBEs**
AAC108 *Challengies (cost of goods - healthcare payers) and opportunities*

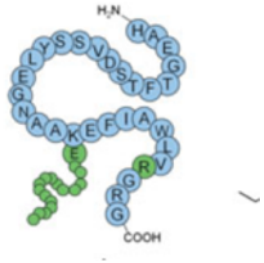
WORKSHOP LISTING - THE MAKING OF AN INNOVATIVE MEDICINE BIO698			
! NON EXHAUSTIVE LISTING - SUGGESTIONS WELCOME !			
sessions	no	workshops	speaker/s
S02 (28-09-23) ! AAC108 !			
historical medicines	1	vaccine discovery : E. Jenner and smallpox	Danica M
with Nobel laureates while	2	penicilin: impact, whose invention ?	
hopping on giant shoulders	3	prozac at the core of psychiatry	
	4	lipitor/statins at last a blockbuster	
	5	artemisinin and malaria	Umair
	6	cyclosporin from soil sample to blockbuster	Umair
S03 (5-10-23) ! AAC014 !			
translational research	7	expanding the scope of targeted therapies	
an emerging field	8	chronotherapy	Pitt
S04 (12-10-23) ! AAC014 !			
therapeutic target identification	9	rare diseases repurposing medicines	Adrien
S04b (19-10-23) ! AAC108 !	10	nocosomial inf/MRSA/phage antibacterials	Georges
therapeutic target identification	11	Crispr/Cas9 gene editing huntington disease	Pitt
	12	AI in drug discovery	Simon
S05 (26-10-23) ! AAC108 !			
structure based drug design	13	macrocycles and non druggable targets	Masota
	14	chemoproteomics - NMEs	Nico G
		my therapeutic target	Roger
S06 (02-11-23) ! AAC108 !			
therapeutic modalities - NBEs	15	Biologicals/biotech production/**incretins**	Tim
	16	armed monoclonal AB medicines	Nico G
	16	RNA therapeutics, antisense medicines	
S07 (9-11-23) ! AAC108 !			
PHC personalized healthcare	17	BRCA1 preventive surgery/tumor board	Nikita
Human genomics	18	SOPHiA Genetics - GWAS	
	19	disease enabling biomarkers/micro RNAs	Isika
S08 (16-11-23) ! AAC014 !			
pharmacogenetic polymorphism	20	NextGenSequencing - precision medicine	Hien
	21	deCODE Inc pharmgenomic/iceland genealogy	
S09 (23-11-23) ! AAC108 !			
in vivo pharmacology	22	organoids come of age	Nathalie B
toxicology	23	thalidomide repurposing	Ekaterina
S10 (30-11-23) ! AAC108 !			
clinical research	24	AI medicine 2.0	
	25	most common genetic defect : cystic fibrosis	.
	26	sex bias in preclicnial and clinical research	Weilin
	27	placebo/nocibo effects	Tim
S11 (07-12-23) ! AAC108 !			
intellectual property/integrity	28	SMA gene therapy - pay for performance	Abtin
	29	biopatents - 23 and Me - my genome	khosiyat
S12 (14-12-23) starts @ 2PM		Hacking medicine	all + invitees
! MED21522!			
S13 (21-12-23) start @ 2 PM		Hacking medicine	all + invitees

Workshops _ The Making Of An Innovative Medicine

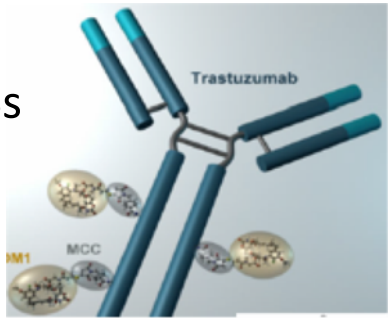
(today's class)

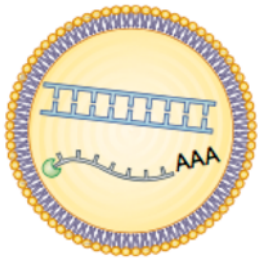
Session 6 : biologicals – biotherapeutics – NBEs : older and emerging therapeutic modalities

Peptides



MABs



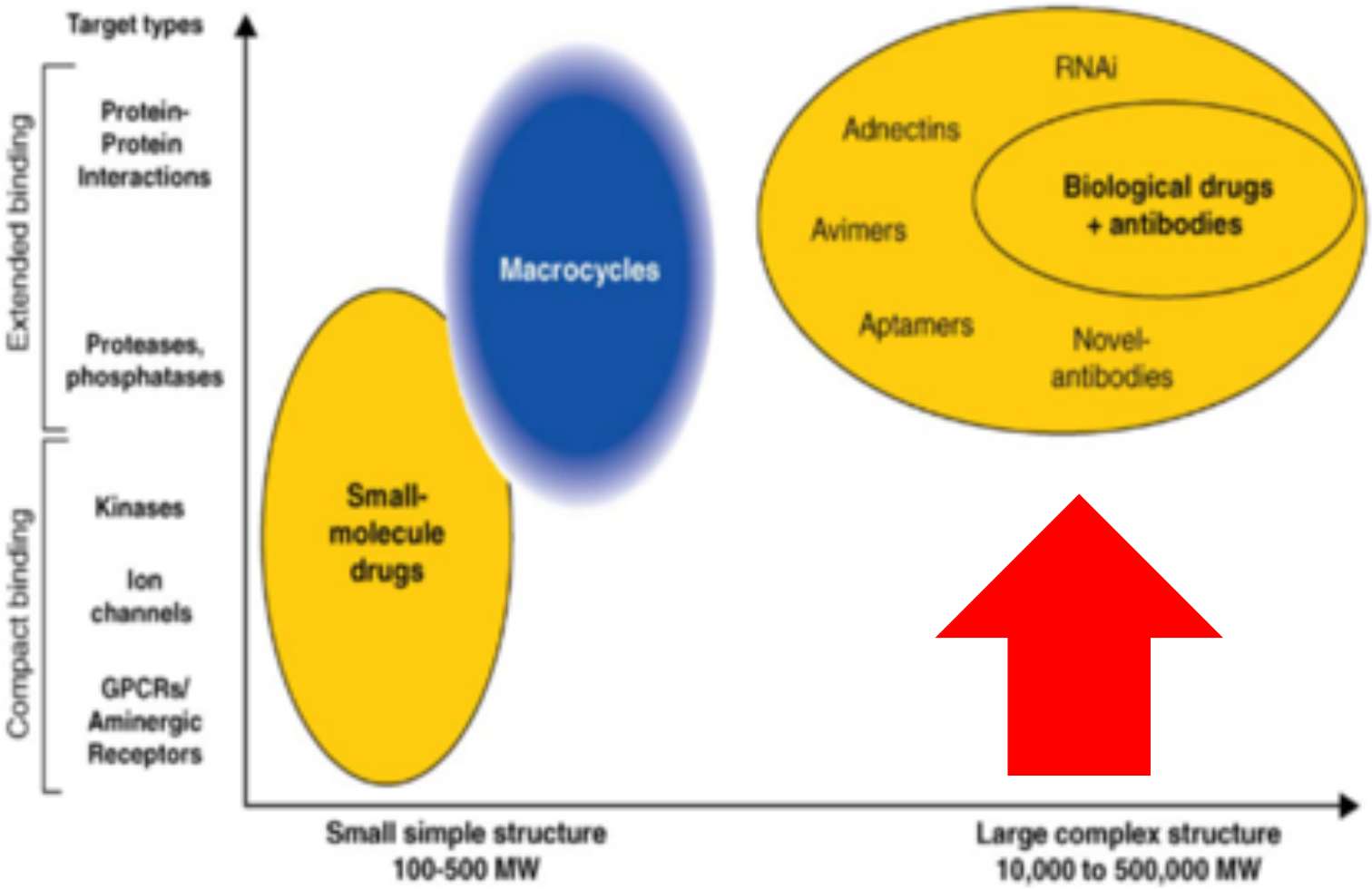


ASOs
(antisense oligonucletides)
RNAs
(miRs, siRNAs etc)

- 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

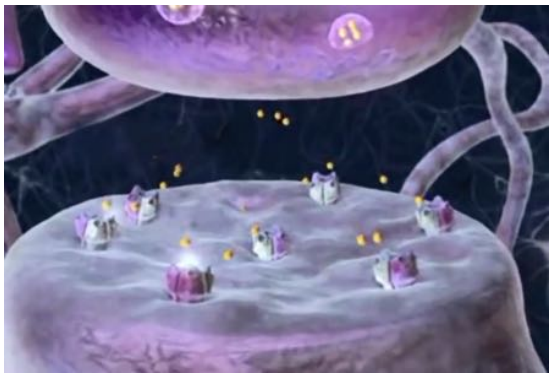
Large complex macromolecules for non small MW druggable targets



Disease biology_Molecular Biology_Drug discovery

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

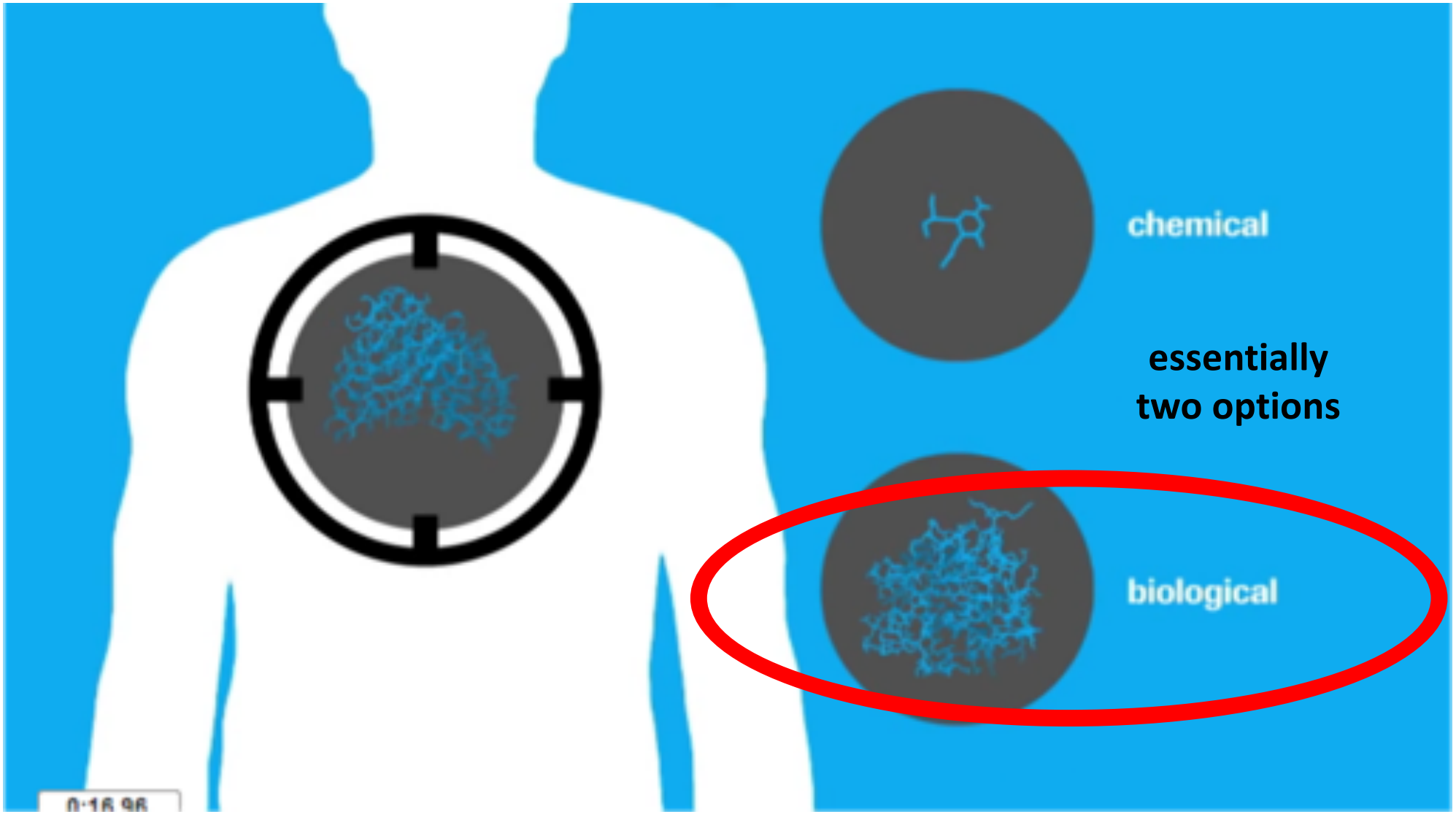
- **DISEASE BIOLOGY IS COMPLEX: SEVERAL THOUSANDS OF ELEMENTS ARRANGE**
- **IN A VERY INTRICATE MANNER**



- **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

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



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"!

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**

Peptides vs small M_R compounds

Table I. Comparison Between Peptides and Small Molecules

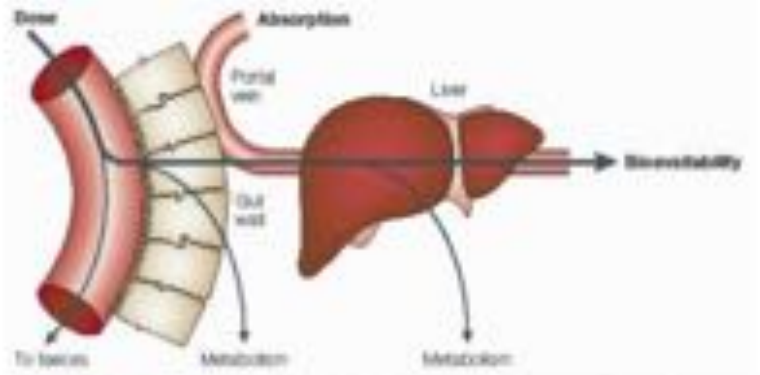
Small molecules	Peptides
• ~80% drug market	• ~2% drug market
• Low cost	• High cost
• Permeable	• Low permeability
• Stable	• Limited stability
• Good oral bioavailability	• Poor oral bioavailability
• Easier synthesis	• More challenging synthesis
• 95% current medicines	• Short half-life
	• Limited 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")

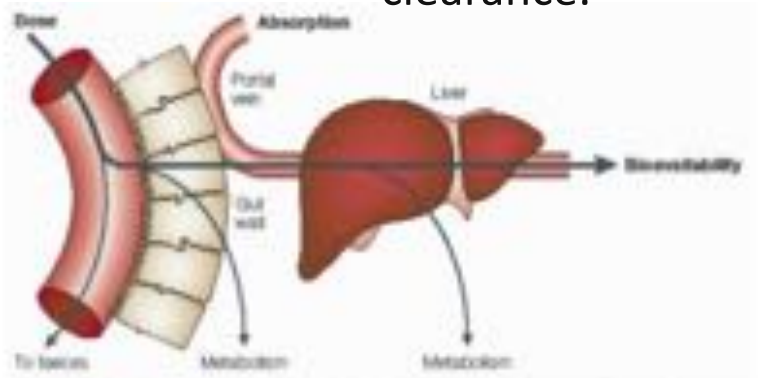
- Solubility
- Lipophilicity (clogP) octanol water partition <5) (partition coefficient)
- Number of H donors (<5)
- Number of H acceptors (<10)
- Molecular weight (generally <500 Da
- Permeability (PAMPA assay)
- CYP450s screen (eg. adverse drug metabolites
- Metabolic stability (PK, PD, ADME)
- Cardiac 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.



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Challenging MDO (multiparameter drug optimization) for peptides

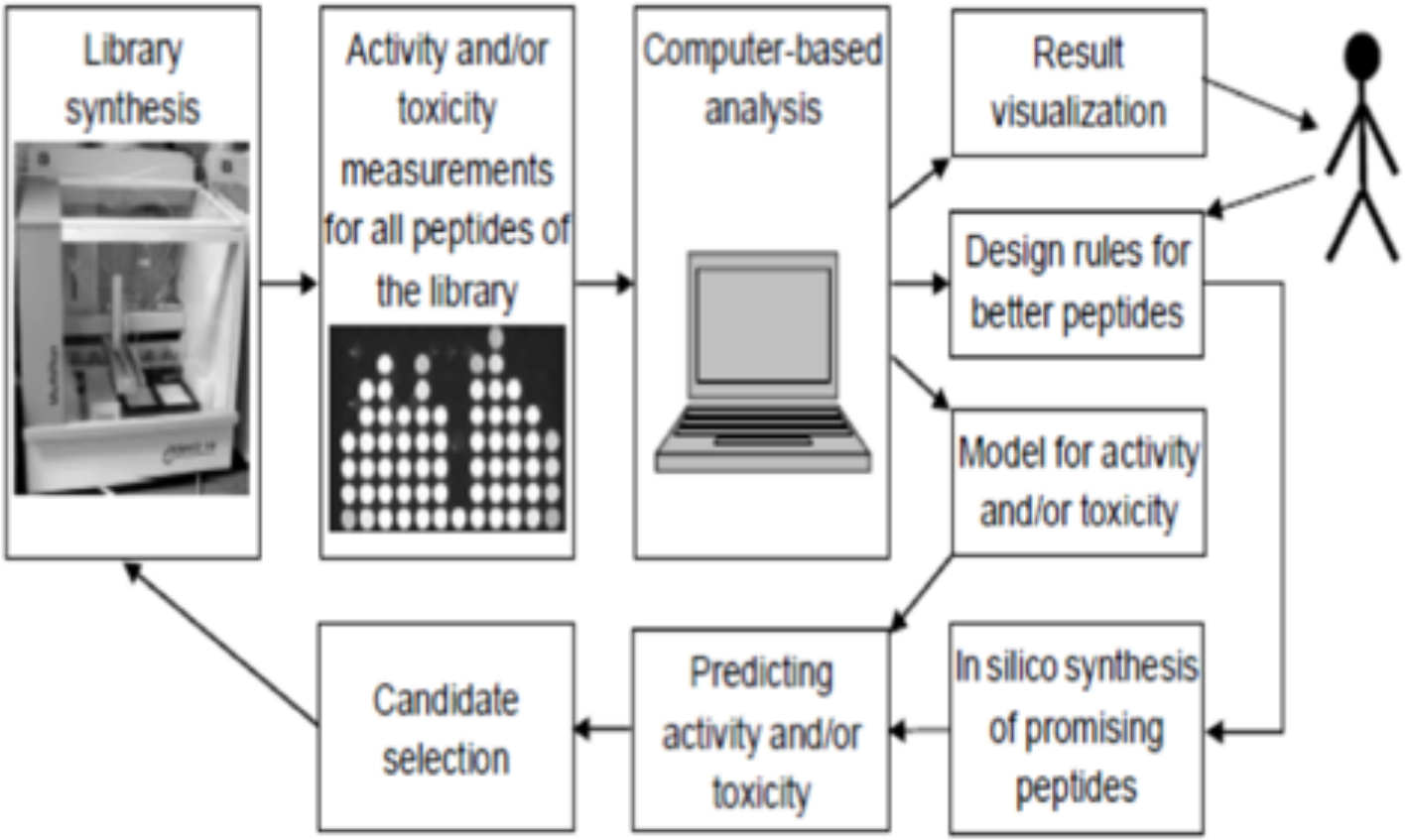
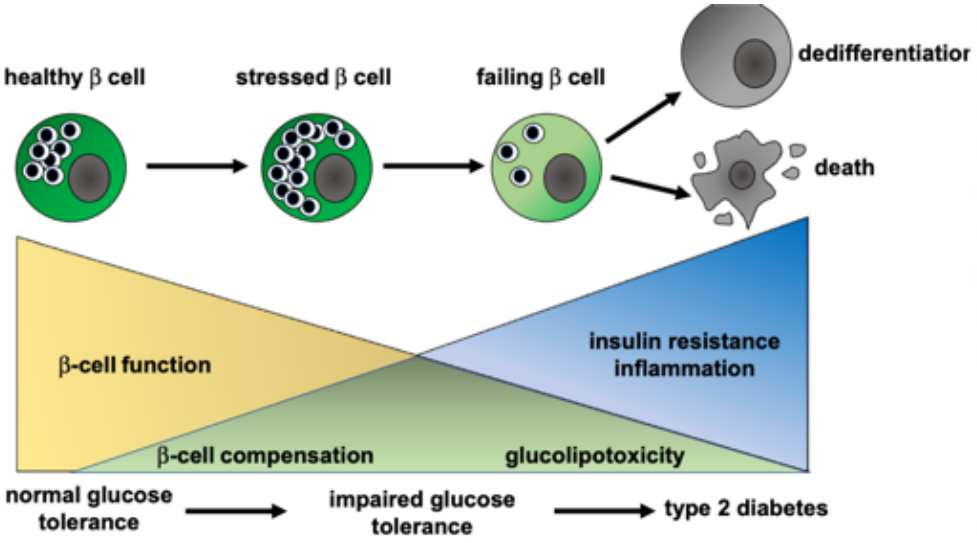
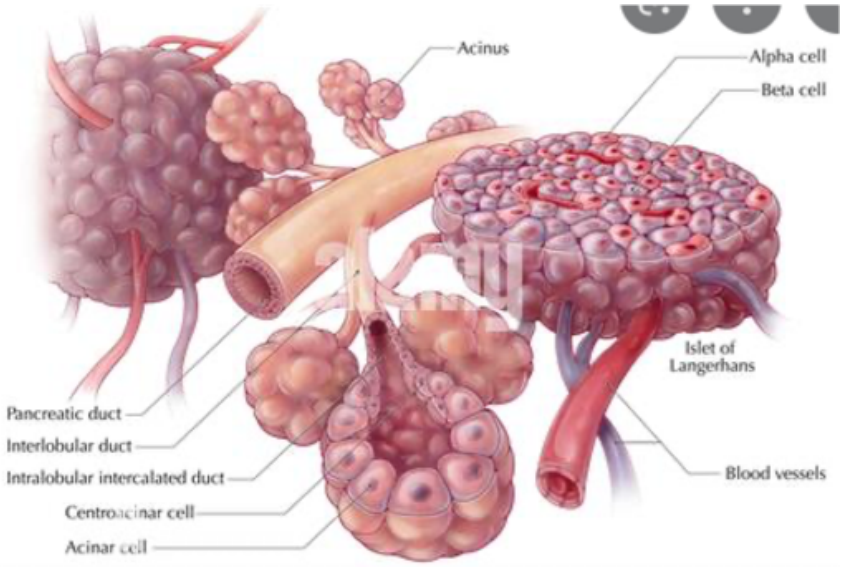
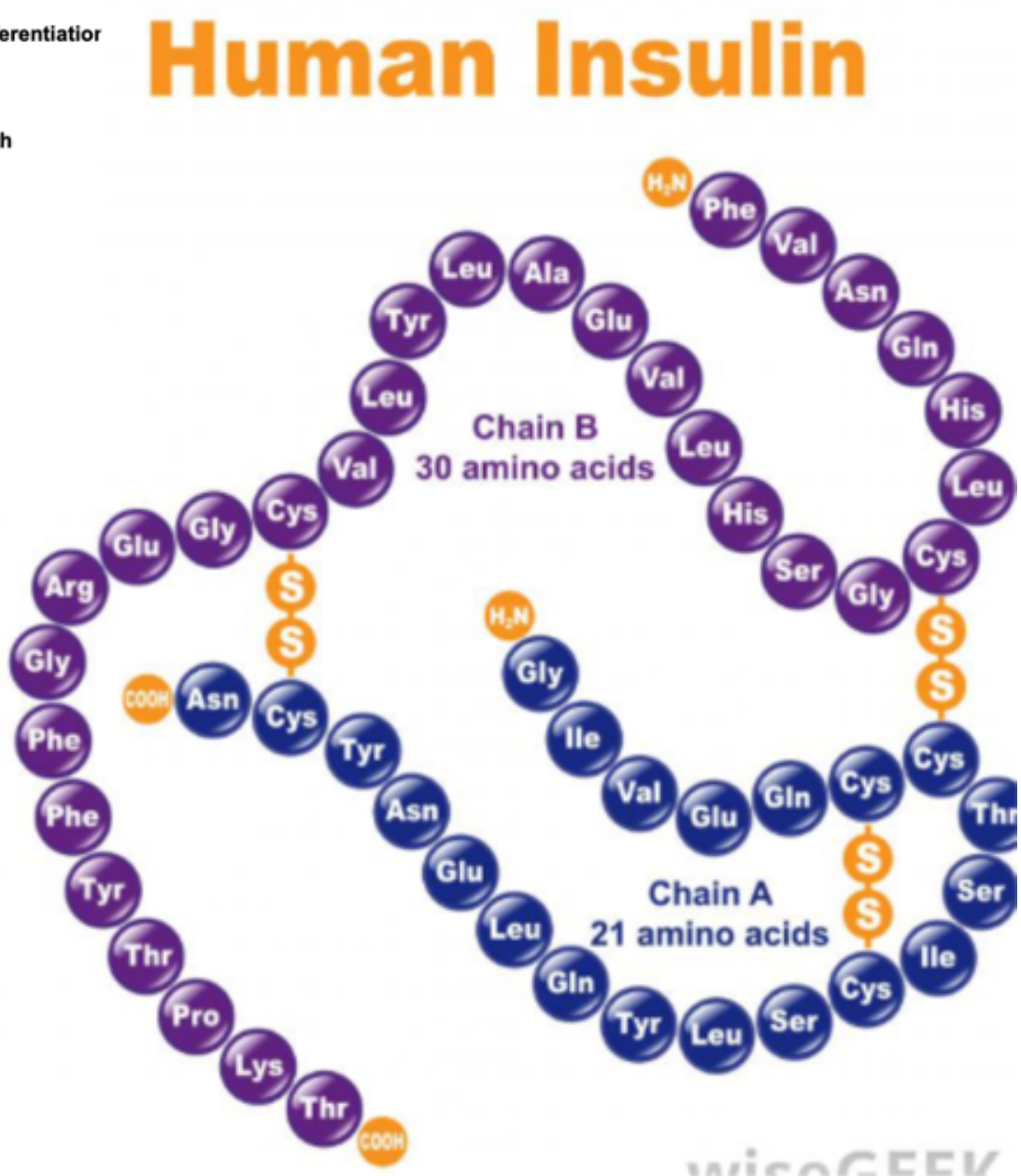


Figure 5. Optimization strategy for peptide libraries (modified from [134])

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







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

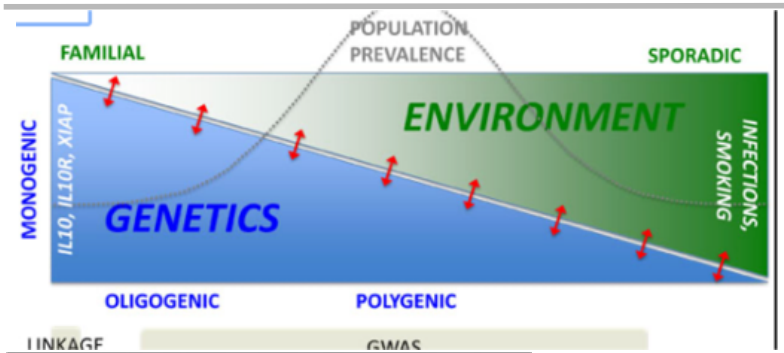
1921
F Banting-C.Best Beagle dogs

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

Prof Banting and McLoad 1929

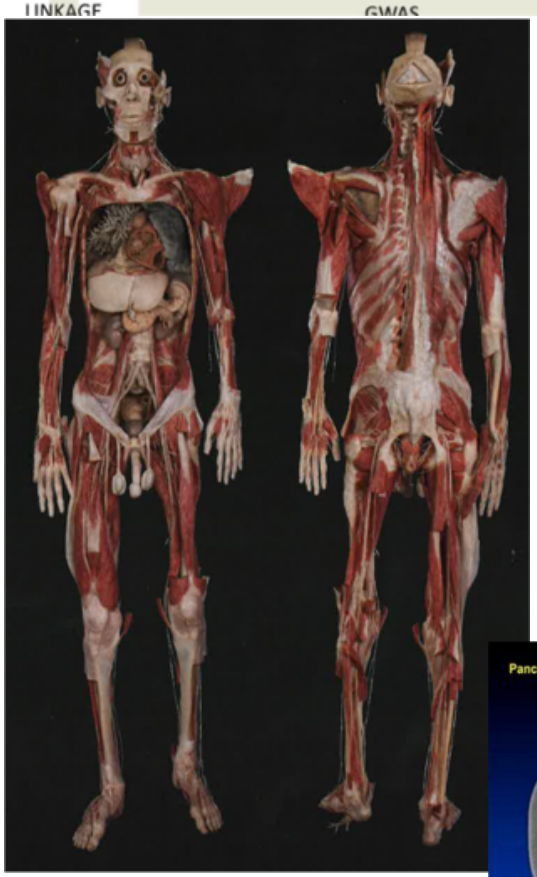
« Inventas vitam iuvat excoluisse per artes »

Metabolic syndrome : when patients become insulin resistant

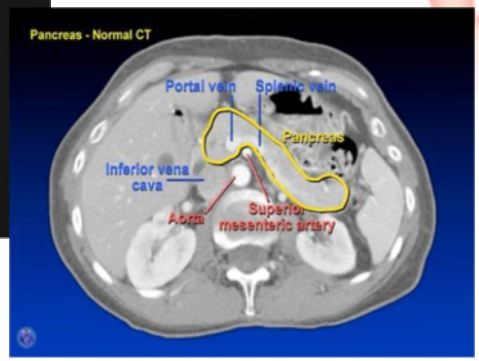


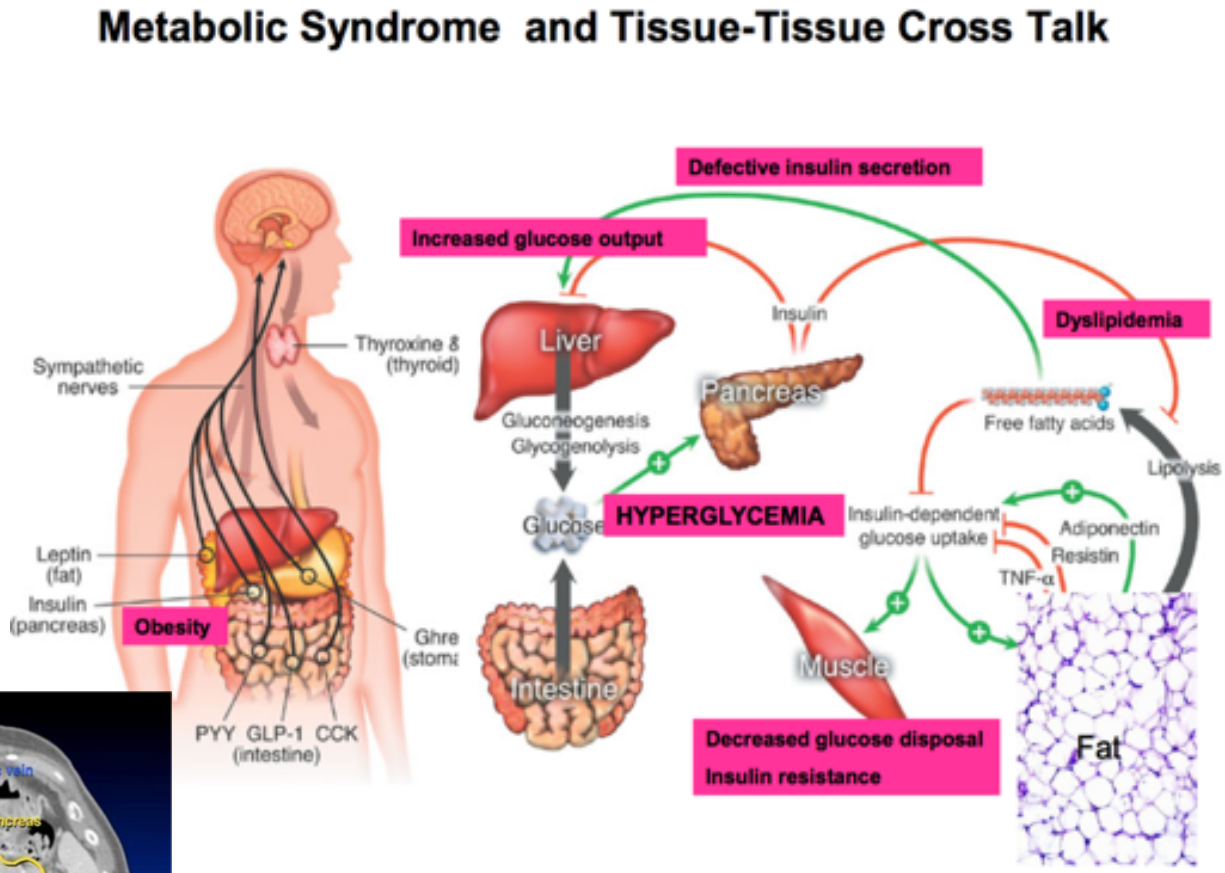
Metabolic Sydrome and Tissue-Tissue Cross Talk

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

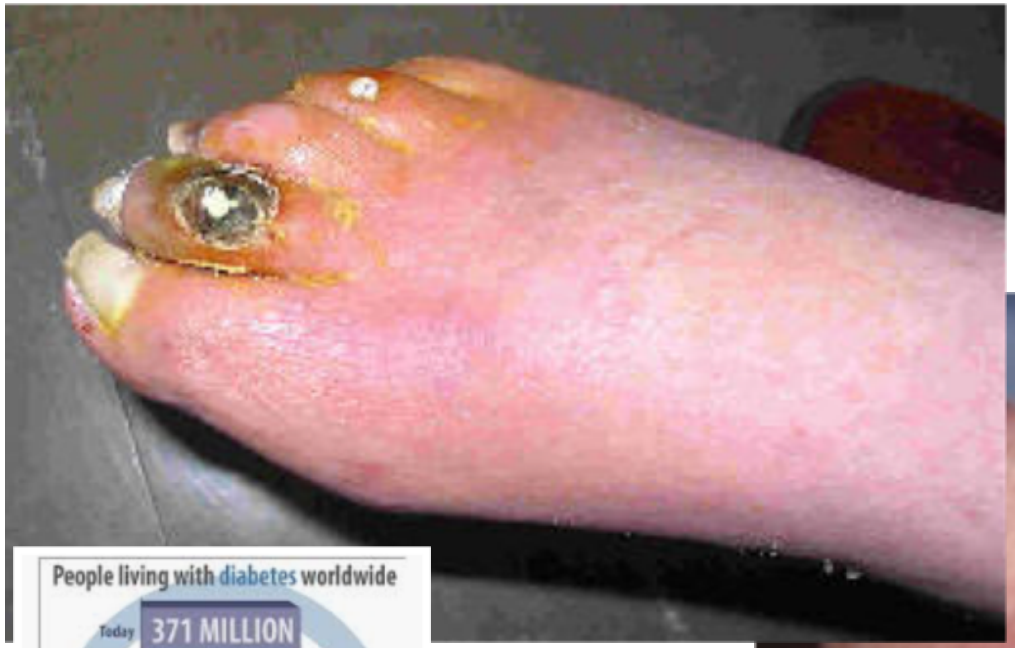


Gubener Plastinate GmbH
Guben Germany

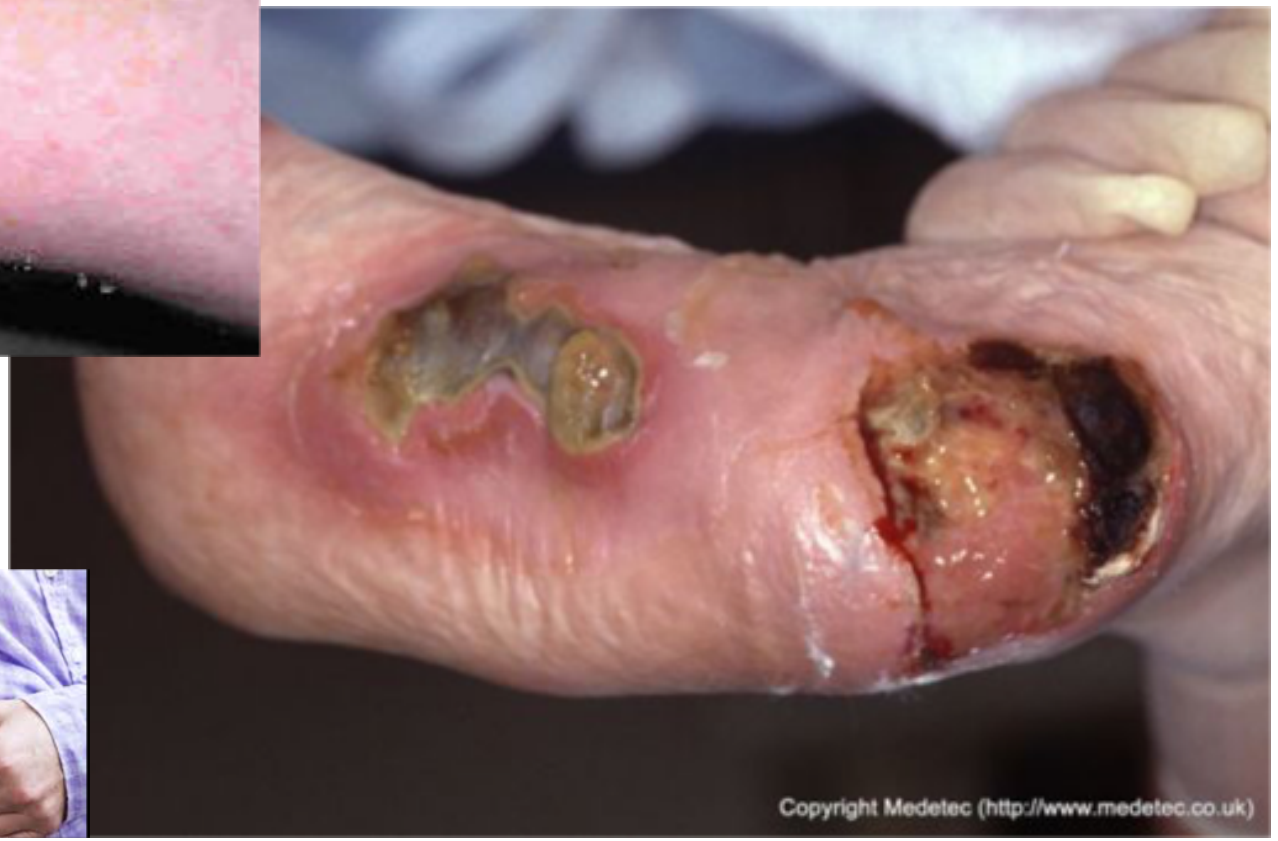




Advanced type II diabetes : peripheral ischaemia-necrosis

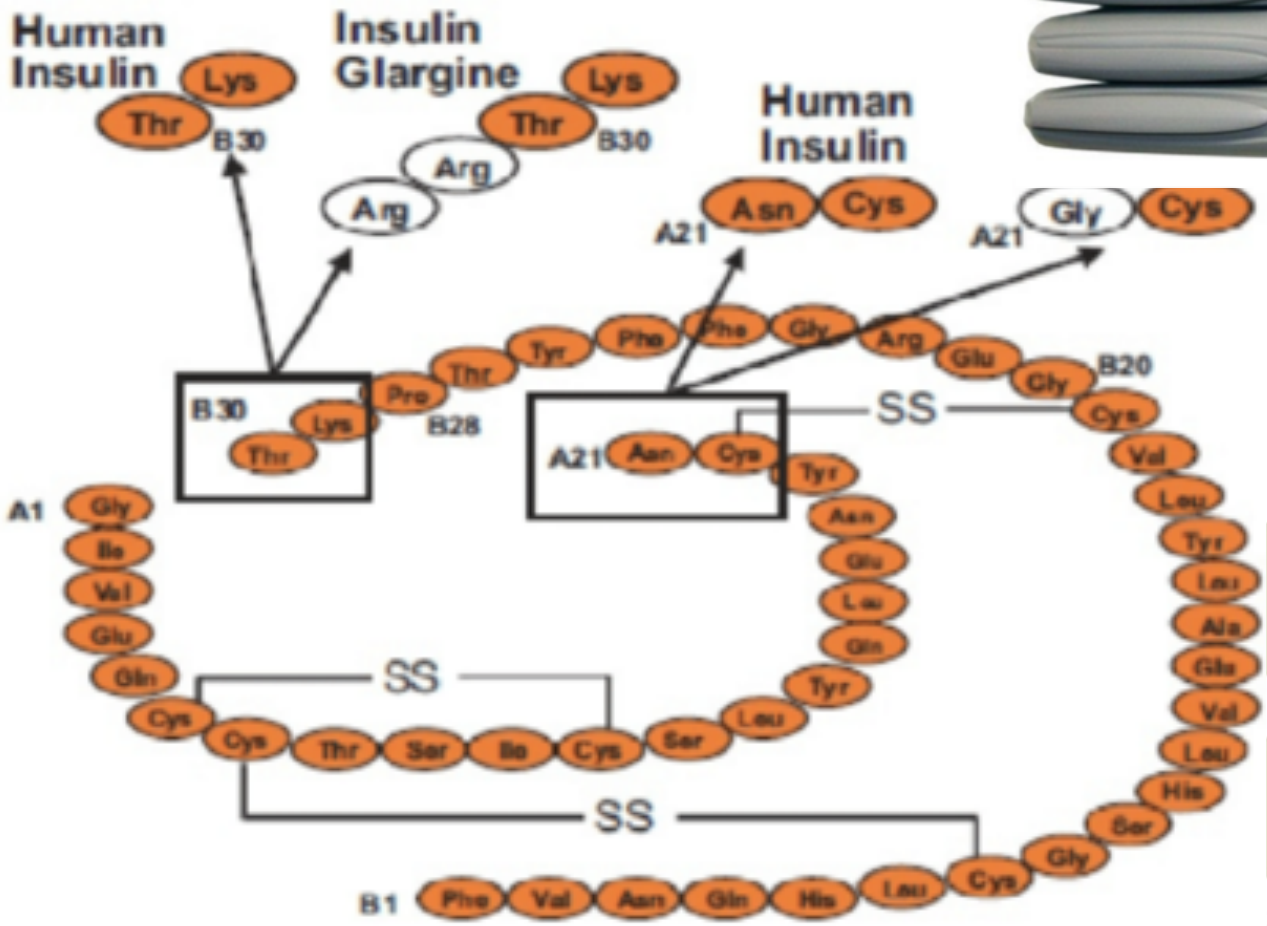


MEDICAL NEED :
IMPROVE INSULIN
SENSITIVITY ASAP !

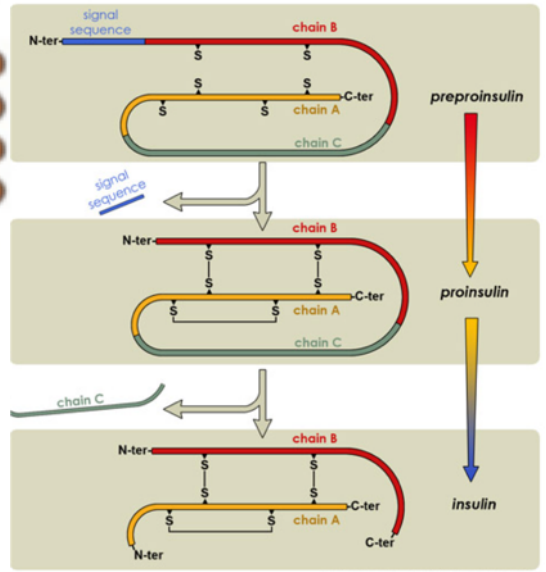


MACRO- AND MICROVASCULARIZATION IMPAIREMENTS

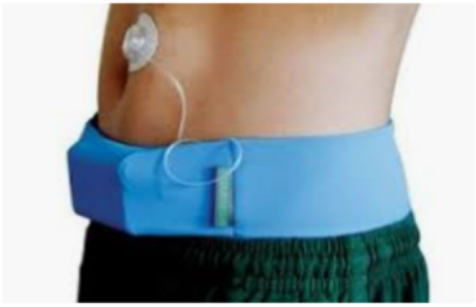
Insulin glargine differs by replacing Asp and Gly positon 21 and Cterm ext of chain B
Medical need ; shifting isoelectric point pH5.4 to 6.7 makes more soluble at pH7 and pharmacodynamic peakless level for 24 hours



Modifications of the Insulin Sequence in Insulin Glargine.

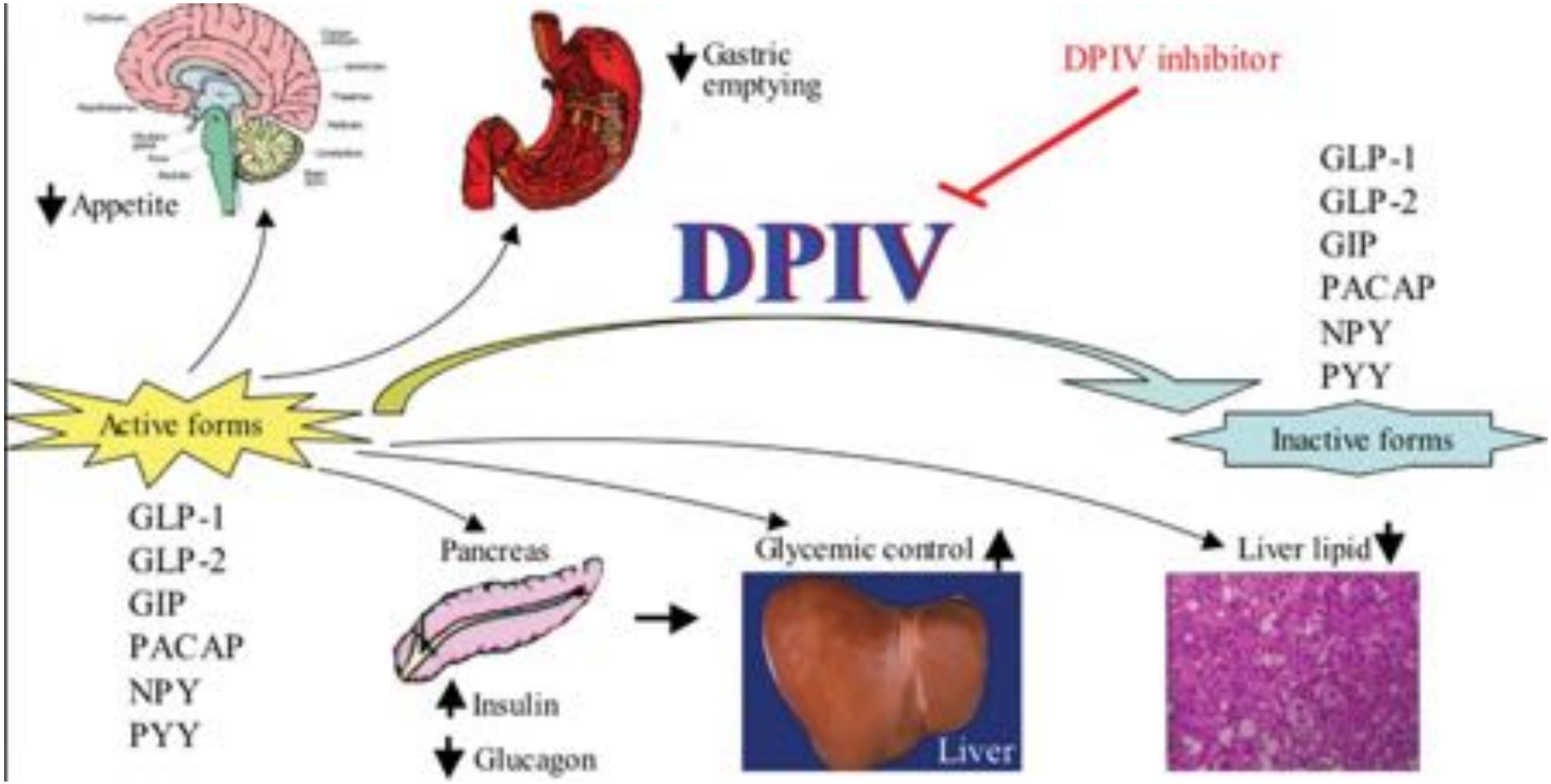


Insulin seringes, automatic pumps connected to acucheck today‘s reality in clinical practice with both type I and II diabetics



Global Insulin pump Accessories Market 2017 …

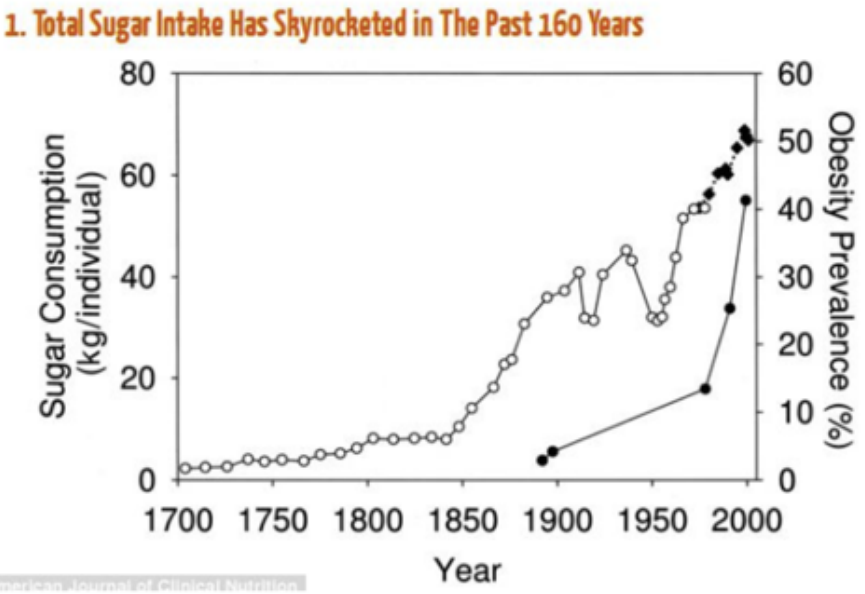
Peptide drugs – incretins in diabetes – vs DPP4 inhibitors

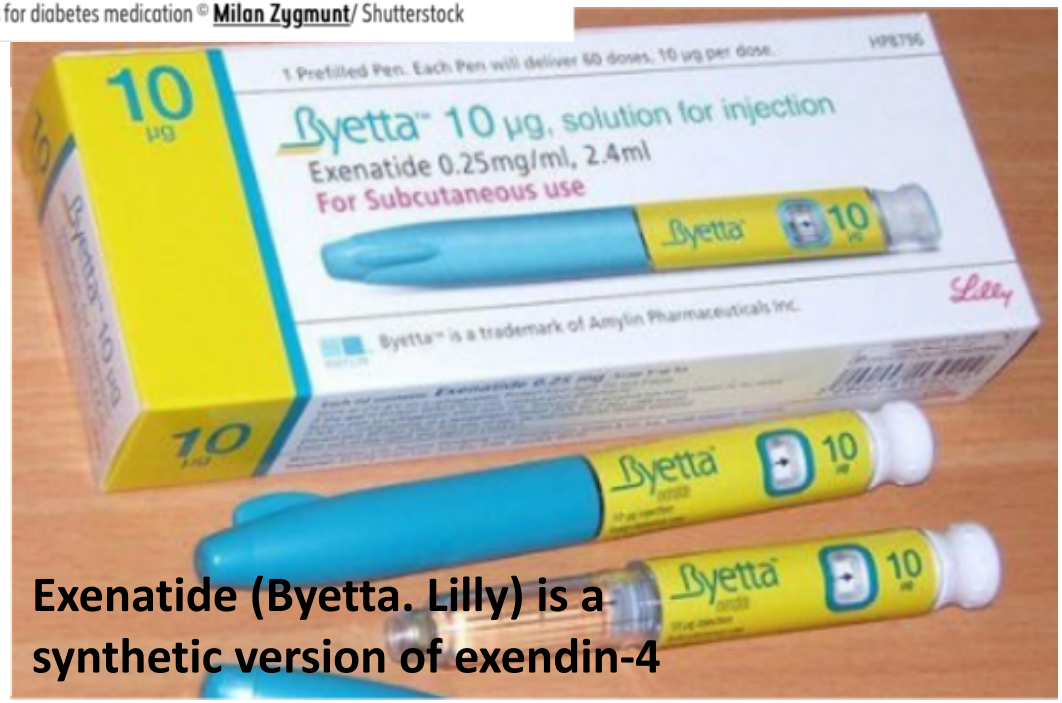


Peptide drugs – when pharmaceutical sciences grab into the wild

- Gila monster: a species of venomous lizard; Mexico
- Eats only 4 times a year
- When fasting, it shuts down the pancreas, stopping insuli
- When its time to eat, it restarts pancreas with exendin-4 in its saliva - a GLP-1R agonist

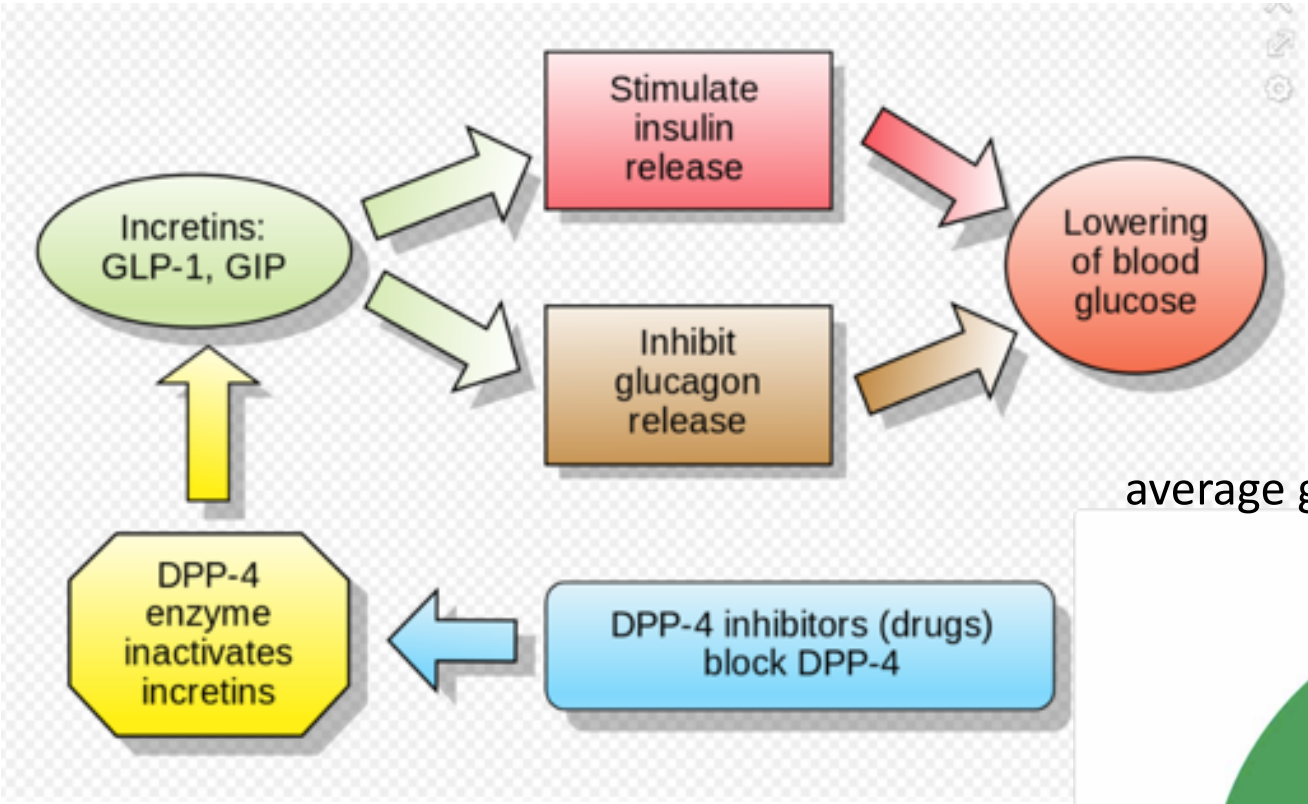
The similarity between a hormone in the Gila monster's saliva and the human hormone GLP-1 makes the lizard's venom suitable as the basis for diabetes medication © Milan Zygmunt/ Shutterstock



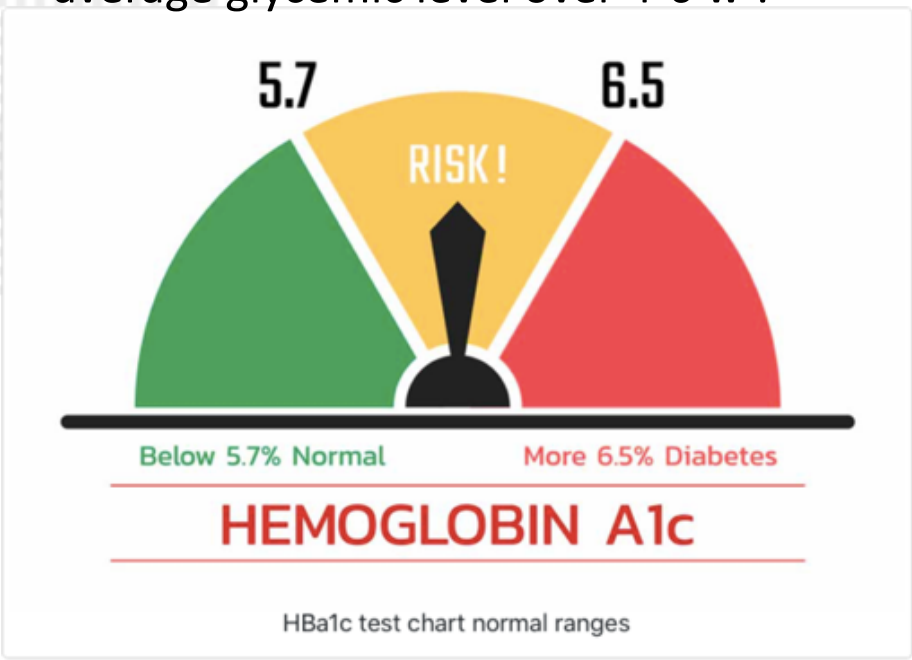


Exenatide (Byetta. Lilly) is a synthetic version of exendin-4

Peptide drugs – GLP1r and incretins



average glycemic level over 4-6 w !



- **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**

GLP1 peptides in drug research and development

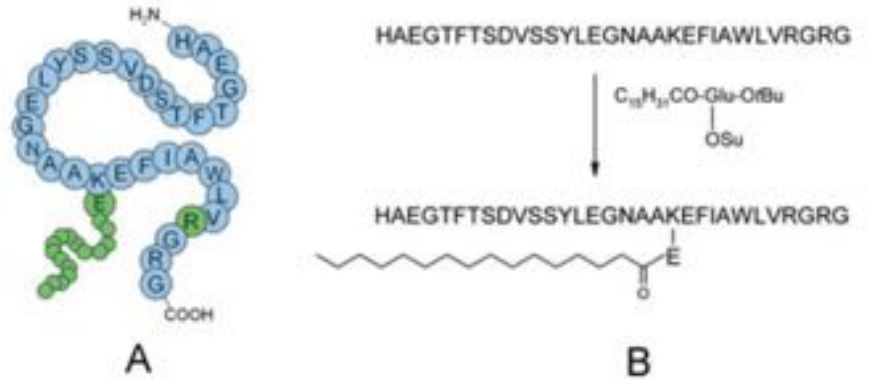
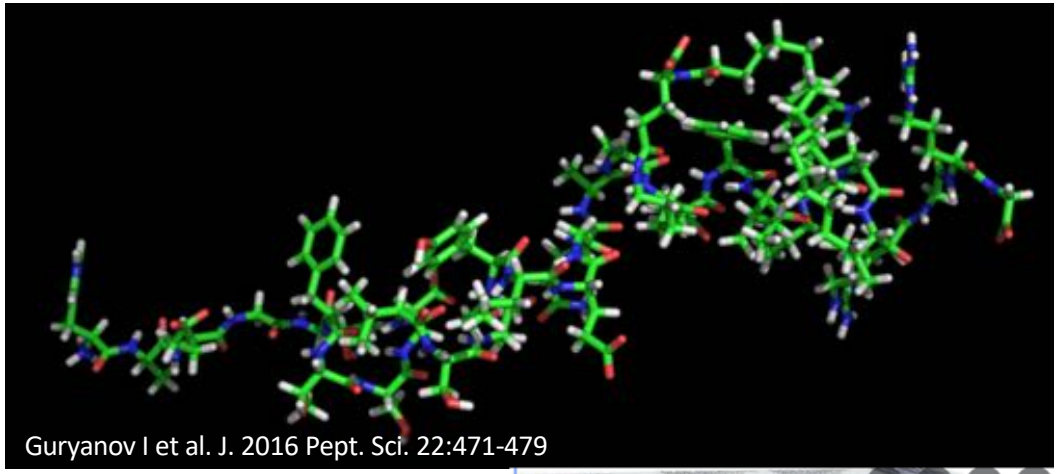


Figure 1. (A) The amino acid sequence of liraglutide. The changes with respect to glucagon-like peptide-1 (7-37) are shown in green; (B) A scheme of preparation of liraglutide by chemical modification of a recombinant peptide precursor (OSu, 1-oxysuccinimidoyl).



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

- 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.

Peptide drugs – GLP1 and incretins

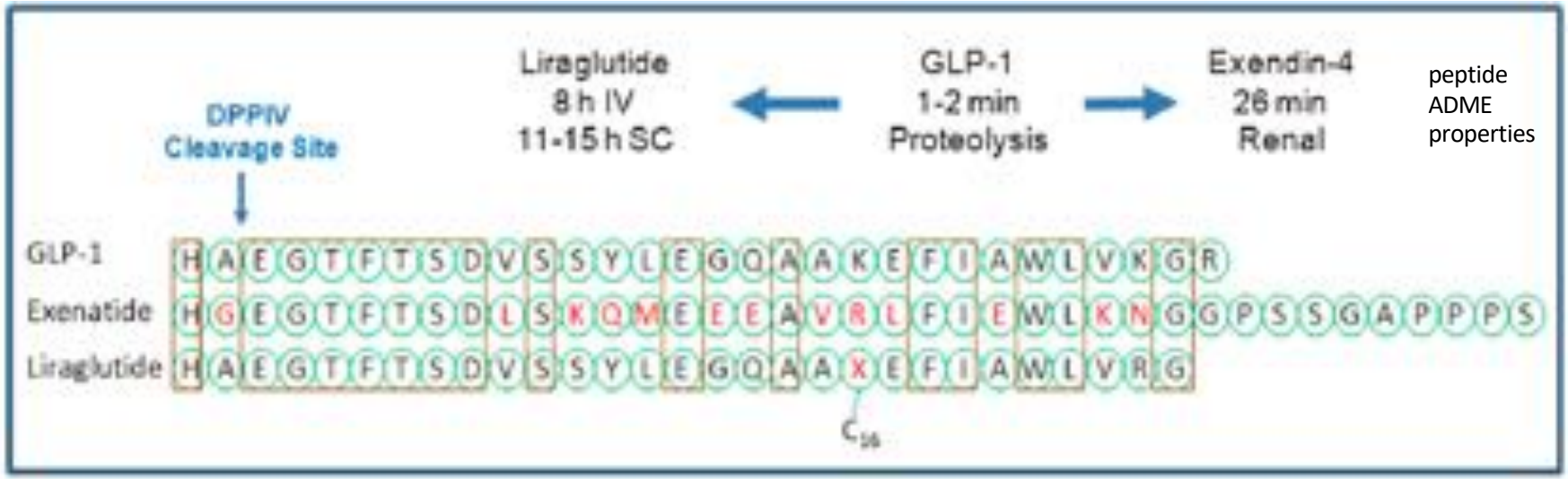


Fig. 2. Strategies to enhance peptide stability: GLP-1 peptide has a half-life of 1–2 min.

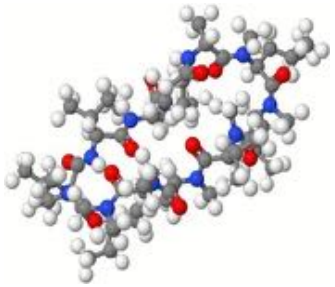
- **GLP1 receptor agonists (incretins) from gut L cells have entered the clinics in the treatment of diabetes type II, drawback: short half life**
- **Exendin-4 (synthetic Exenatide) has improved PK PD properties (lower clearance) allowing once a week dosing as compared to GLP1 (rapidely cleaved by peptidase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

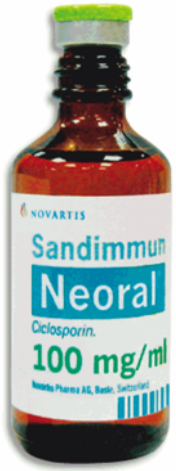
Cyclic Peptides in drug research_pioneering therapeutic modalities

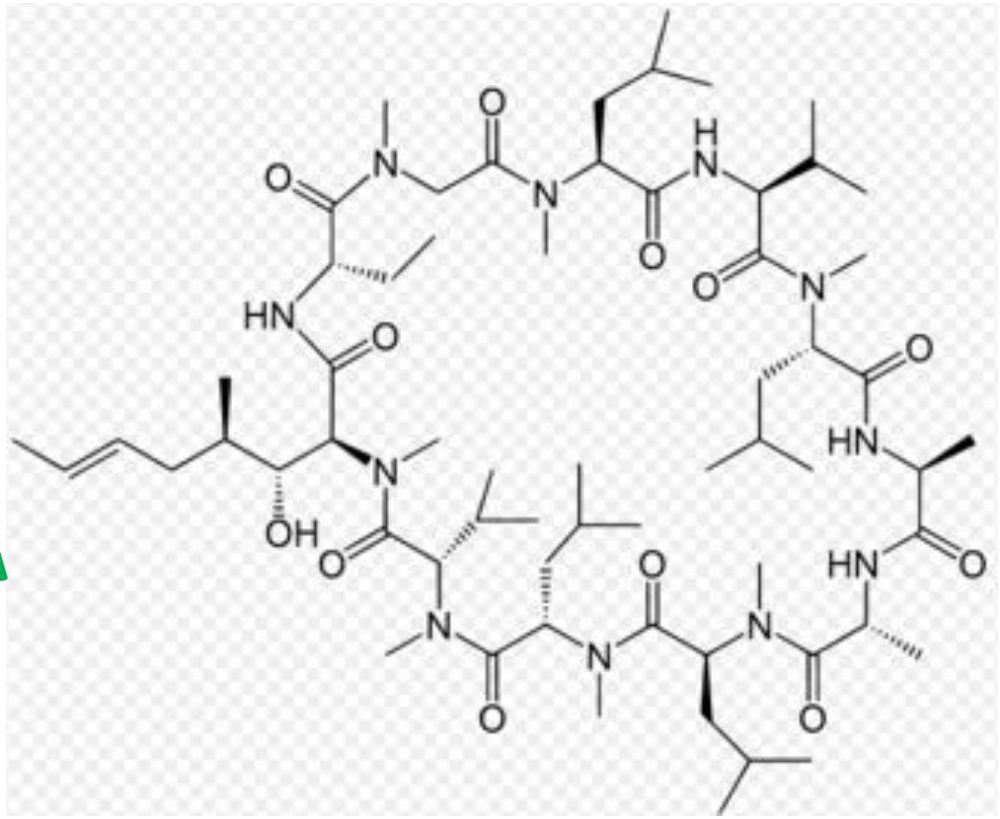
- **Only 2% of the marketed medicines**
- **200 peptide drugs are currently in clinical development**
- **Advantage: high selectivity, low amount need for optimal exposure, agonists**
- **Disadvantage, formulation issue, (oral no option) stability, cost of goods**

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.

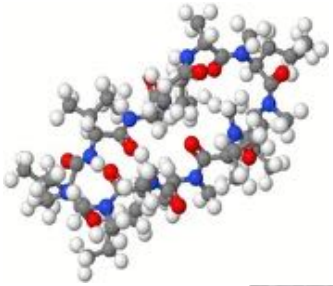
Guryanov I et al. J. 2016 Pept. Sci. 22:471-479

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

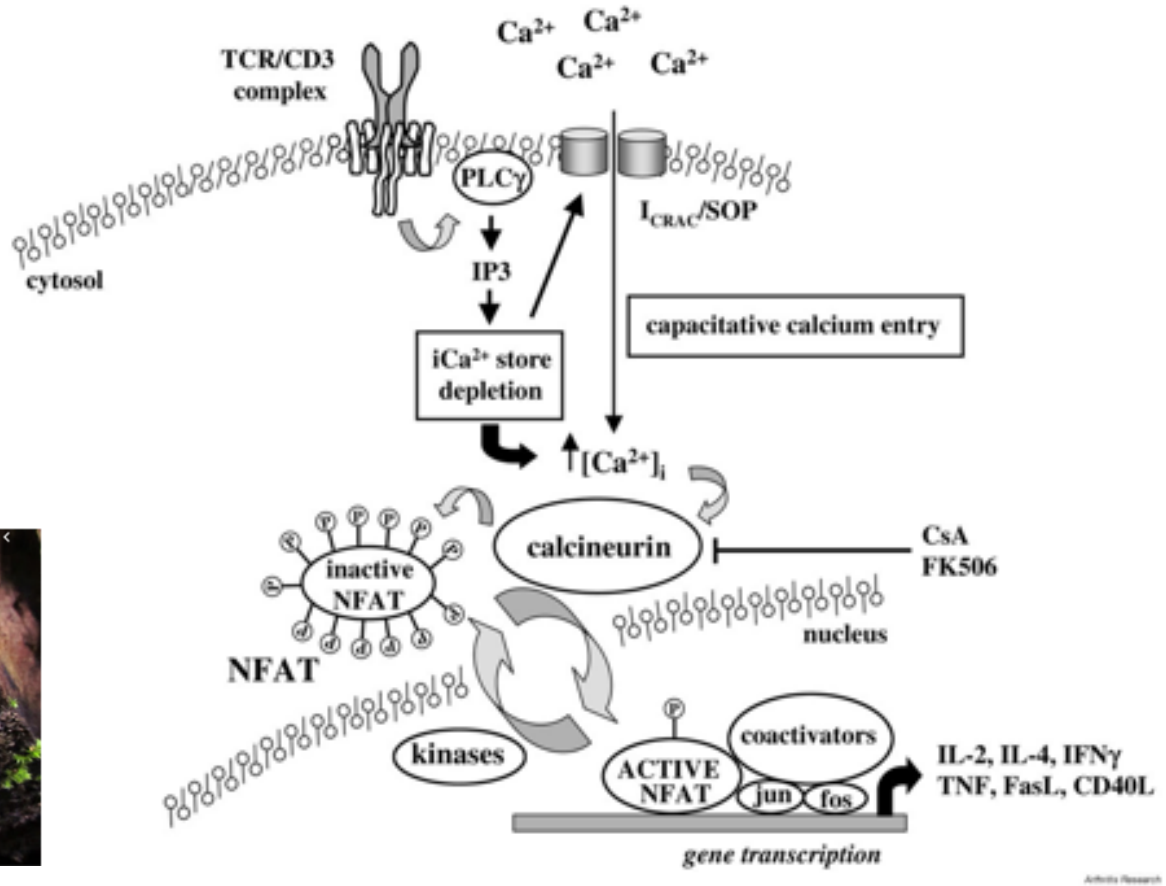
Cyclosporin A

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

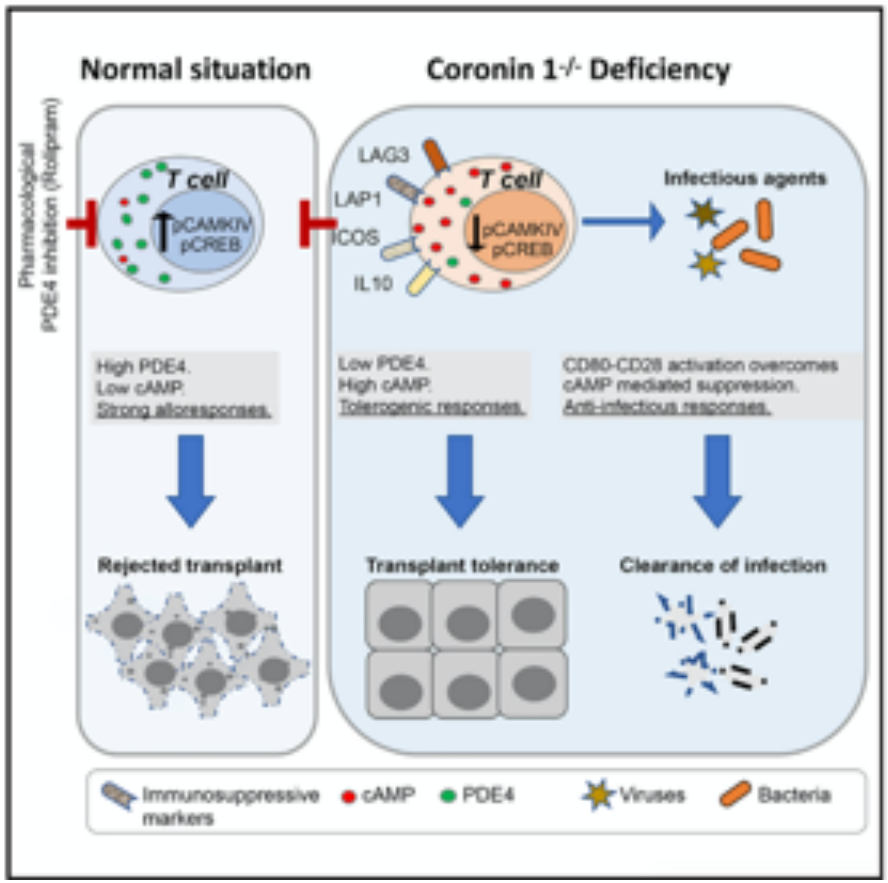
Immunosupression in clicnics-opportuinties for coronin1 inhibitors

Article

Immunity

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

Graphical Abstract



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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

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)

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 shoul ADCC activity of other therapeutic IgGs.

Keywords: glycosylation, effector function, antitumor antibody, t

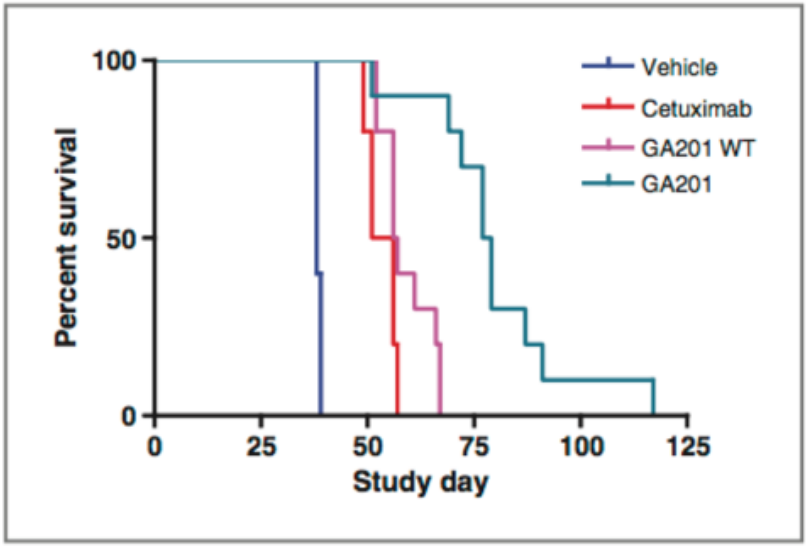


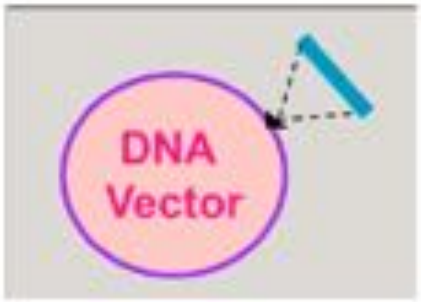
Figure 2. Superior efficacy of GA201 versus cetuximab and the wild type, nonglycoengineered version of GA201 in the A549 lung adenocarcinoma xenograft model in SCID/beige mice. All animals ($n = 10$ per

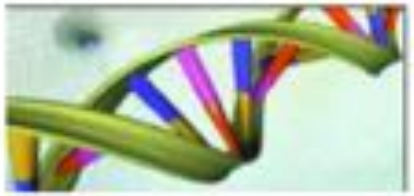
Glycosylation pattern may influence the biology and therapeutic index of mABs

Biologicals - large scale production

GMP PRODUCTION : A MULTISTEP PROCESS THAT REQUIRES STRICT CONTROLS

BIOTECHNOLOGY: KING IN THE KINGDOM OF UNCERTAINTY ?

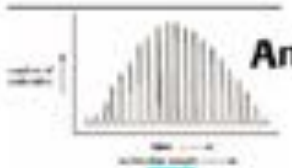




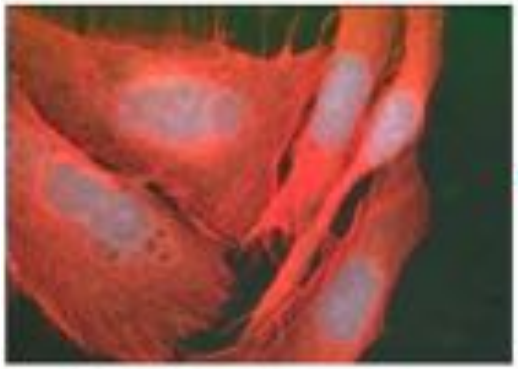
Cloning genes into
DNA Vector(s)

Downstreaming (purify)

Transfer into Host Cell
for Expression



Analytics



e.g., bacterial or mammalian cell

Large-Scale Fermentation

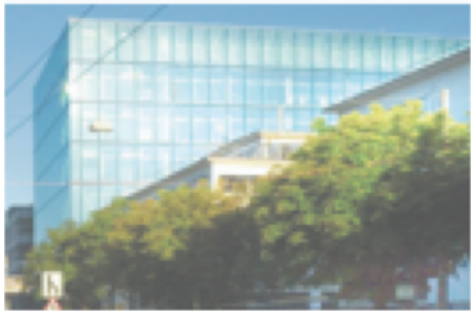
COST OF GOODS: who is going to support the financial burden of biologicals ?

Formulation

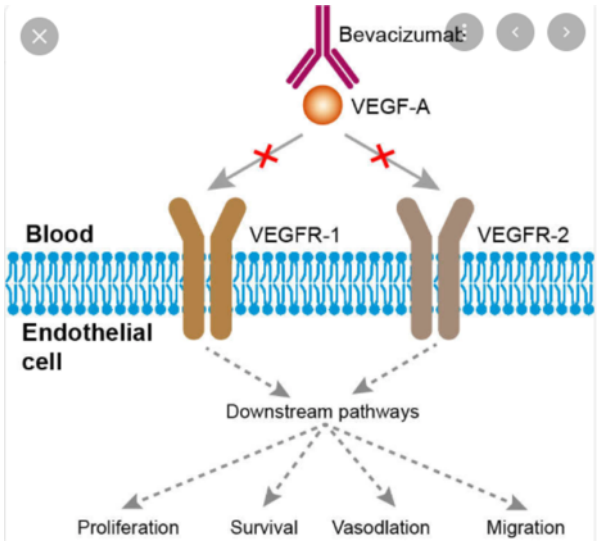
Biologicals - large scale production in Basel – Avastin production

100mg

GMP PRODUCTION OF AVASTIN (hVEGF MAB)



oncology
ophtalmology AMD
(controversy Lucentis)

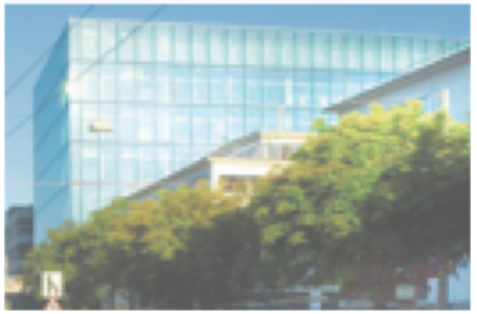


Cancer patient life extension : average 4.7 months (phase 3 trials)
20’000.-US $ /y/patient
5mg/kg bi-monthly iv dose, 120mg/y

Biologicals - large scale production in Basel – Avastin production

GMP PRODUCTION OF AVASTIN (hVEGF MAB)

Controversy Lucentis vs Avastin off label



oncology
ophtalmology AMD
(controversy Lucentis)

SANTÉ

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

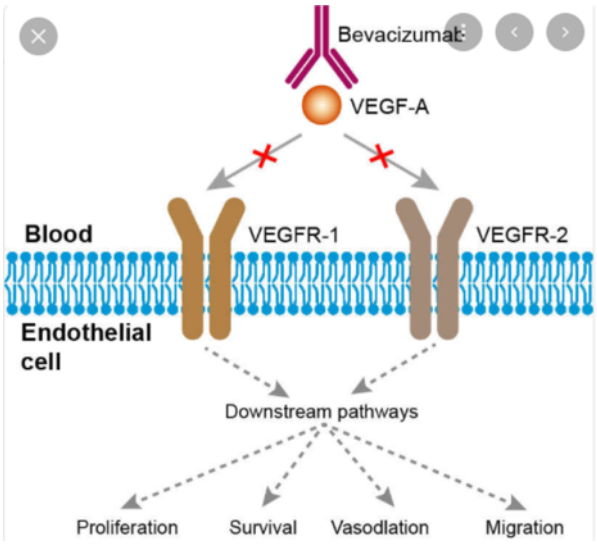
Publié le 09 septembre 2020 | Imprimer la page

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

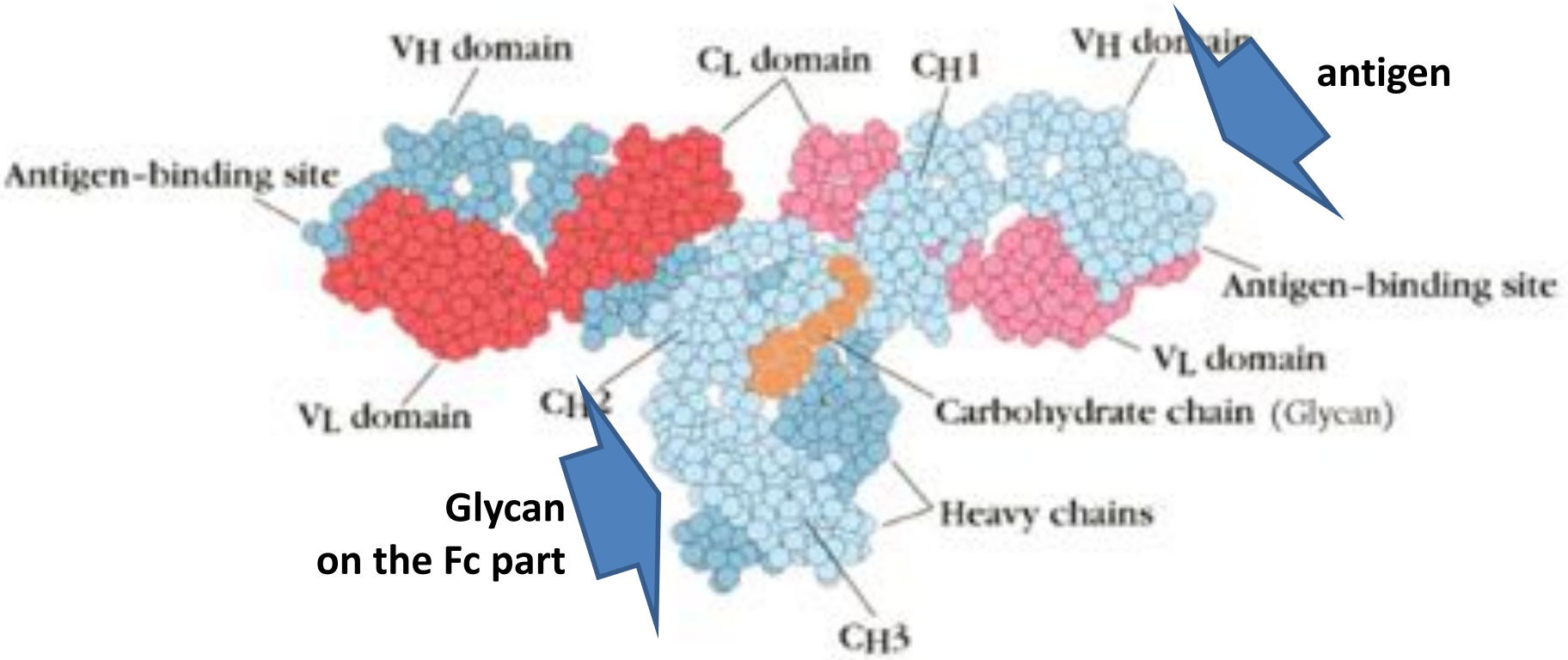
La dégénérescence maculaire liée à l'âge (DMLA), est la principale cause de malvoyance chez les sujets de plus de 50 ans dans les pays industrialisés. Elle entraîne une altération sévère de la vision centrale, qui se présente notamment sous la forme de taches sombres perçues par le patient au milieu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

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



Monoclonal antibodies _ mABs

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

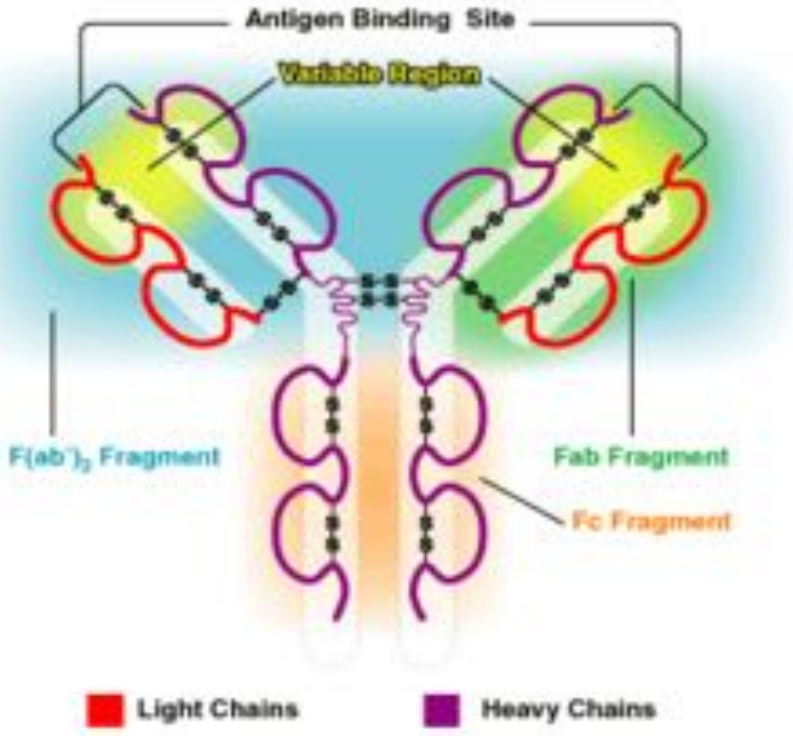


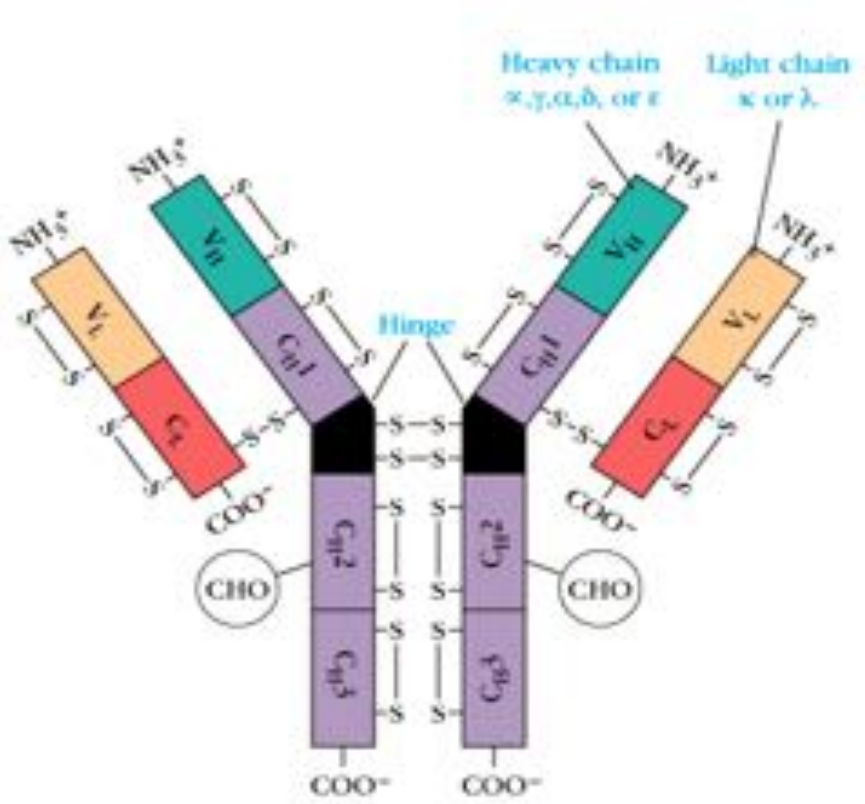
Immunglobulins are glycoproteins

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)

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)

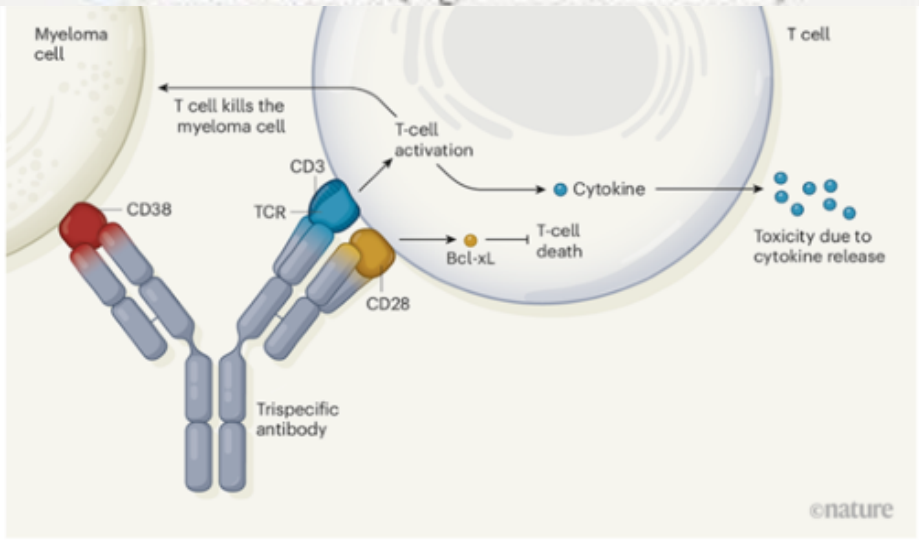
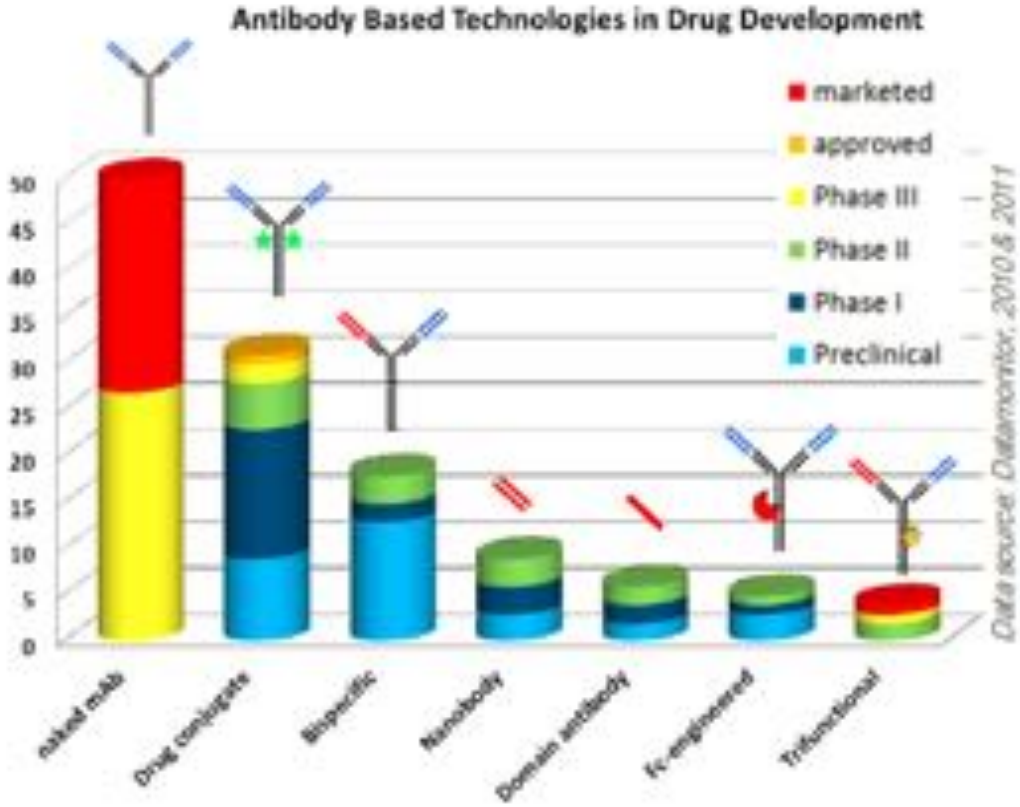


Figure 1 | An antibody that helps immune cells to target cancer cells. Wu *et al.*[1] report the



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

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

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 !

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®)

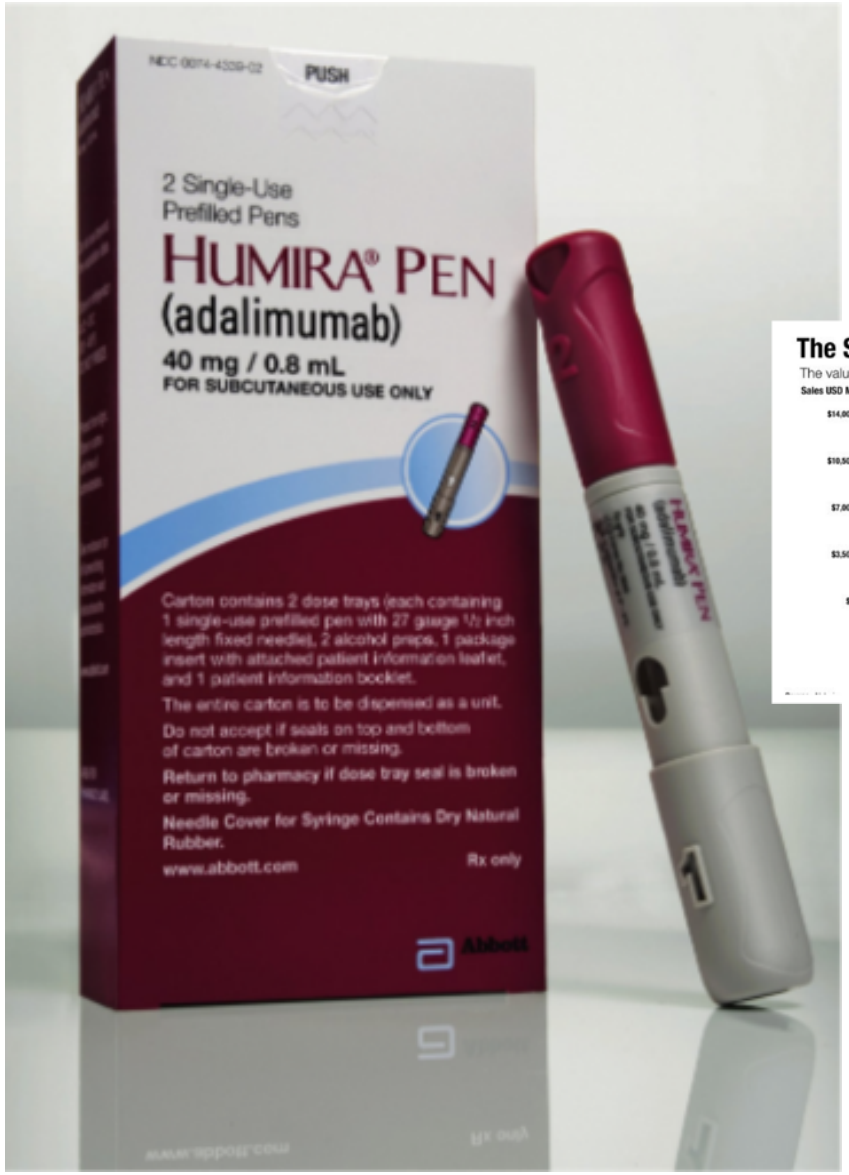
mABs in clinical practice oncology, neurology, inflammation (01. 2019)

Onkologie-Marktführer

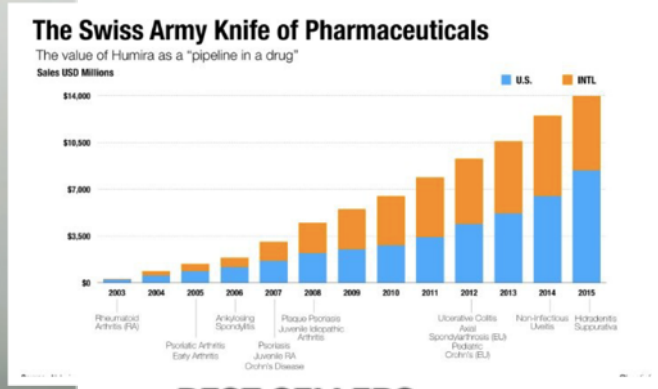
Umsätze 2017 mit Krebsmedikamenten in Milliarden US-Dollar		Veränderung zu 2016, in %
Roche	26,2	+3,7
Bristol-Myers + Celgene	8,4 + 10,8 = **19,2**	+18,6
Novartis	12,3	–4,1
Johnson & Johnson	7,3	+25,0
Pfizer	6,1	+32,7
Merck & Co	4,6	+107,5
Astra-Zeneca	4,0	+19,1
Eli Lilly	3,5	–3,3
Abbvie	3,4	+28,2

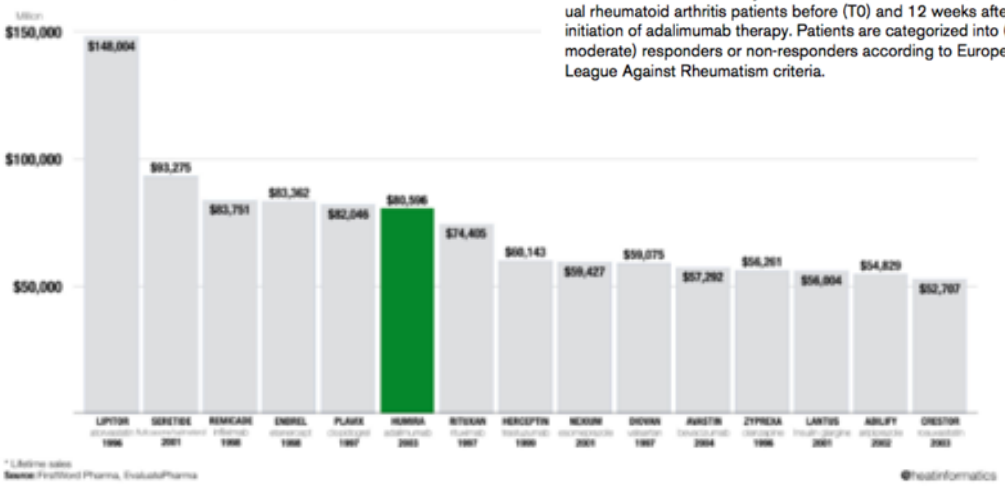
Grafik: ake/Quelle: «Handelsblatt»

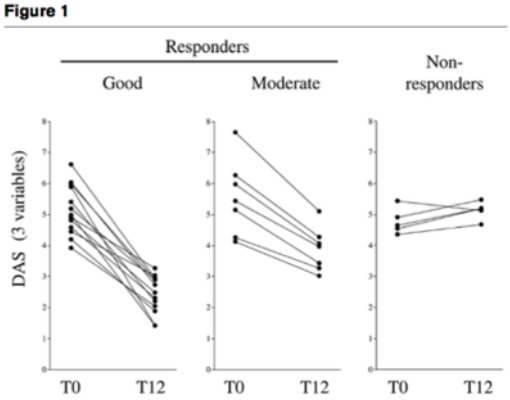
Anti TNF : rhumatoid arthris-Crohn bowel disease-ulcerative collitis



- 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



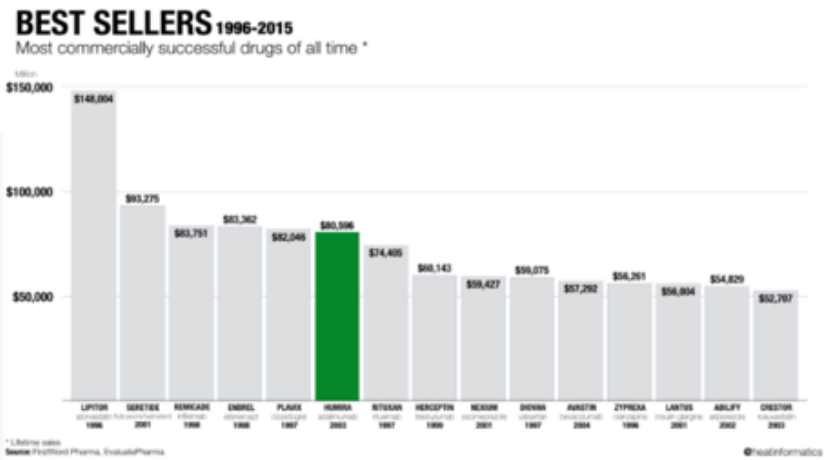




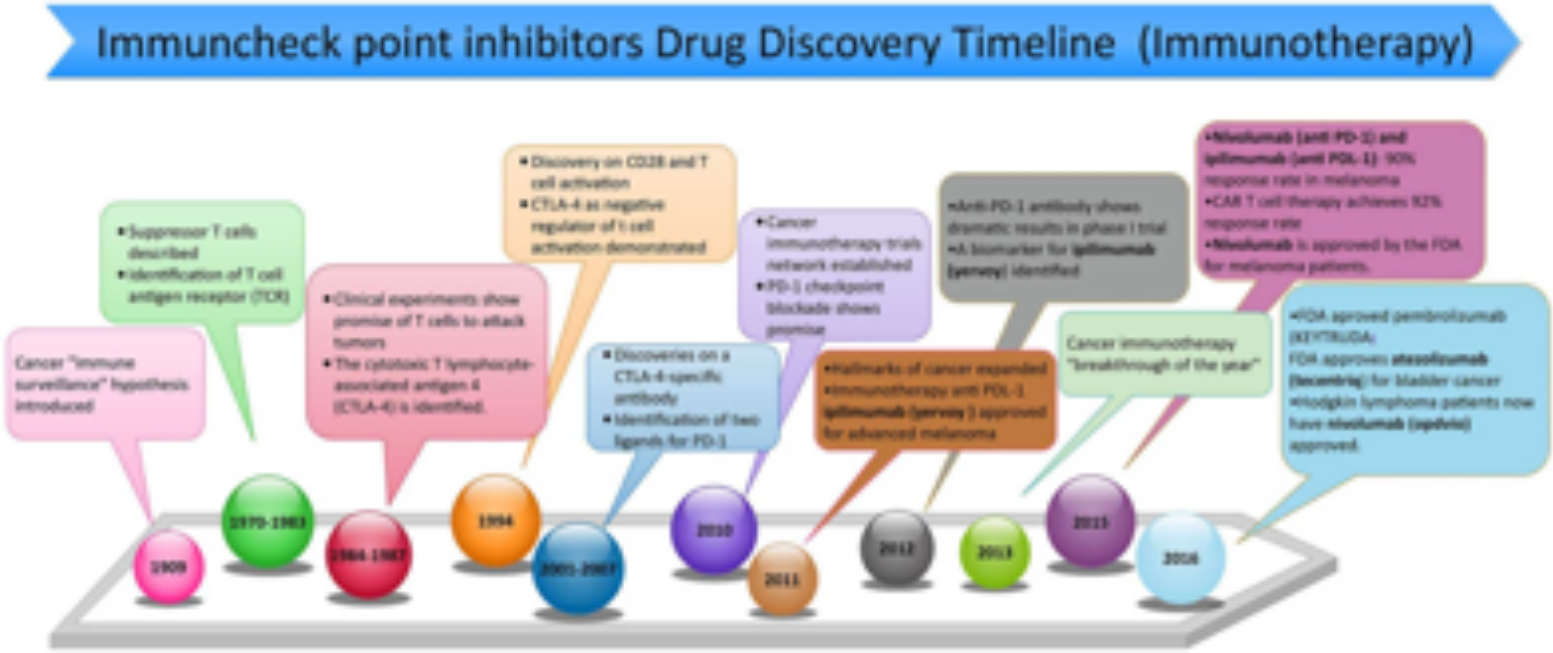
Evolution of disease activity score (DAS) (three variables) in 25 individual rheumatoid arthritis patients before (T0) and 12 weeks after (T12) initiation of adalimumab therapy. Patients are categorized into (good or moderate) responders or non-responders according to European League Against Rheumatism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

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



Immune checkpoint blockade : time line



Alsaab & al. frontiers in Pharmacology, 2017

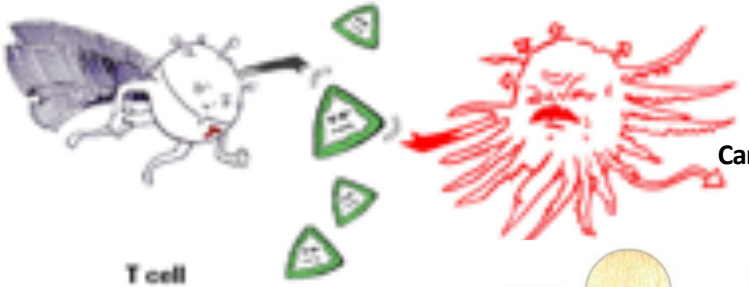
Important Dates for PD-1/PDL-1:

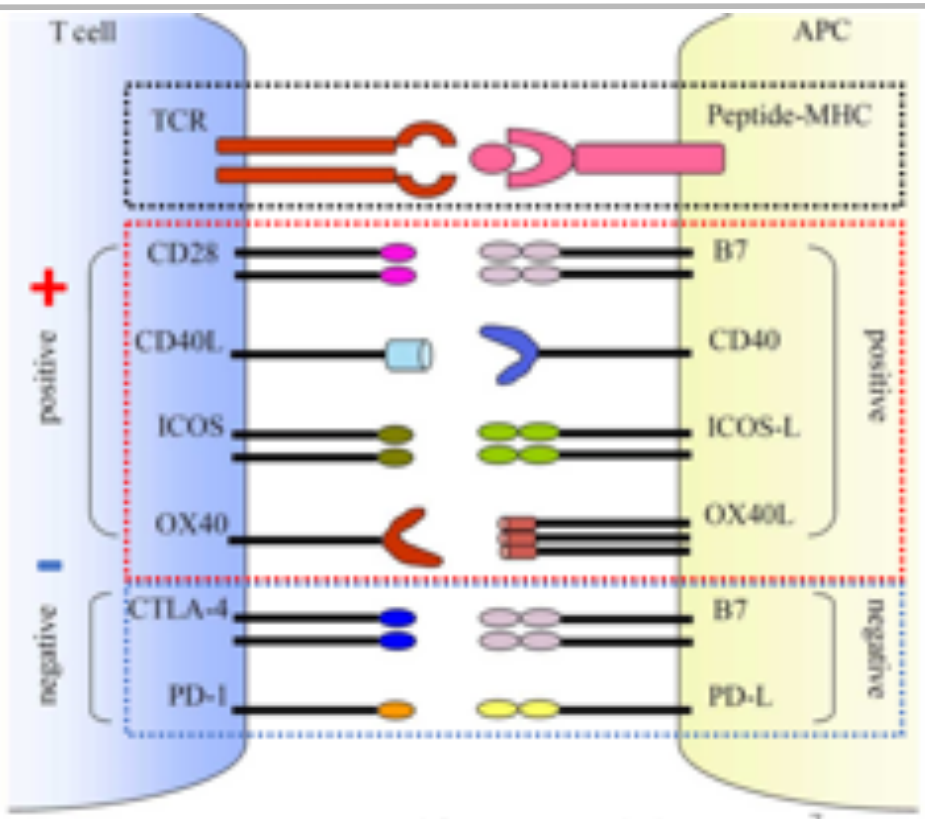
- 1992: Discovery of PD-1
- 1999: Discovery of B7-H1 (PDL-1)

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

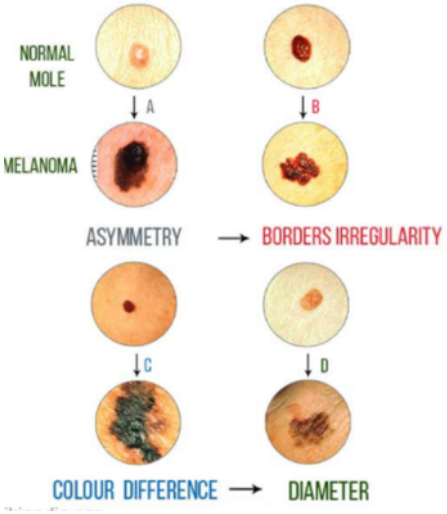
- Activation of T cell upon foreign antigen encounter **+ "Go"**

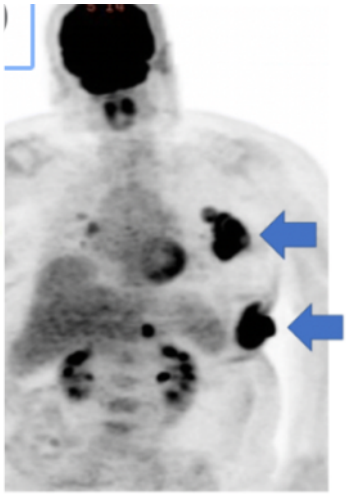
- Regulation and blocking of self-antigen recognizing T cells (that avoided self-tolerance check) **- "No Go"**





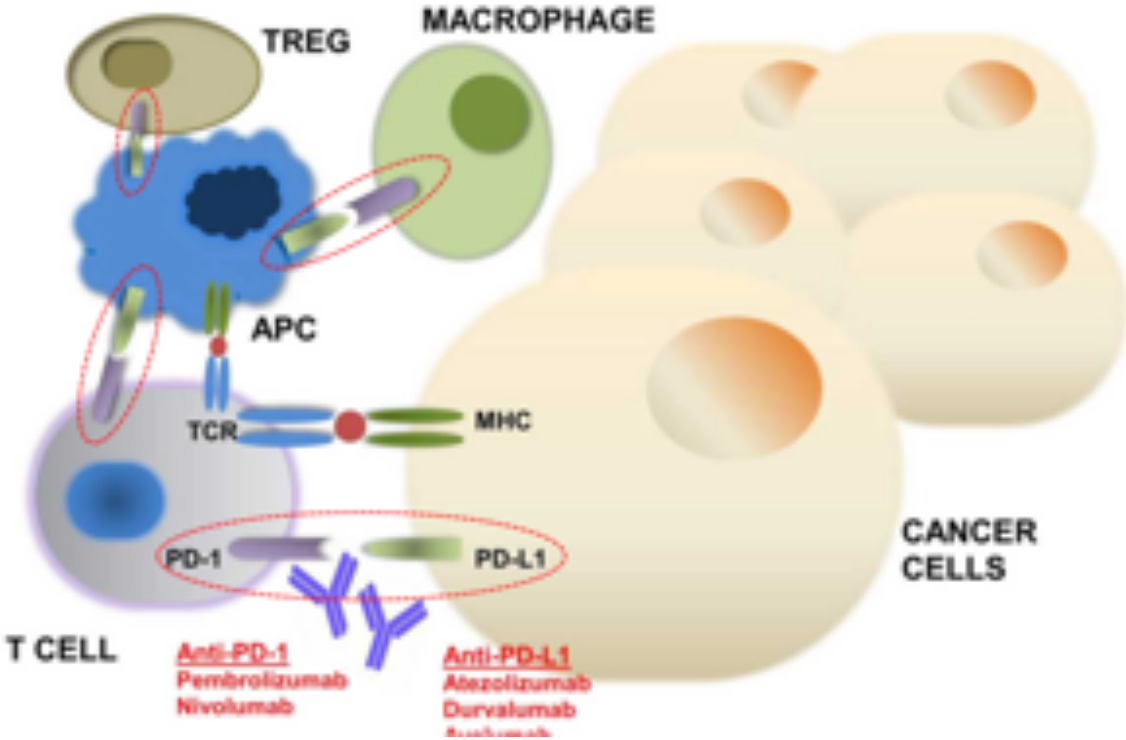
Huang & al. frontiers in Microbiology, 2011





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…)



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

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 !)

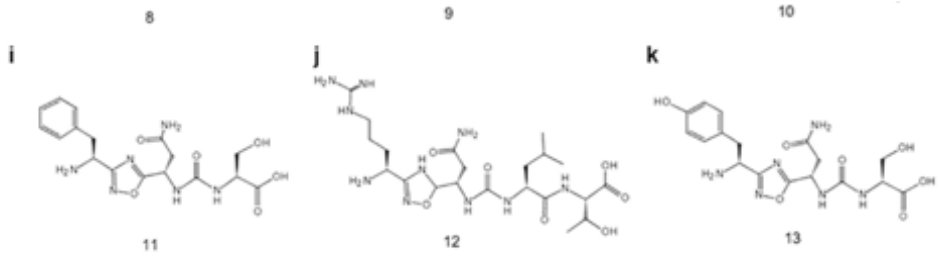
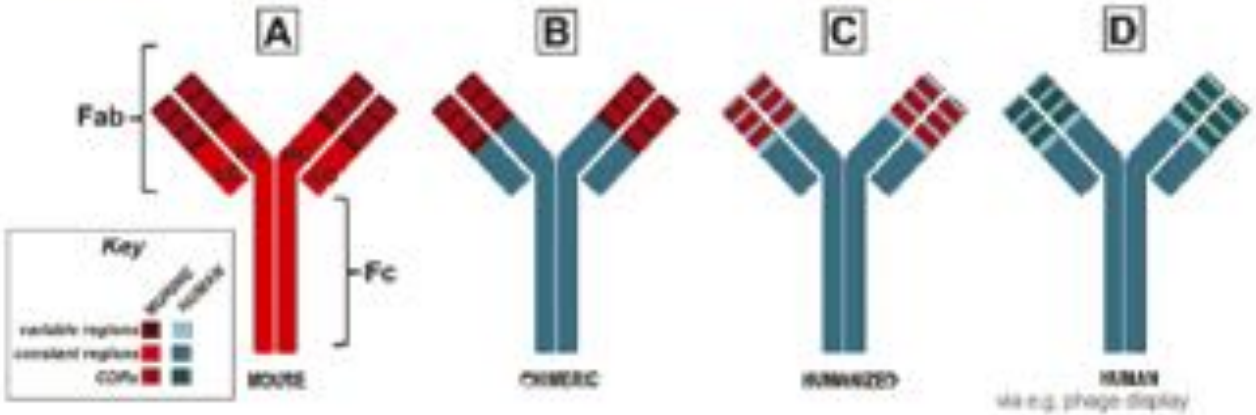


Fig. 3 Nonpeptide-based small molecule inhibitors targeting PD-1/PD-L1

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

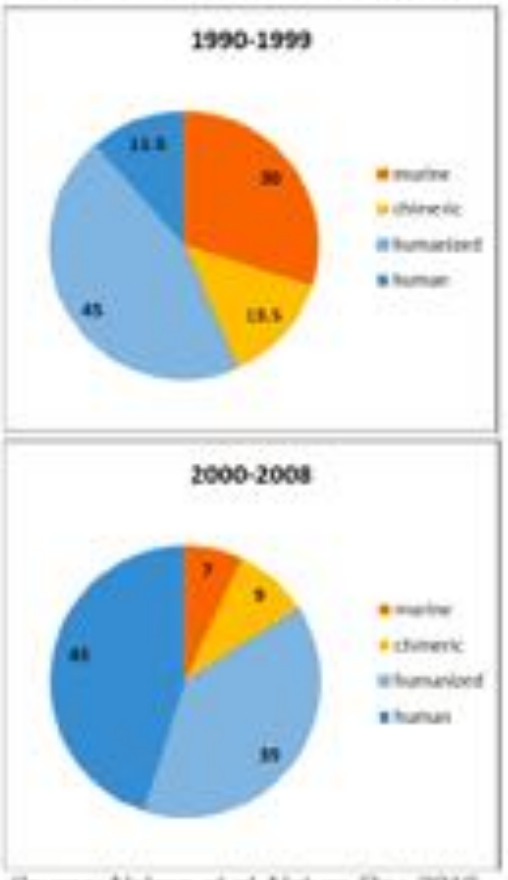
Classification of mAbs by level of humanisation



mAbs Nomenclature

Types of mAbs	Description	Nomenclature
Murine	Entirely murine amino acids	'o' = mouse e.g. muromonab
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

mABs in clinical practice and nomenclature

Type d'Ac

Seules les IgG seront utilisées en thérapeutique.

- **Les anticorps murins (suffixe -omab)** sont des anticorps produits chez la souris. Le principal défaut de ces anticorps est la production d'anticorps humains anti-souris (HAMA) lorsqu'ils sont utilisés comme agent thérapeutique chez l'homme. Aujourd'hui leur utilisation est limitée.

- **Les anticorps chimériques (suffixe -ximab)** sont humains à 60%. Les parties constantes des chaînes lourdes et légères (CH et CL) d'anticorps humain sont greffées sur les parties variables respectives (VH et VL) d'un anticorps murin.

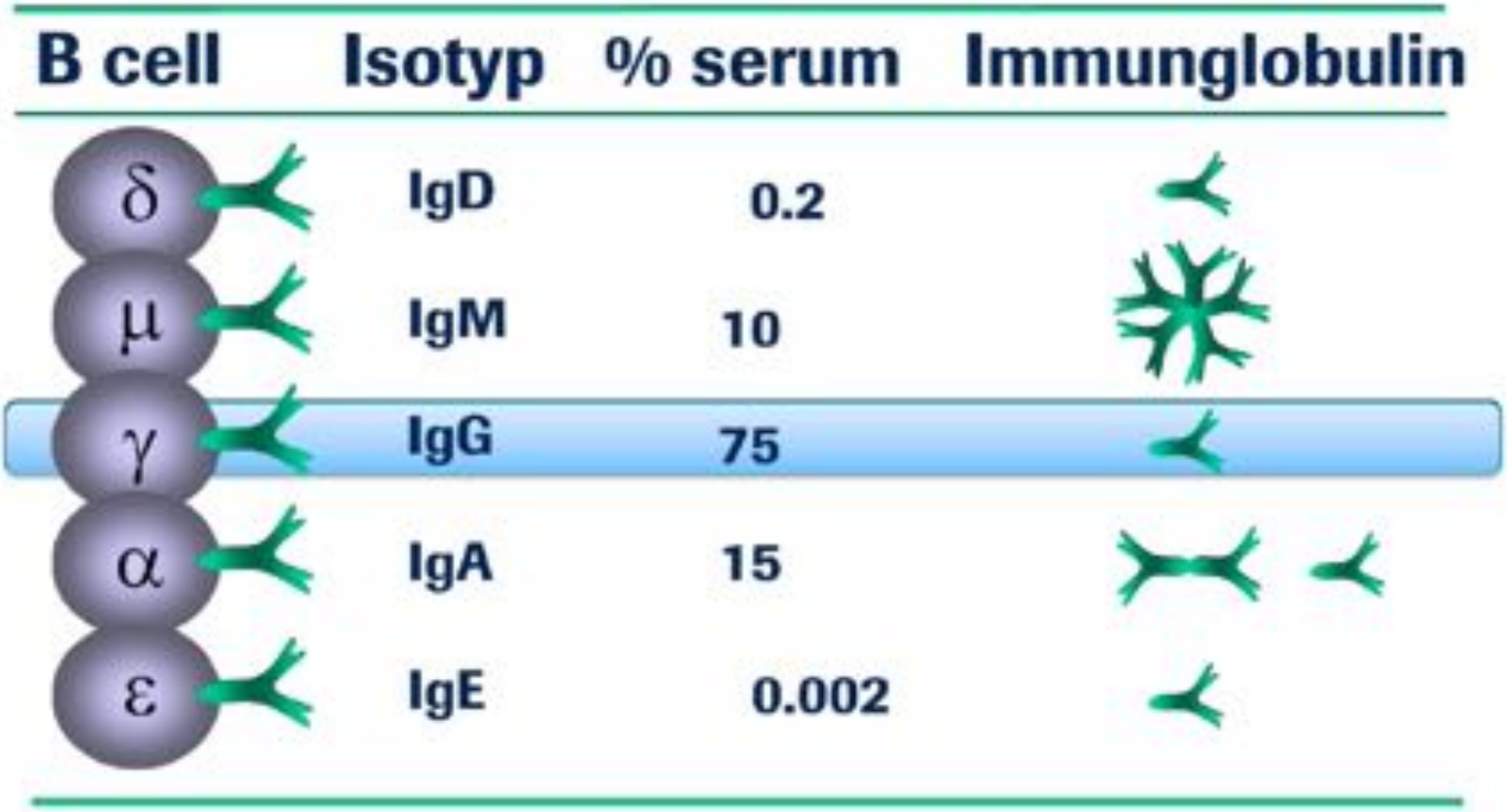
- **Les anticorps humanisés (suffixe -zumab)** sont humains à 90%. Des parties hypervariables (CDR) d'un anticorps murin sont greffées sur une immunoglobuline humaine. L'anticorps humanisé est mieux toléré par l'organisme humain car ressemble plus « au soi ». Son efficacité est renforcée car sa demi-vie est plus longue dans l'organisme.

- **Les anticorps humains (suffixe -umab)** sont humains à 100%. Ils ont l'avantage de limiter l'immunogénicité et diminue le risque de synthèse d'anticorps humains anti-souris retrouvés lorsque des anticorps chimériques et humanisés sont utilisés.

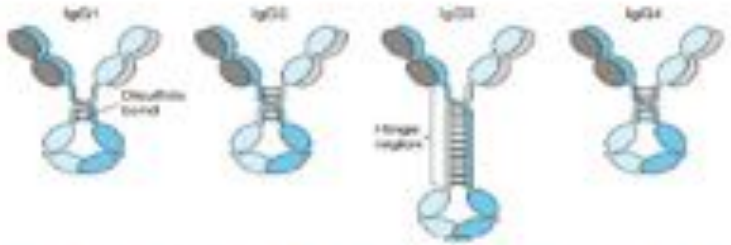
Le choix de l'**isotype** est important à considérer lorsque l'on veut utiliser l'AcM en thérapeutique car les propriétés structurales des différents isotypes vont avoir un impact sur les propriétés effectrices de l'anticorps. Il existe 4 isotypes d'IgG (IgG1 à IgG4). Les IgG1 sont les plus abondantes dans le sang (66%). Viennent ensuite les IgG2 (23%), les IgG3 (7%) et enfin les IgG4, les moins abondantes (4%). Les sous-classes d'IgG se différencient par le nombre de ponts S-S entre les chaînes lourdes avec 2 pour IgG1 et IgG4, 4 pour l'IgG2 ou 15 pour l'IgG3. Elles présentent également des différences sur l'organisation des ponts disulfures intercaténaires.

Les IgG3 ont une demi-vie très courte (8 jours) comparée aux autres isotypes (IgG1, IgG2, IgG4) qui ont une demi-vie de 21 jours. L'isotype IgG3 n'est donc pas utilisé en thérapeutique. La capacité à activer la voie classique du complément est également différente entre les 4 isotypes : IgG1 > IgG3 > IgG2, l'IgG4 n'ayant pas la capacité d'activer le complément. Ainsi, l'IgG1 sera utilisée lorsqu'une activité cytotoxique sera recherchée (ADCC et CDC) tandis que l'IgG2 ou l'IgG4 seront préférées lorsque l'on voudra privilégier la neutralisation.

mABs in drug research



IgG1 is the most common in serum

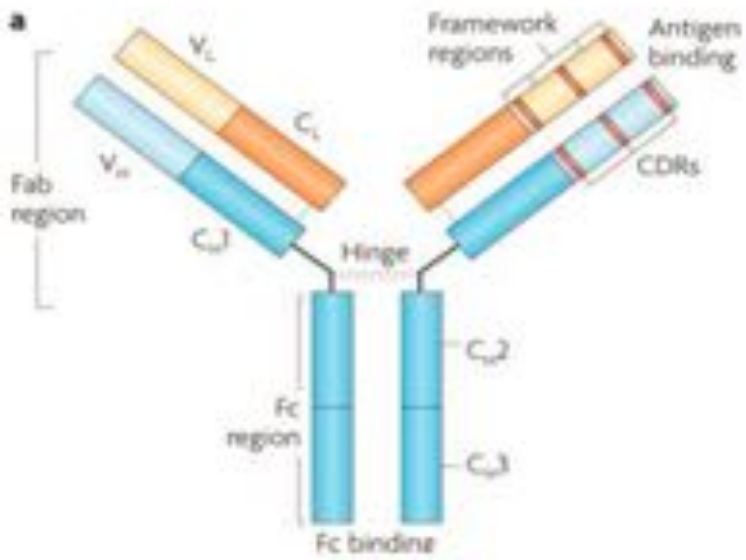


	IgG_1	IgG_2	IgG_3	IgG_4
Serum concentration (%)	60-65	20-25	5-10	<4
Serum half life (days)	21	20	7	21
Placental passage	++	±	++	++
Complement binding	+++	++	++++	±
FcγR binding	RI, RII, RIII	RII	RI, RII, RIII	
ADCC	+++	+	+++	±

antibody dependent cell cytotoxicity

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

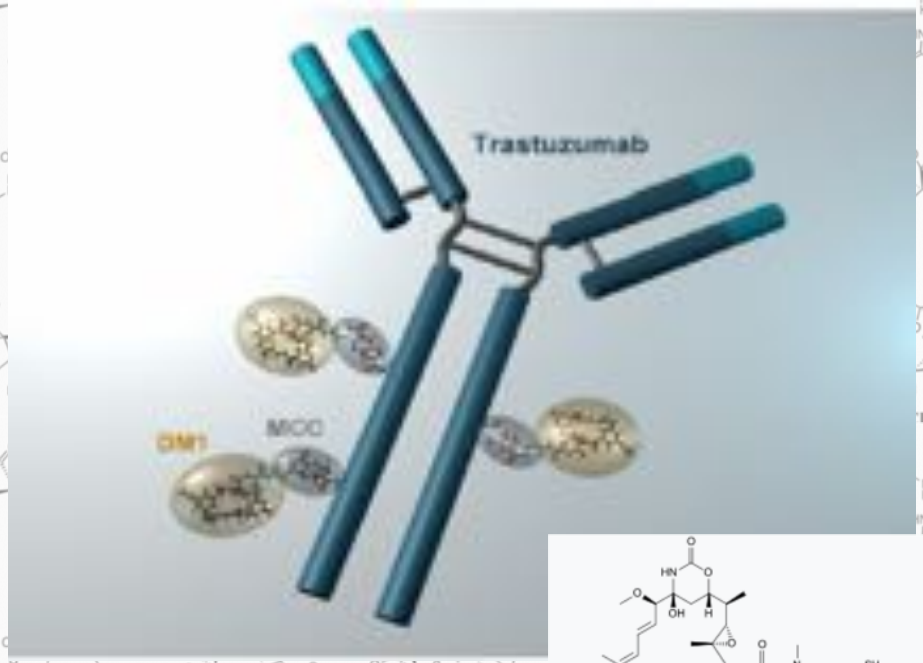
“armed” biologicals – “smart” biopharmaceuticals



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 – 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 »

Common metastatic sites in breast cancer

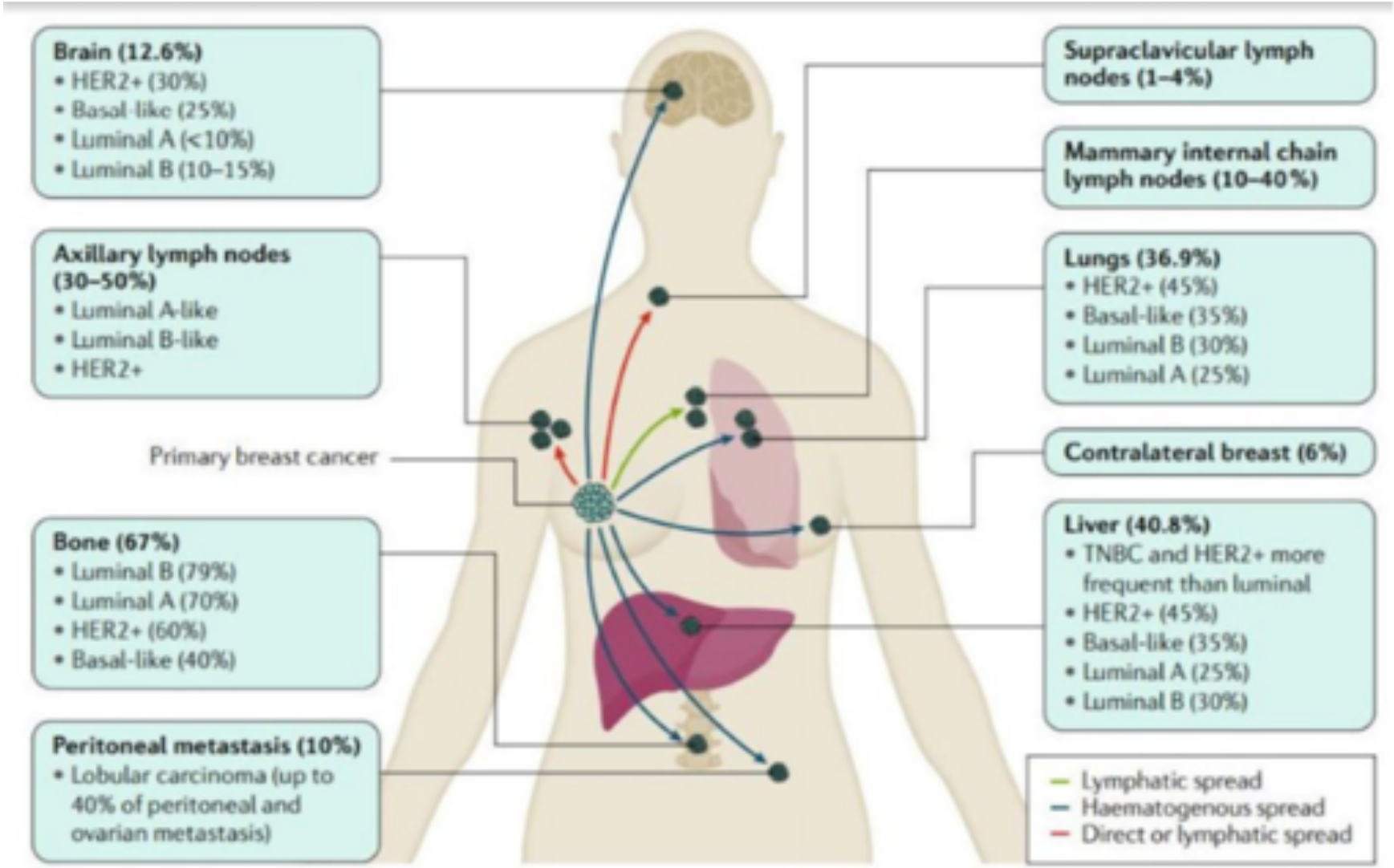
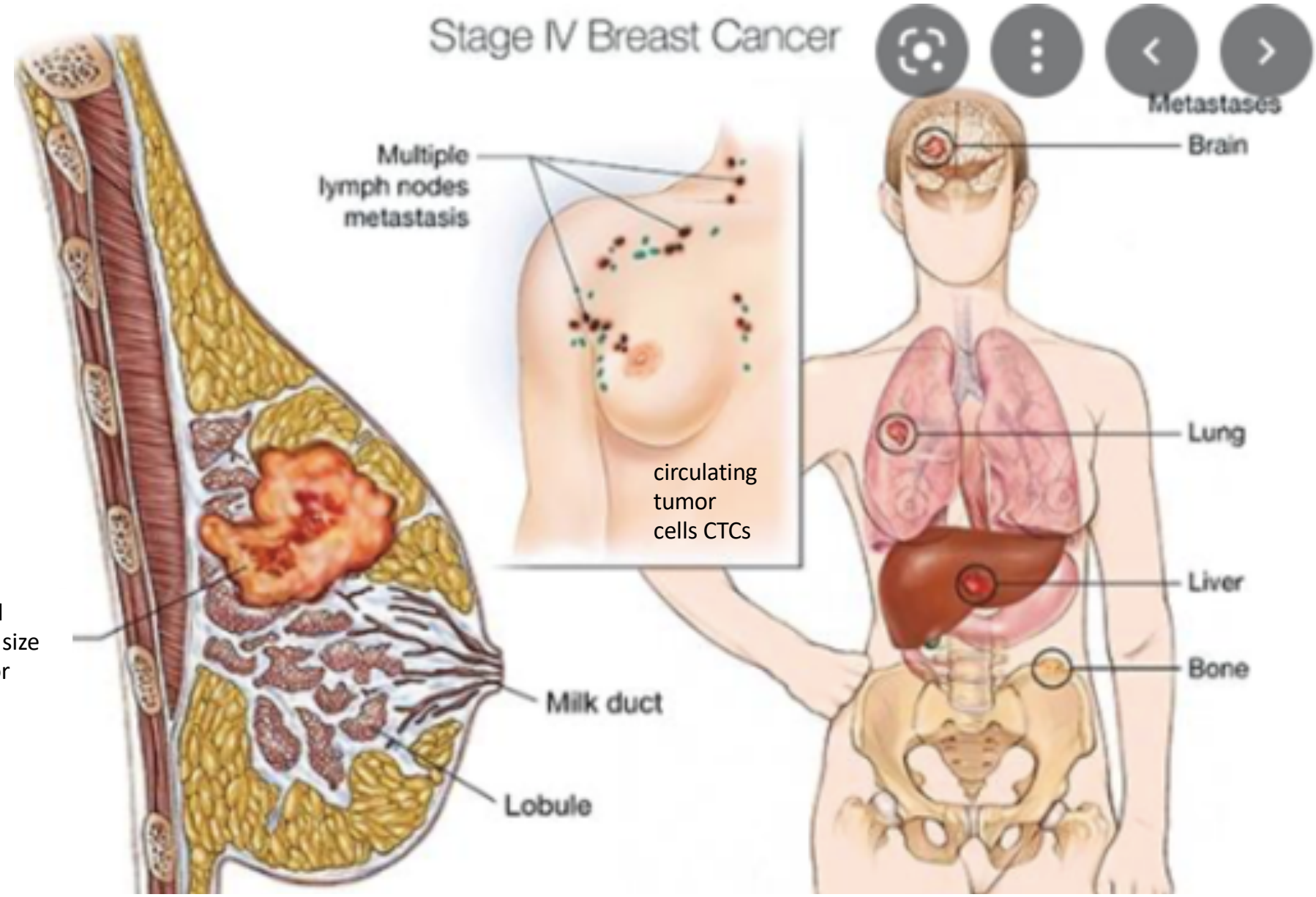


Illustration of common metastatic sites in breast cancer [2]. The most common metastatic sites for breast cancer are the bones, axillary lymph nodes, liver, and lungs. Approximately 10-40% of breast cancer tumors have

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 – ADCs -combining precision medicine with chemo-radio-therapy

DM1

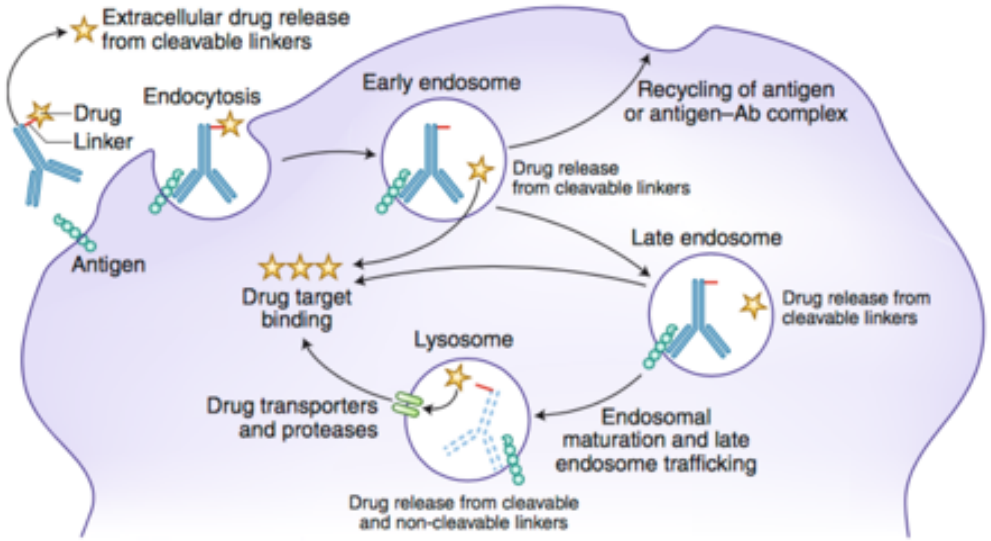


Fig. 1 | Mechanisms for drug release from antibody–drug conjugates. Depending on the linker used,

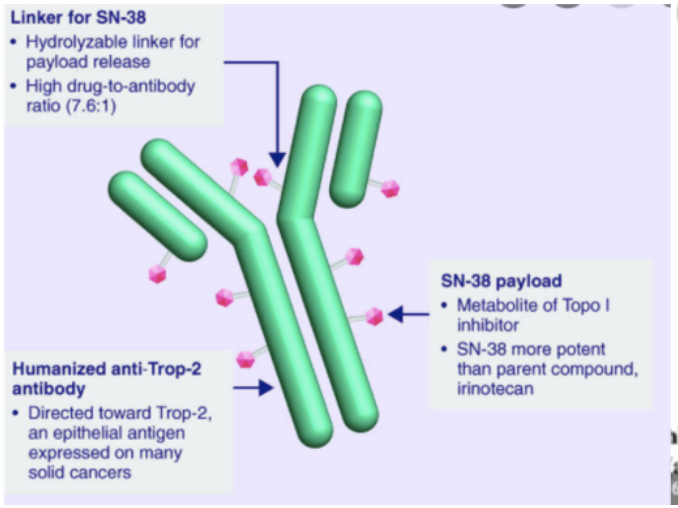
Van Epps and Senter PD. 2019. Nature Chemical Biology 15:934-

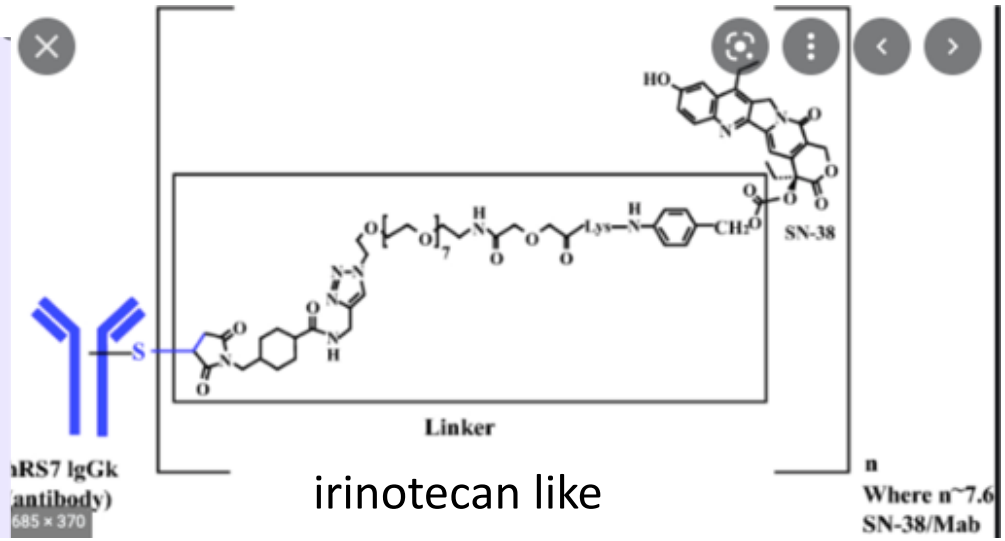
Bioactive compound
Oxaliplatin
p53 DNA
RB94 DNA
RRM2 siRNA
Doxorubicin
Paclitaxel
Doxorubicin
Docetaxel
Melanoma antigens + IFNγ

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

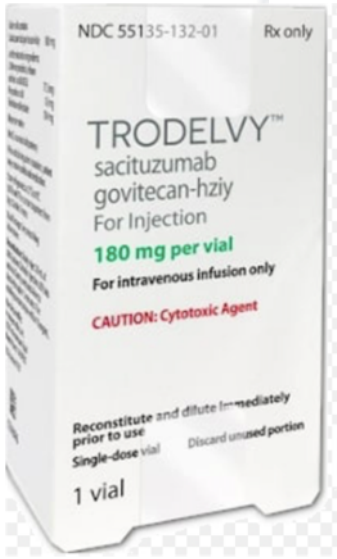
“armed” biologicals – eg. in triple negative breast cancer TNBC
(FDA fast track sacizutumab 2021 when Kadcyla fails)

7 molecules of highly toxic topoisomerase inhibitor irinotecan/hu IgG1

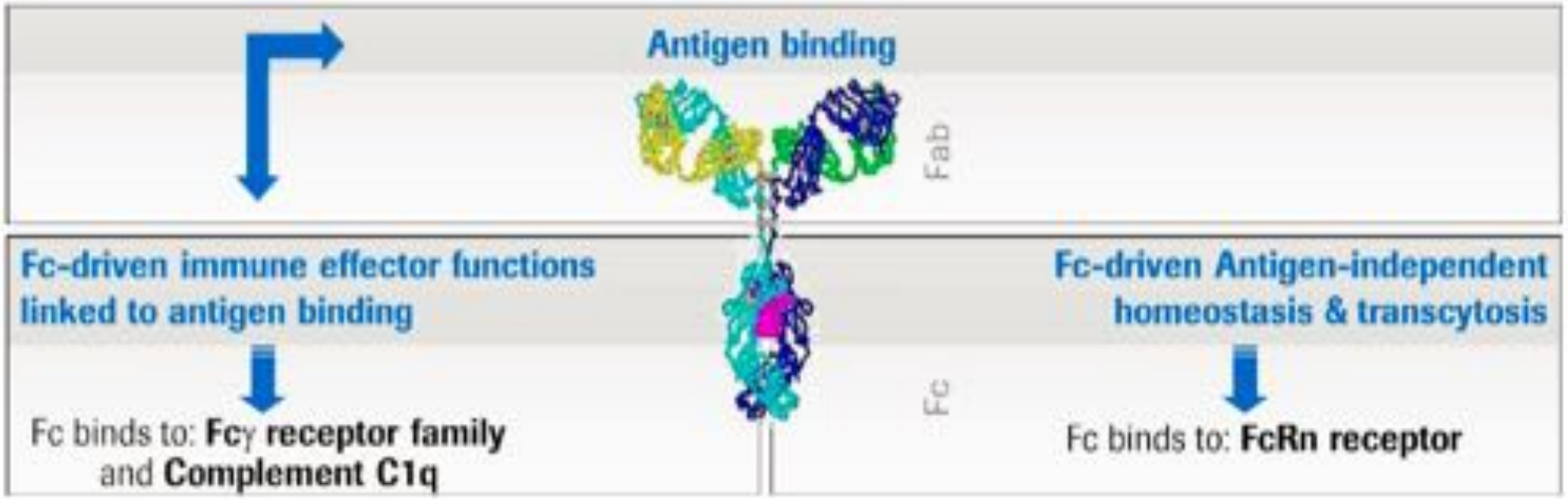




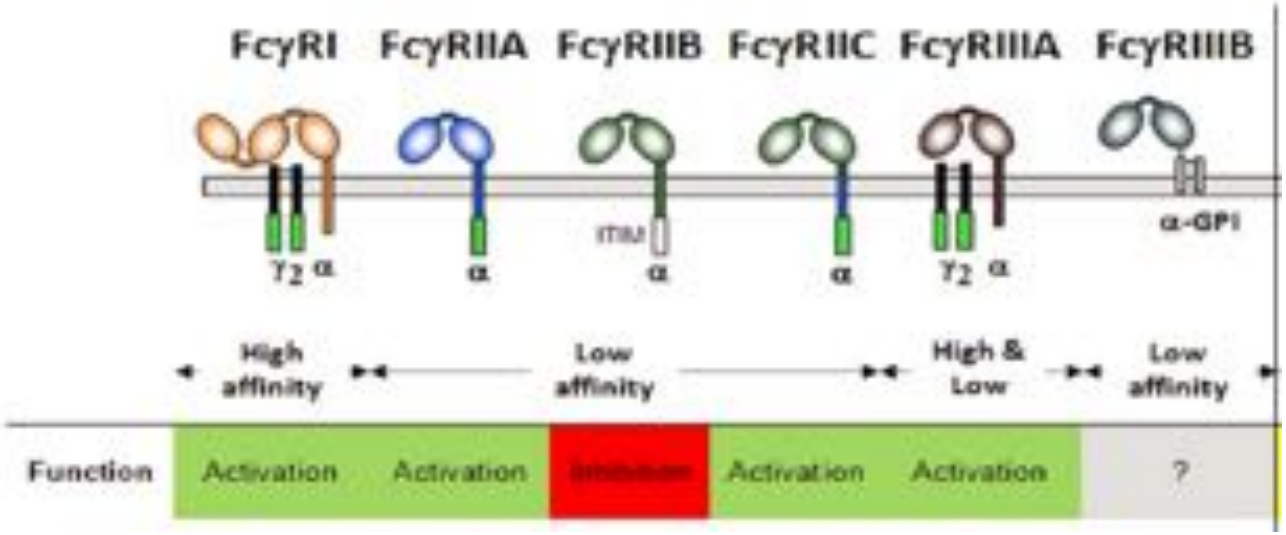
irinotecan like



Engineering IgG can be used to modulate FcR interaction

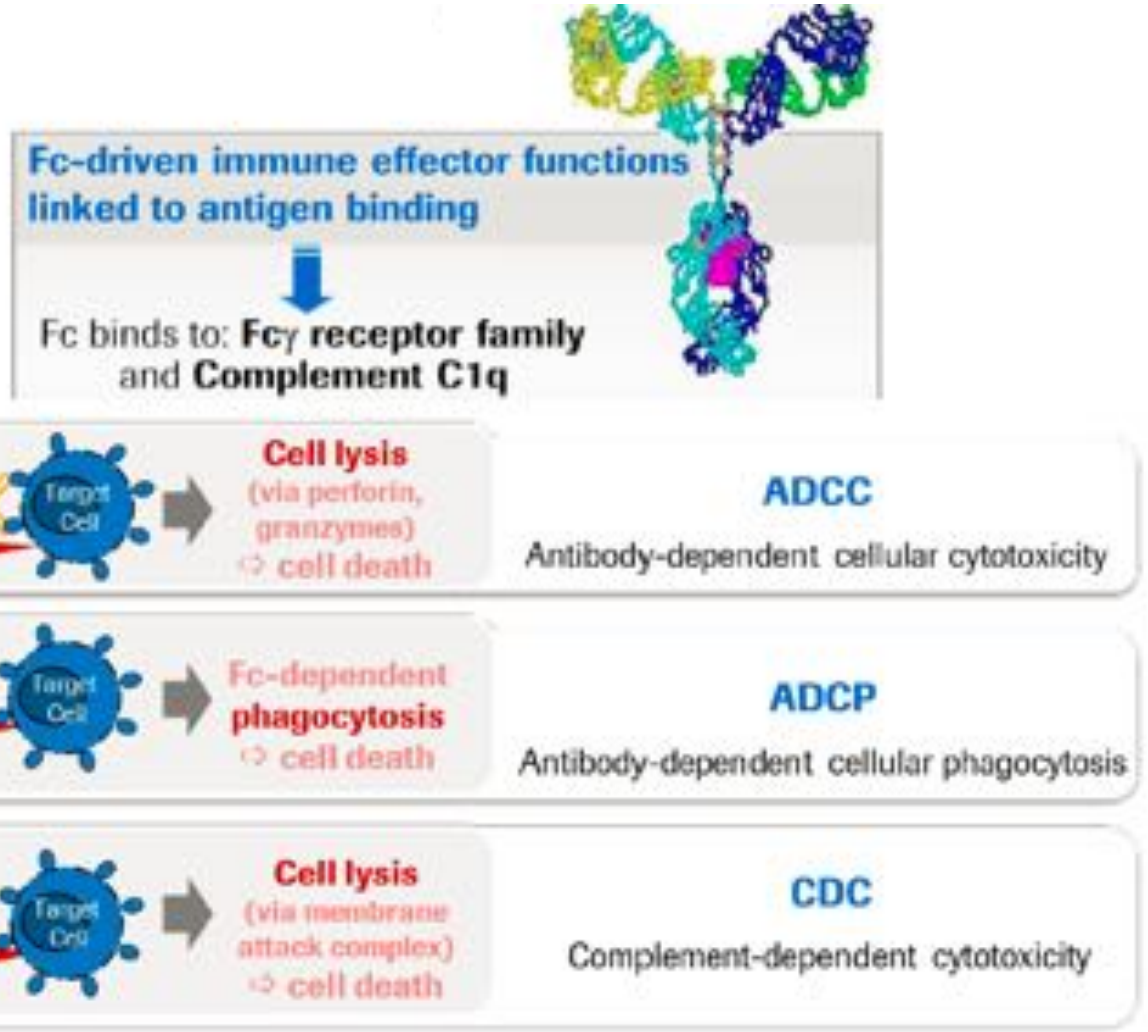


FcγR = Fc gamma-receptor family



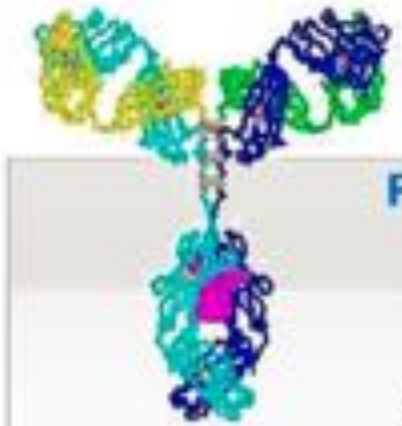
Silencing of the Fc part (mutagenesis) in situation where you do not want to eg activate NK cells

Fc receptor driven effector functions linked to antigen binding



Fc part has two complement binding sites

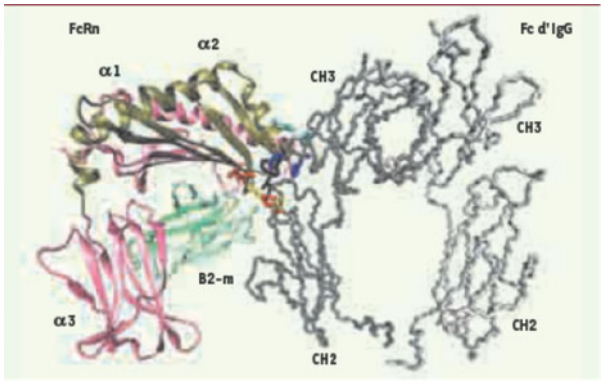
Fc receptor driven effector functions independent to antigen binding



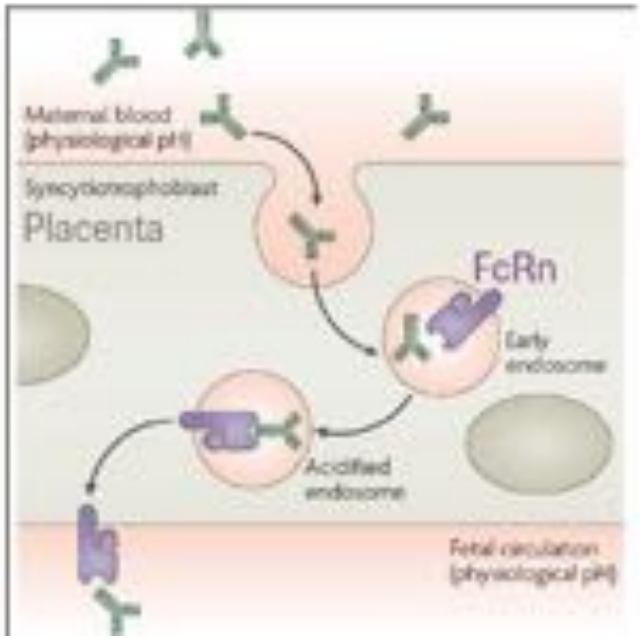
contribute to host defense : long half life of IgG

Fc-driven Antigen-independent homeostasis & transcytosis

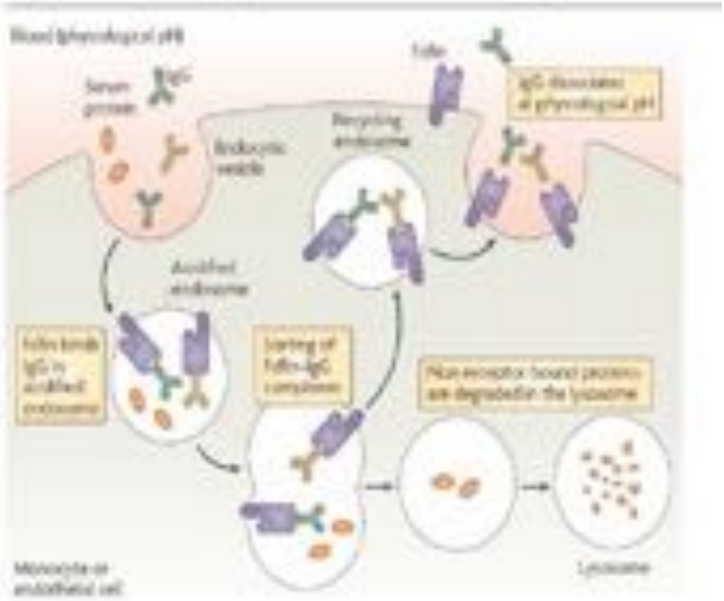
Fc binds to: **FcRn receptor**



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

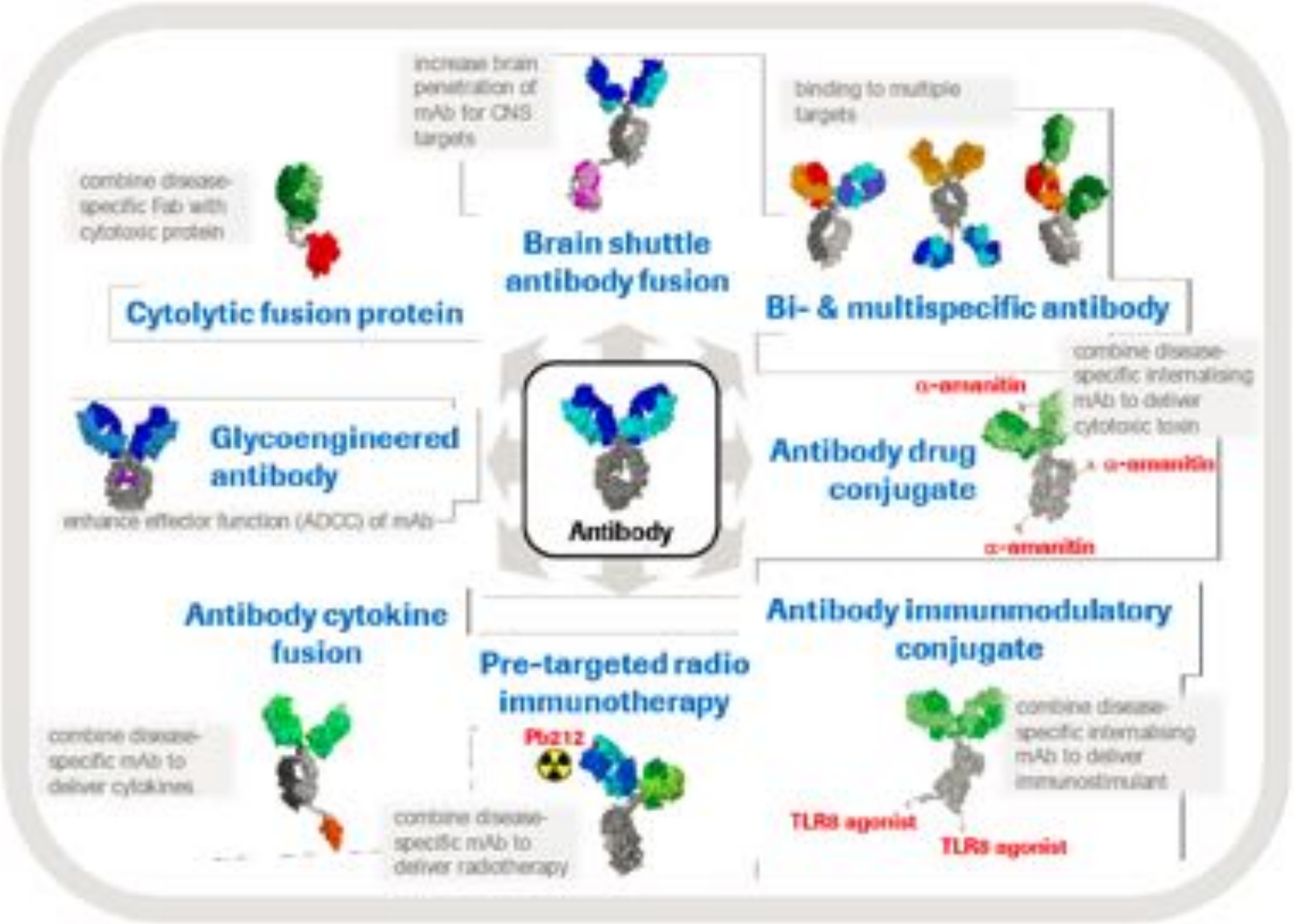


- **FcRn prevents lysosomal catabolism** of IgG by recycling via endosomal pathway (confers long plasma half life of IgG)



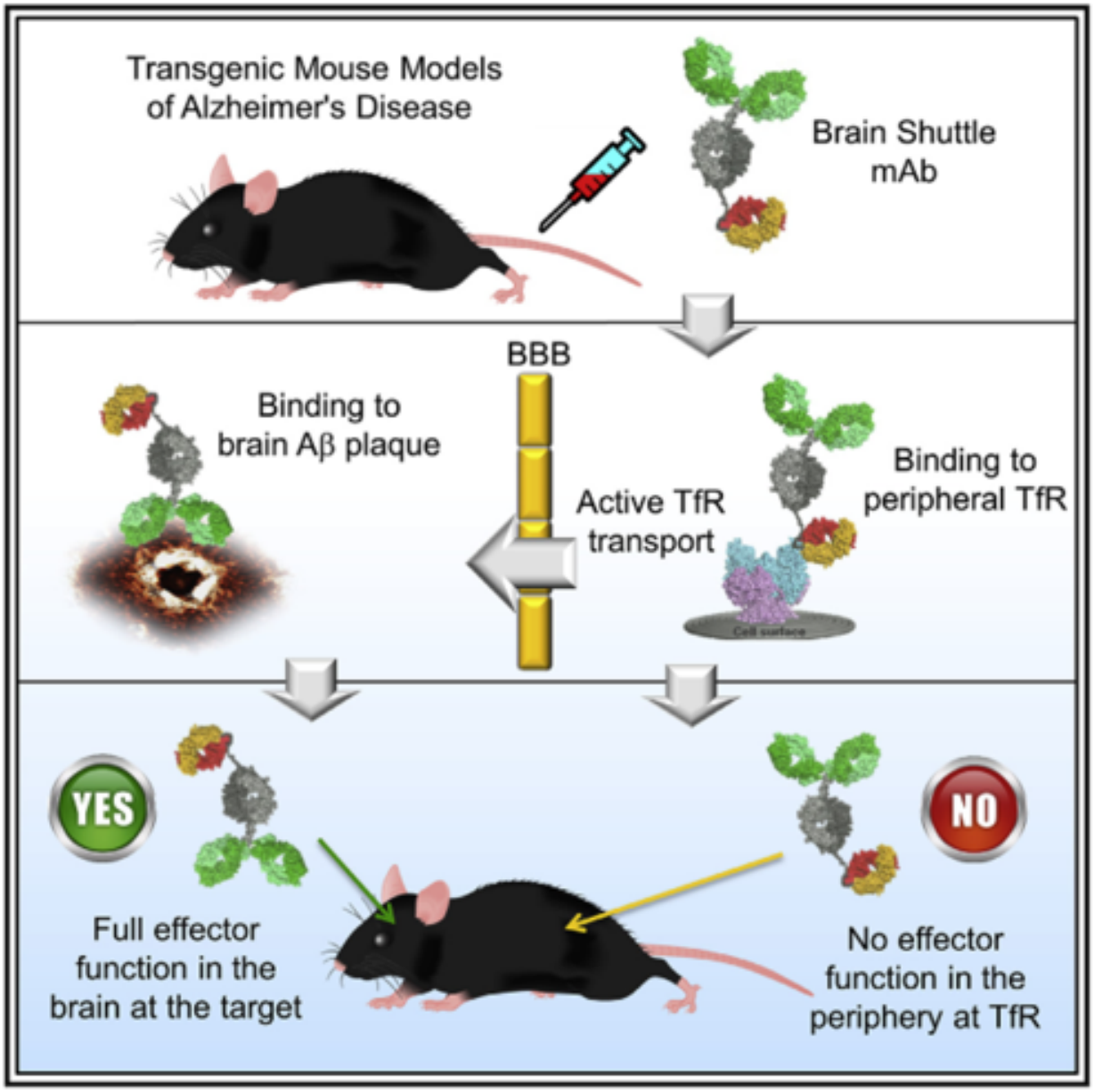
Fc receptor driven effector functions independent to antigen binding

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

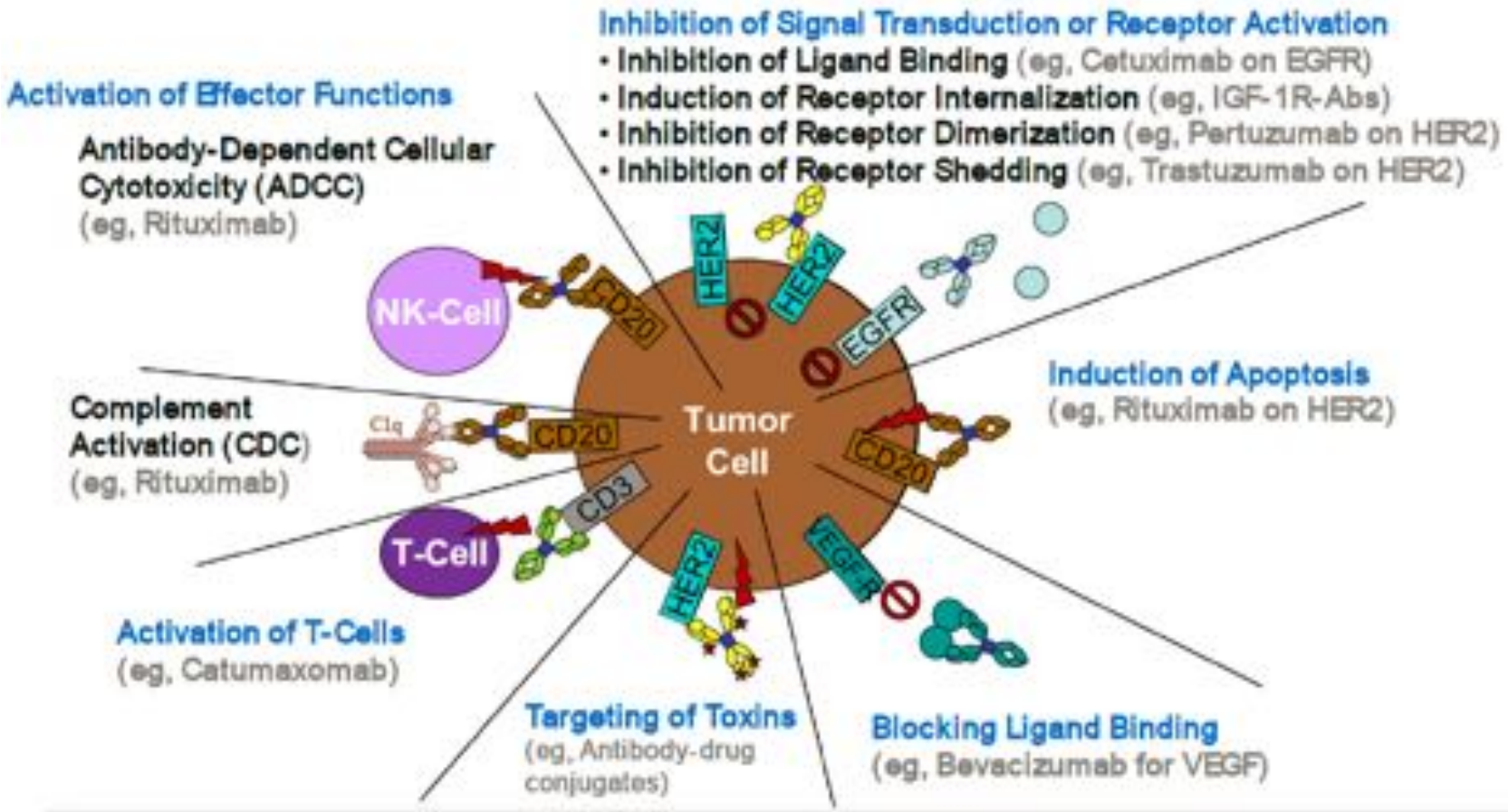


No first infusion Fc portion dependent adverse reaction in periphery, yet BBB transport via Ferritin receptor and clearing of brain Aβ plaques in mouse model by active Fc dependent effector function (eg cellular cytotoxicity)

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

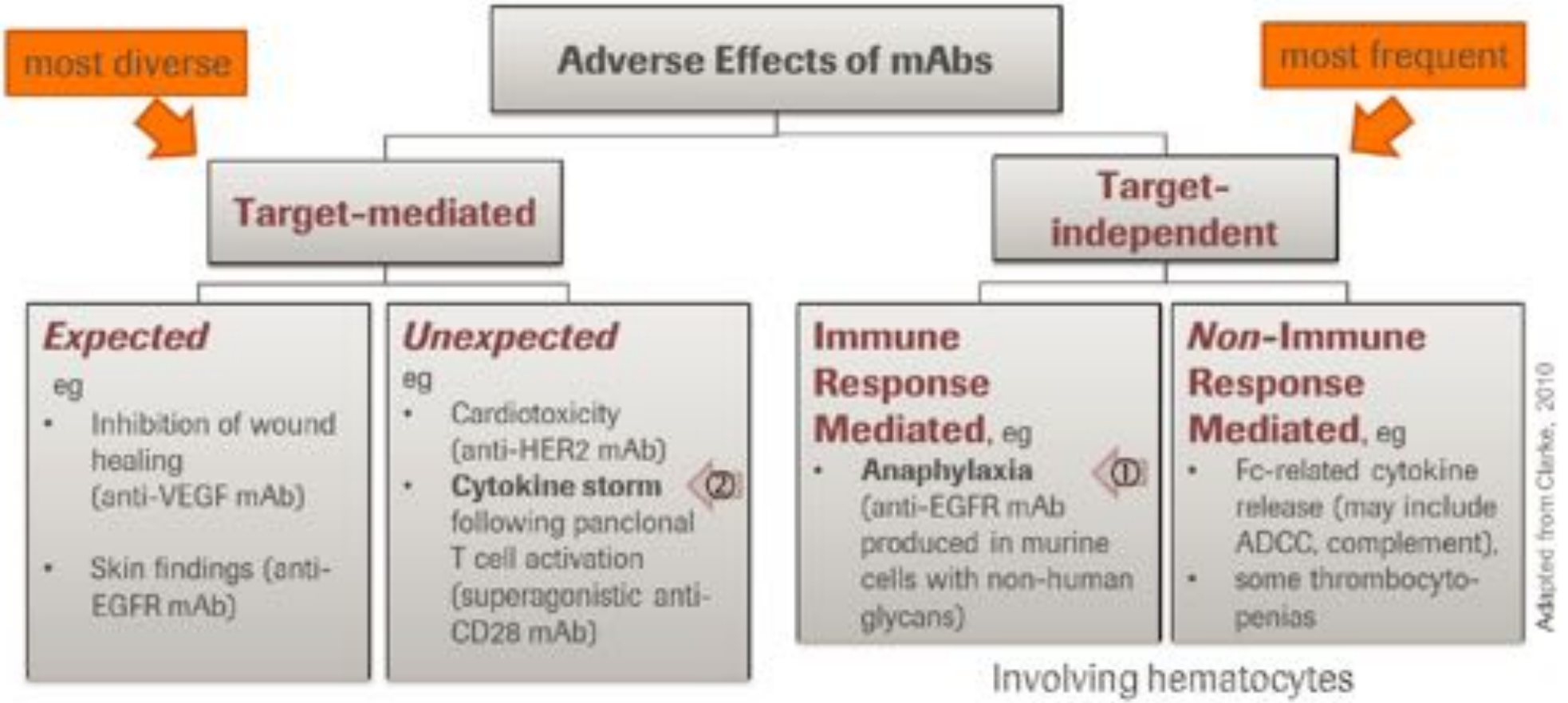
mABs in drug development

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

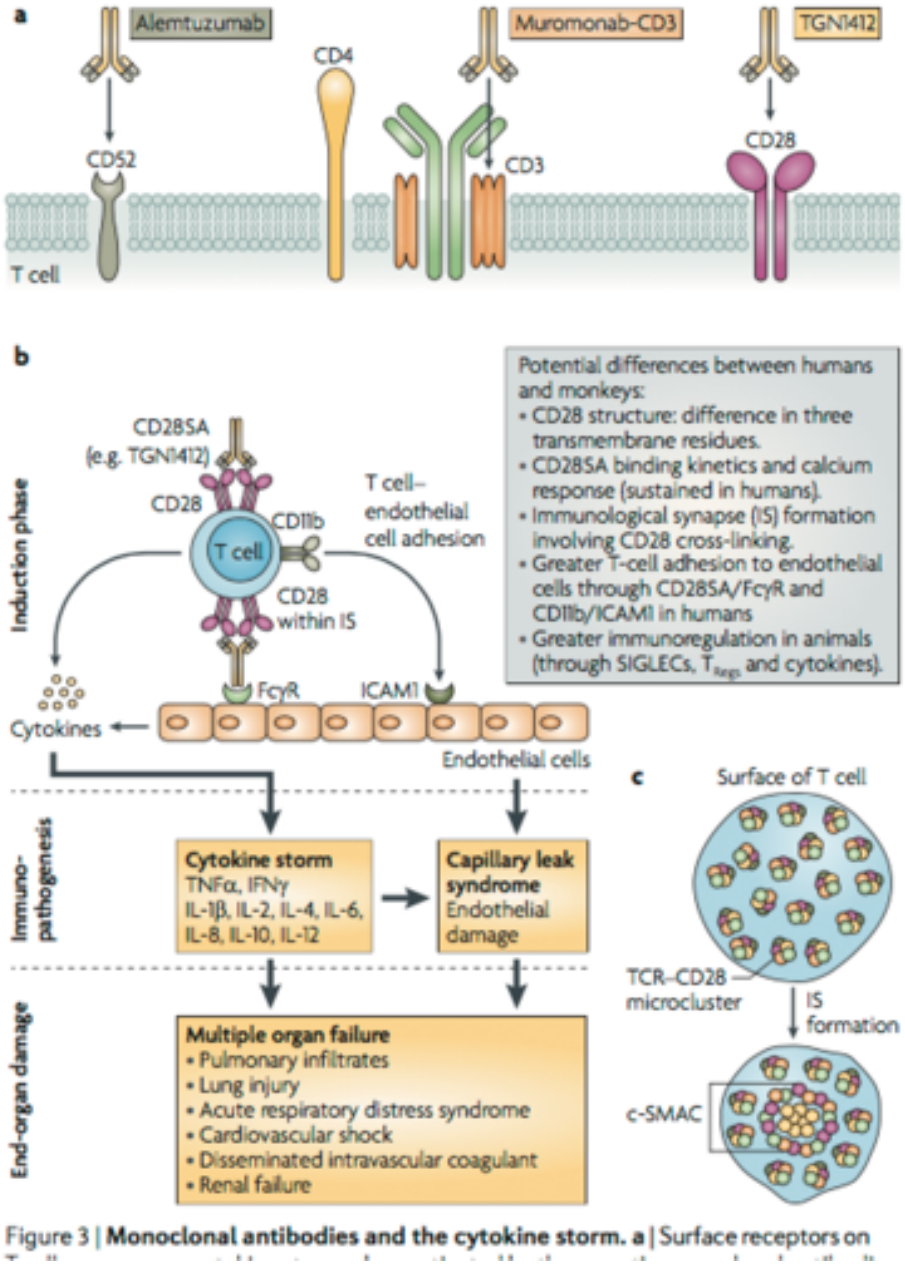
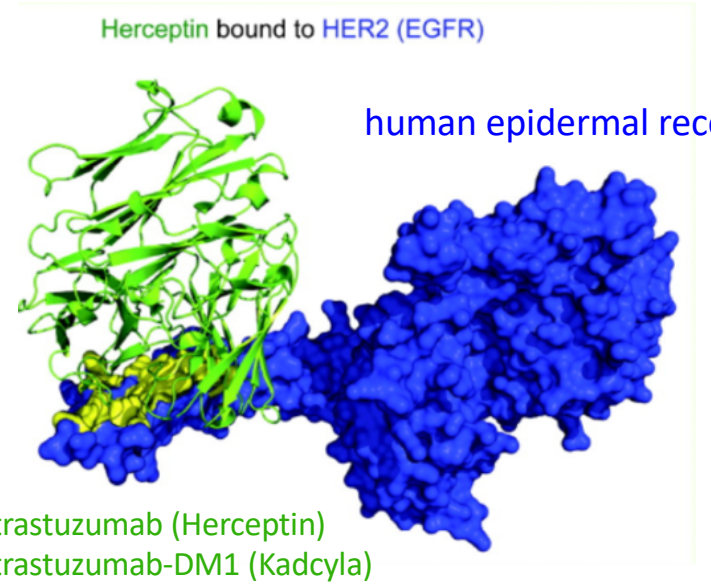


Figure 3 | **Monoclonal antibodies and the cytokine storm. a** | Surface receptors on

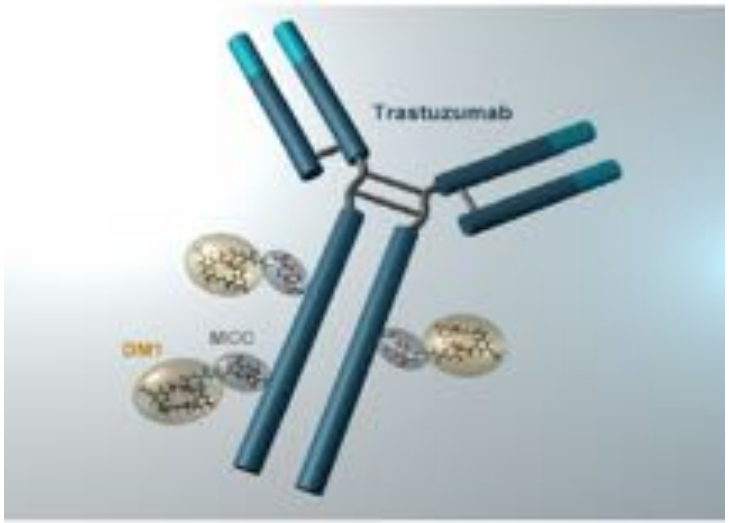
Mechanisms of adverse effects with mABs

- **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)



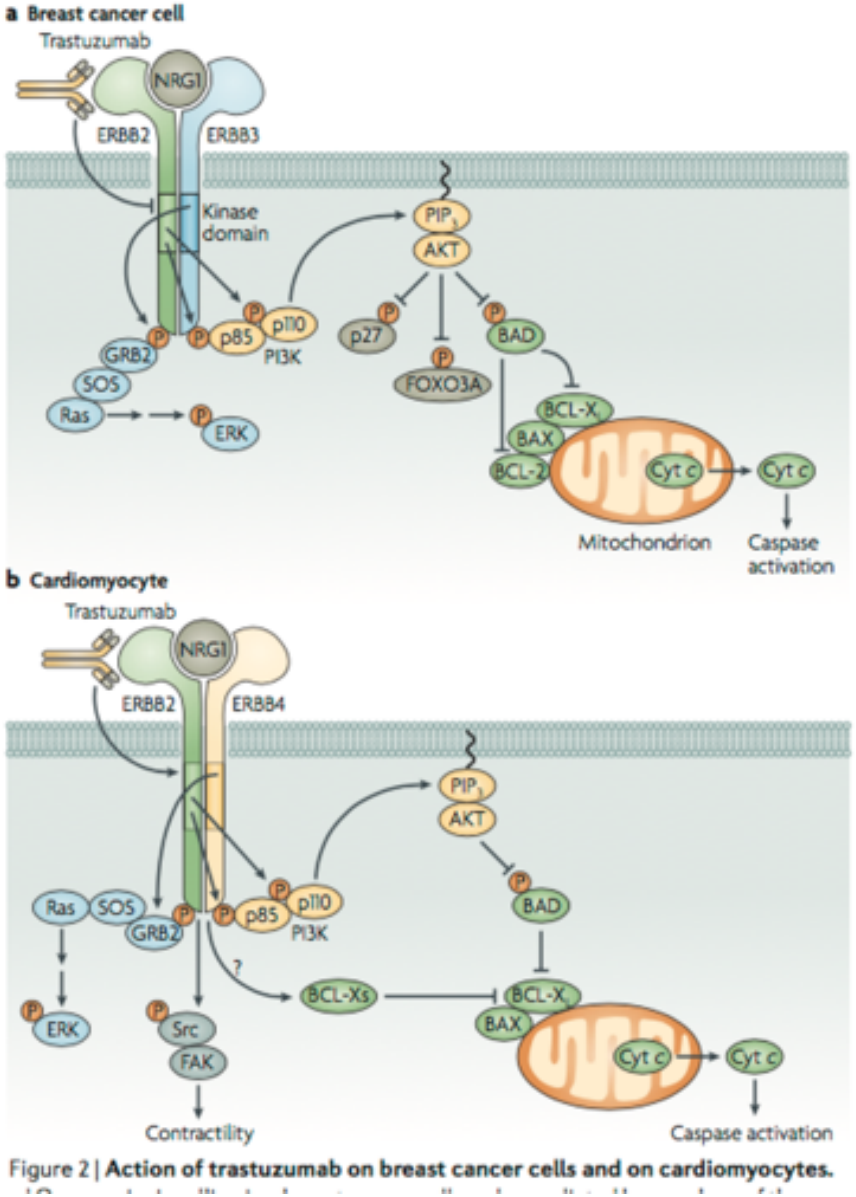


Figure 2 | **Action of trastuzumab on breast cancer cells and on cardiomyocytes.**

MABs are subjected to intense scrutiny in drug safety and development

MOLECULAR BIOLOGY COMPLEX INCLUDING PATIENT HETEROGENEITY SAFETY HURDLE !

Clinical signs of cytokine storm

- Early onset after 1st infusion with a mAb
- Signs and symptoms
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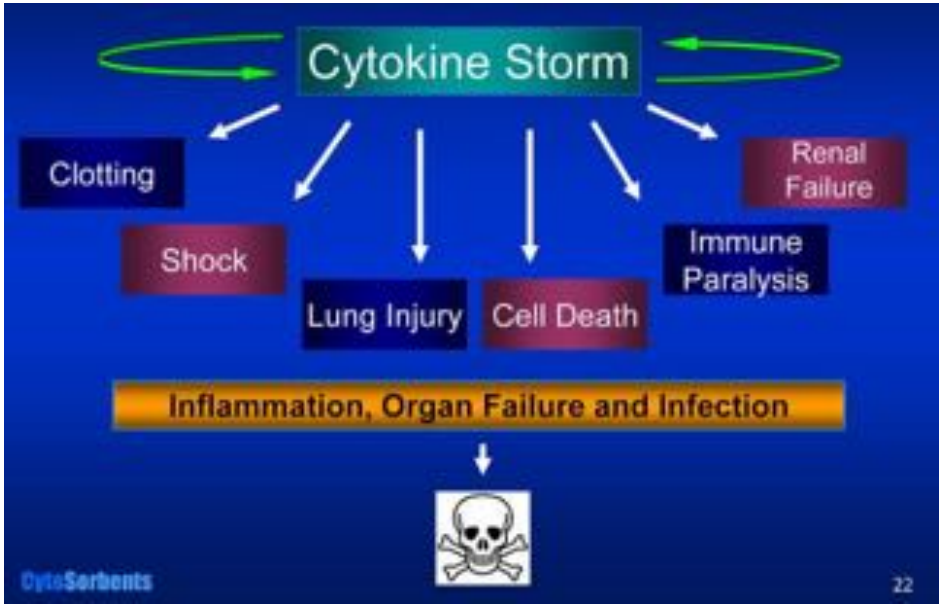
- Clinical signs: Severe symptoms, tissue damage, multiorgan failure
- Retrospectively, the mAb was shown to be a superagonist to CD28 with activation of all CD28⁺ immune cells



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

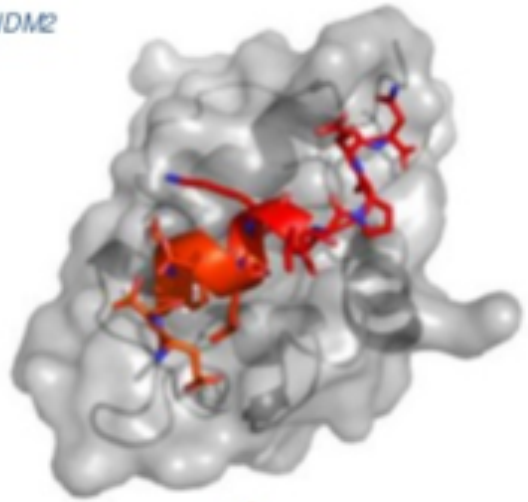
Moellering RE et al. 2002 Nature 462:182-188

„ Drugging the undruggable therapeutic targets“

Fragment Linking – Protein-Protein Interactions

Why protein-protein interactions as targets?

p53-HDM2

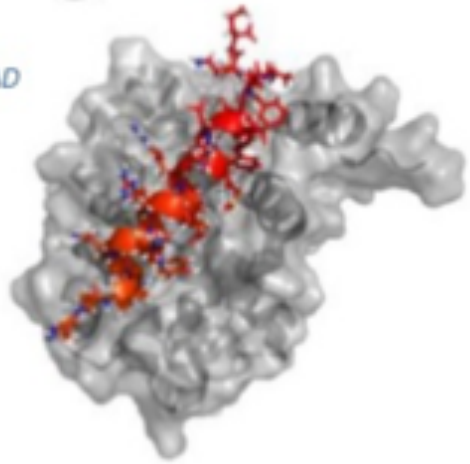


Protein-Protein interactions (PPI's) are found throughout biological systems. Typically these are defined as ***difficult targets*** *as success rates in targeting these has been low especially using HTS approaches.*

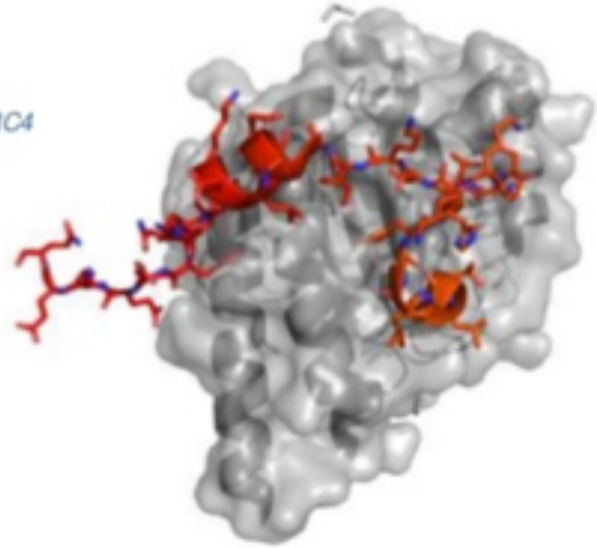
Unlike conventional targets they do not have distinct binding pockets however they have what is known as 'hot-spots' typically on the surface of the protein

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

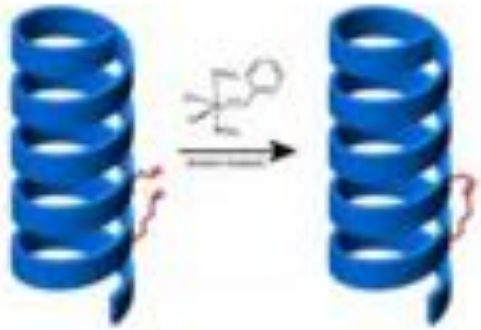


Drugging undruggable targets – peptide stapling

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).



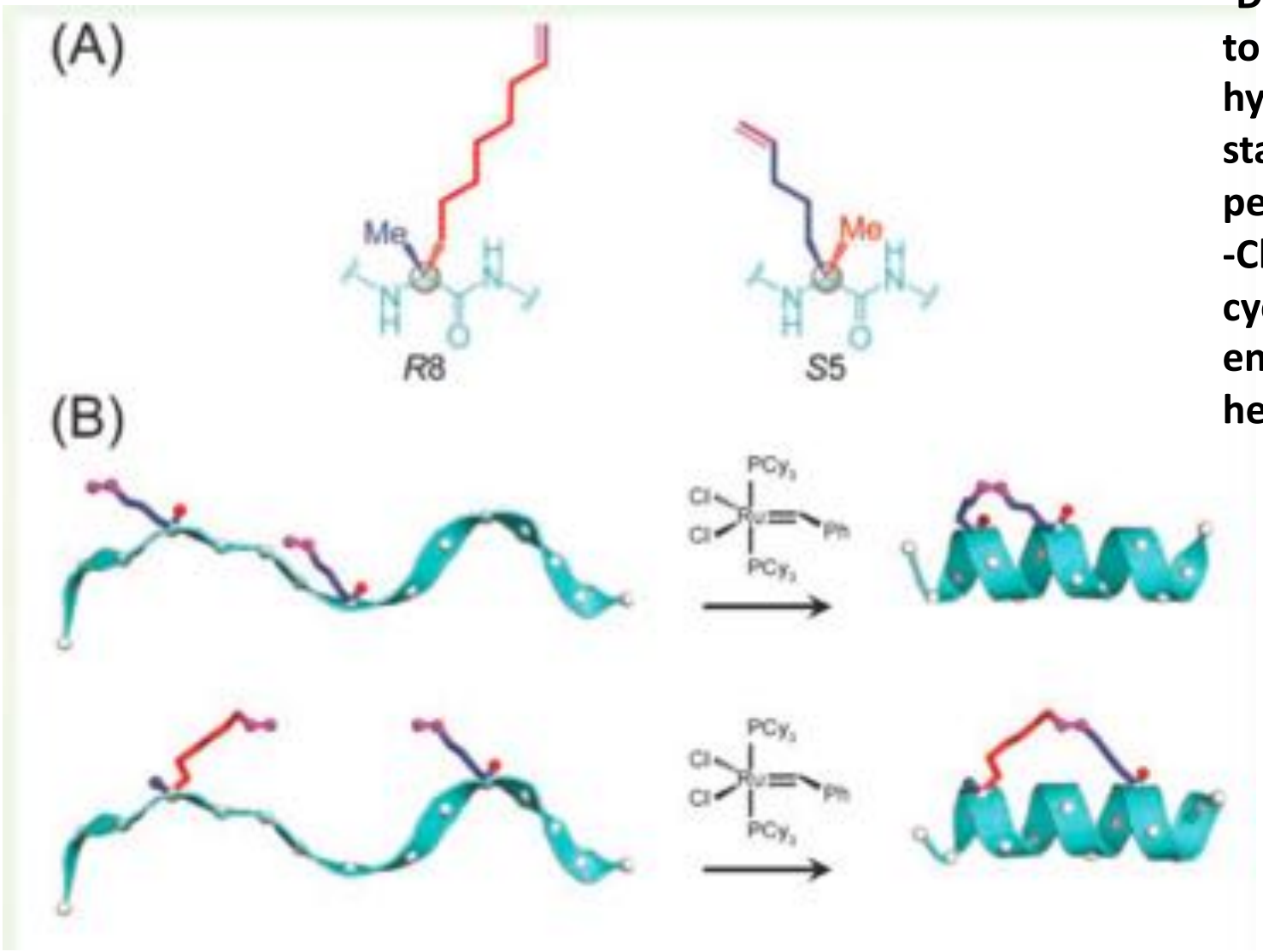
AAPPTec supplies a wide selection of high purity Fmoc-alkenyl alanines used to prepare stapled peptides through a ring-closing metathesis reaction. **AAPPTec's products are affordably priced and are also available in bulk quantities for additional savings. Please send an e-mail to sales@aapptec.com for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

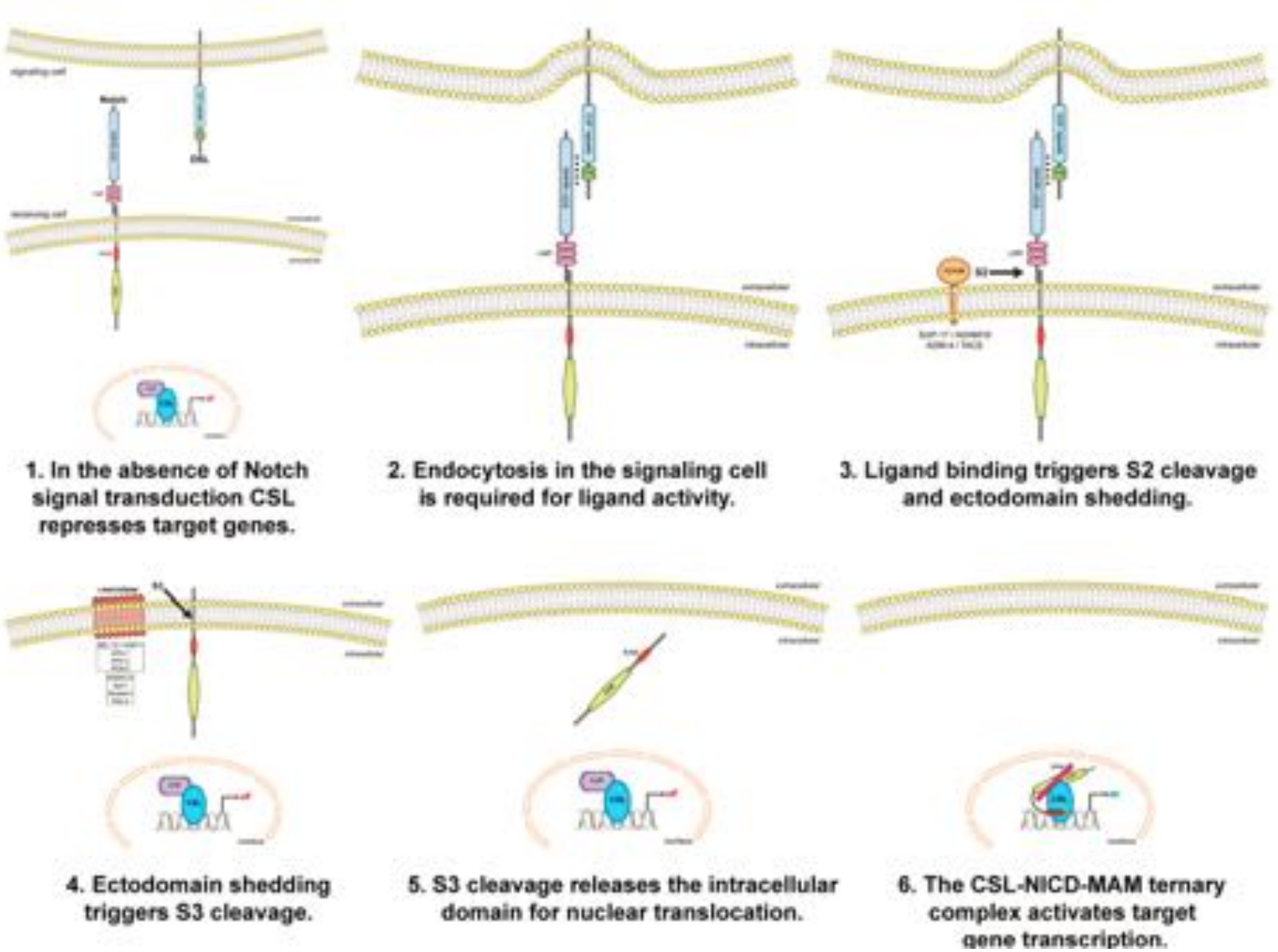
Raymond E. Moellering[1,2,3], Melanie Cornejo[4], Tina N. Davis[6], Cristina Del Bianco[5], Jon C. Aster[5], Stephen C. Blacklow[5], Andrew L. Kung[6], D. Gary Gilliland[4,7], Gregory L. Verdine[1,3] & James E. Bradner[2,3,8]

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

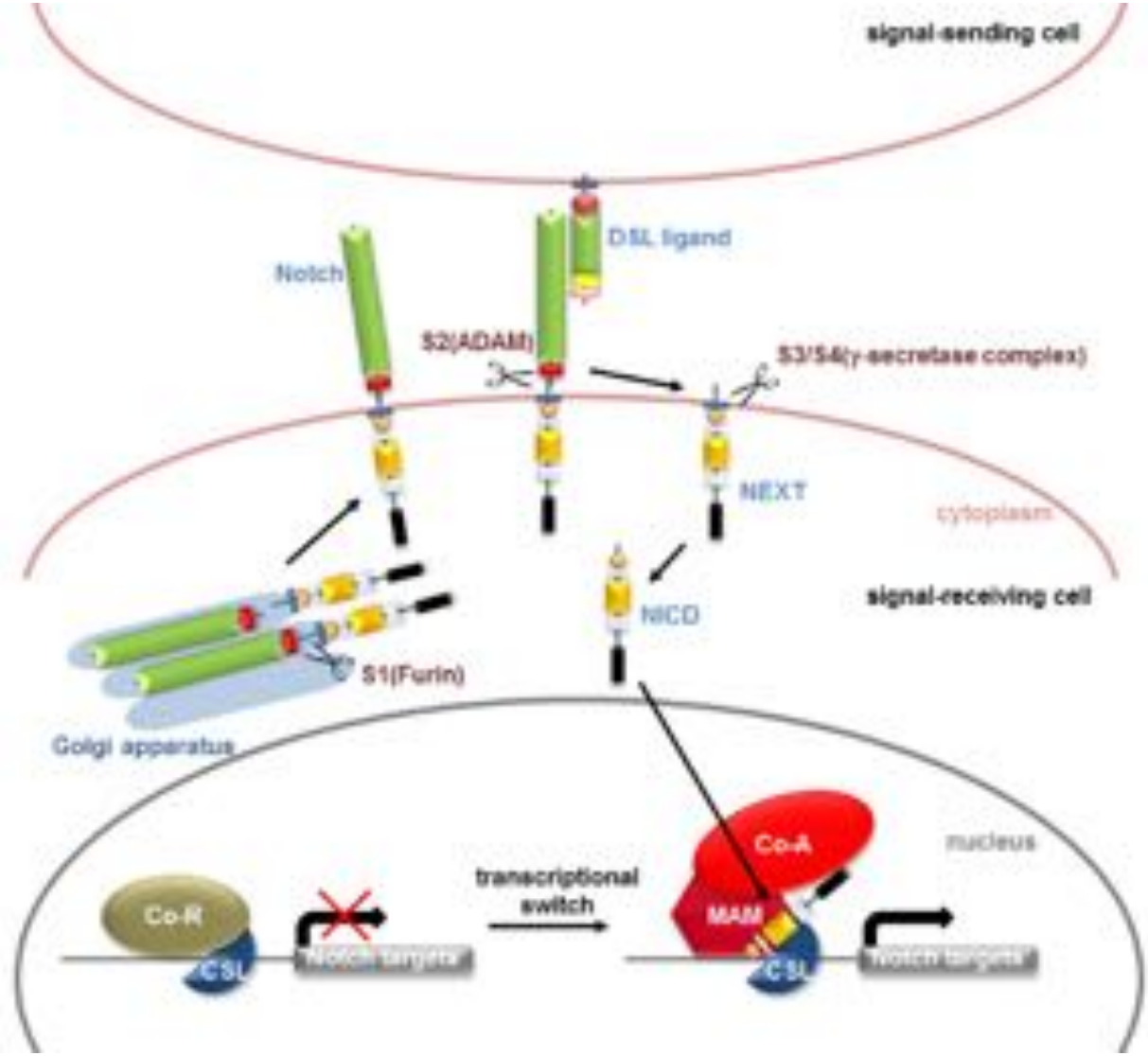
Moellering RE et al. 2002 Nature 462:182-188

NOTCH cellular signal pathway : cell to cell communication



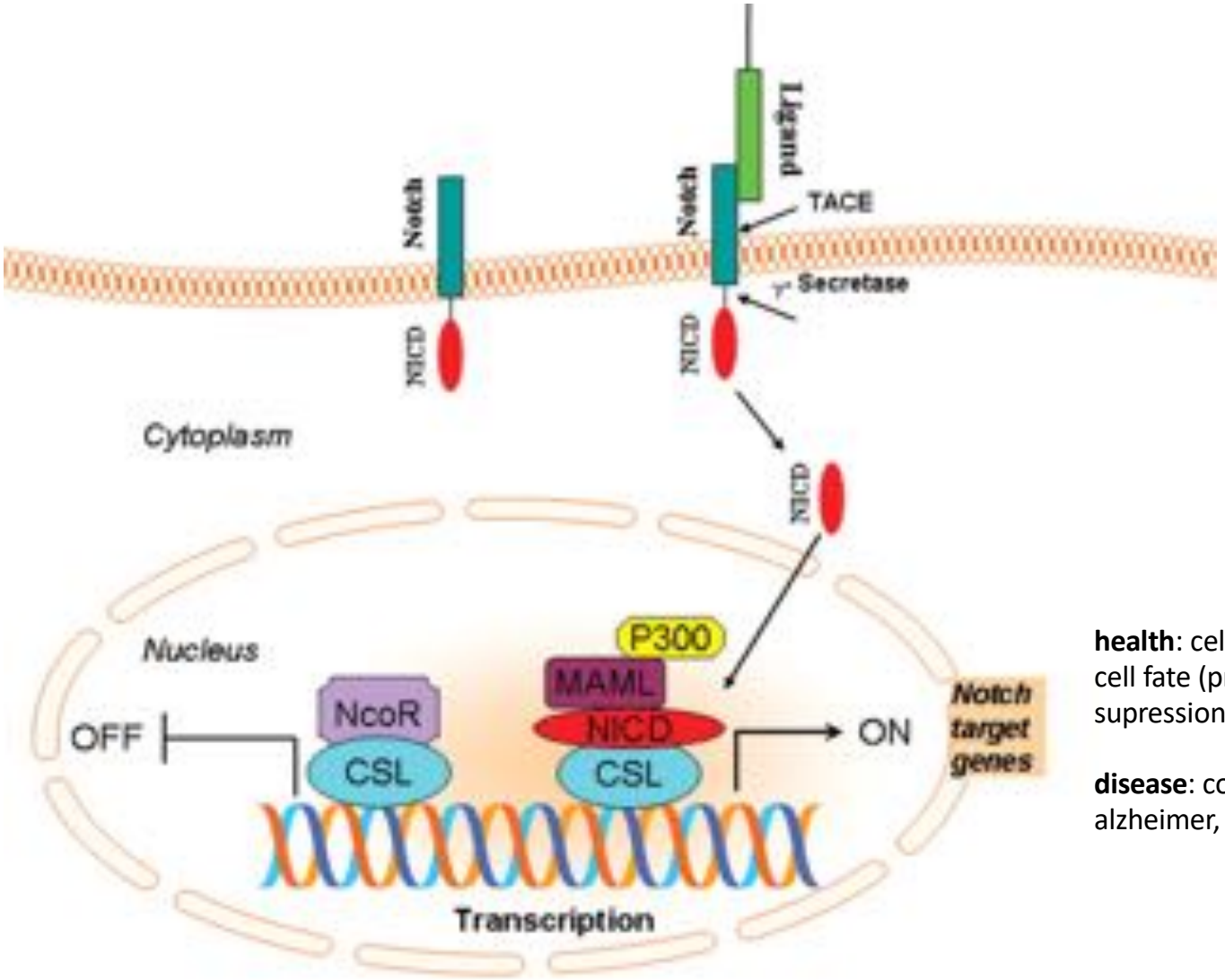
Greenwald I et al 2012 Wormbook.com

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



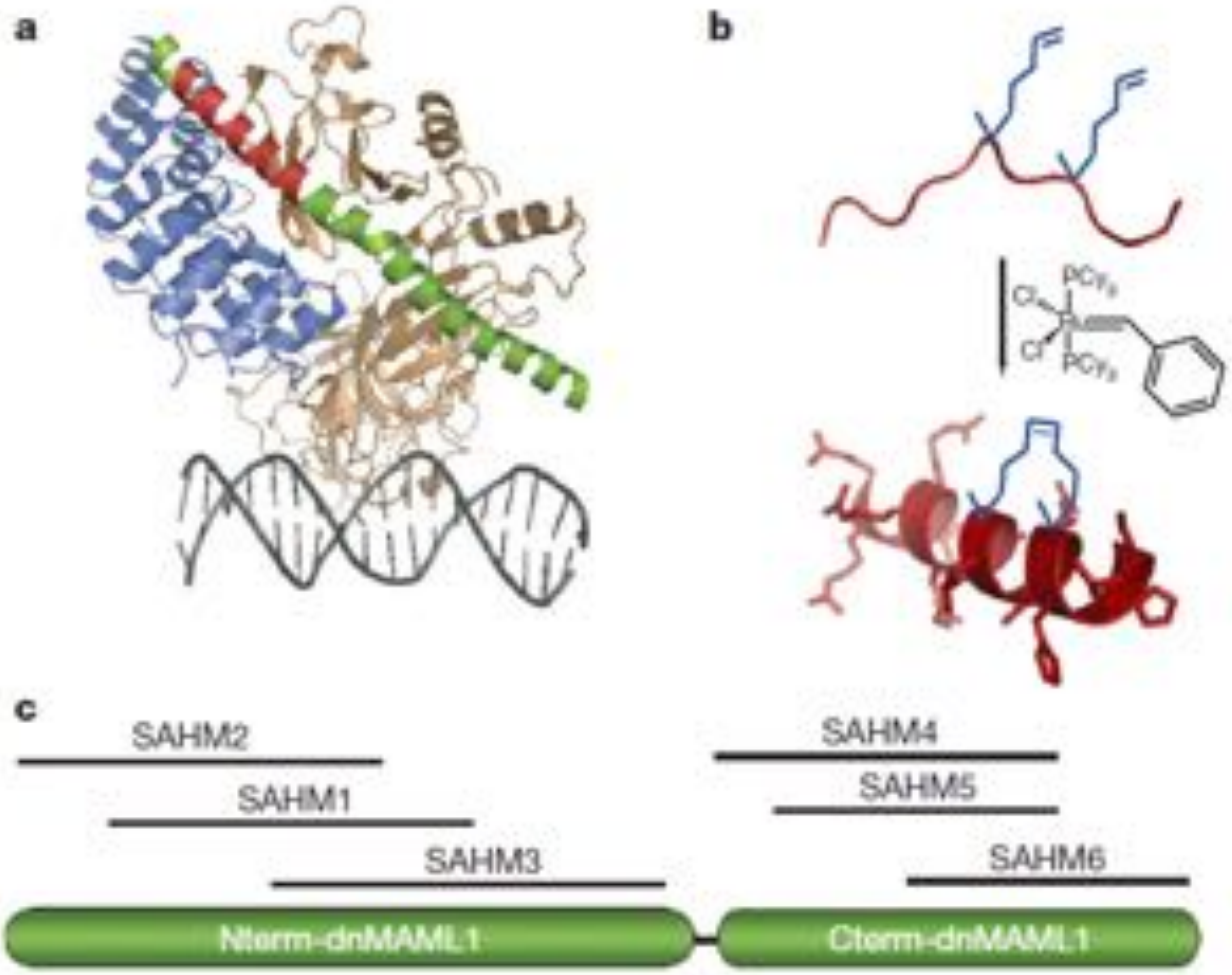
health: cell-cell com, cell fate (promotion supression of proliferation

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

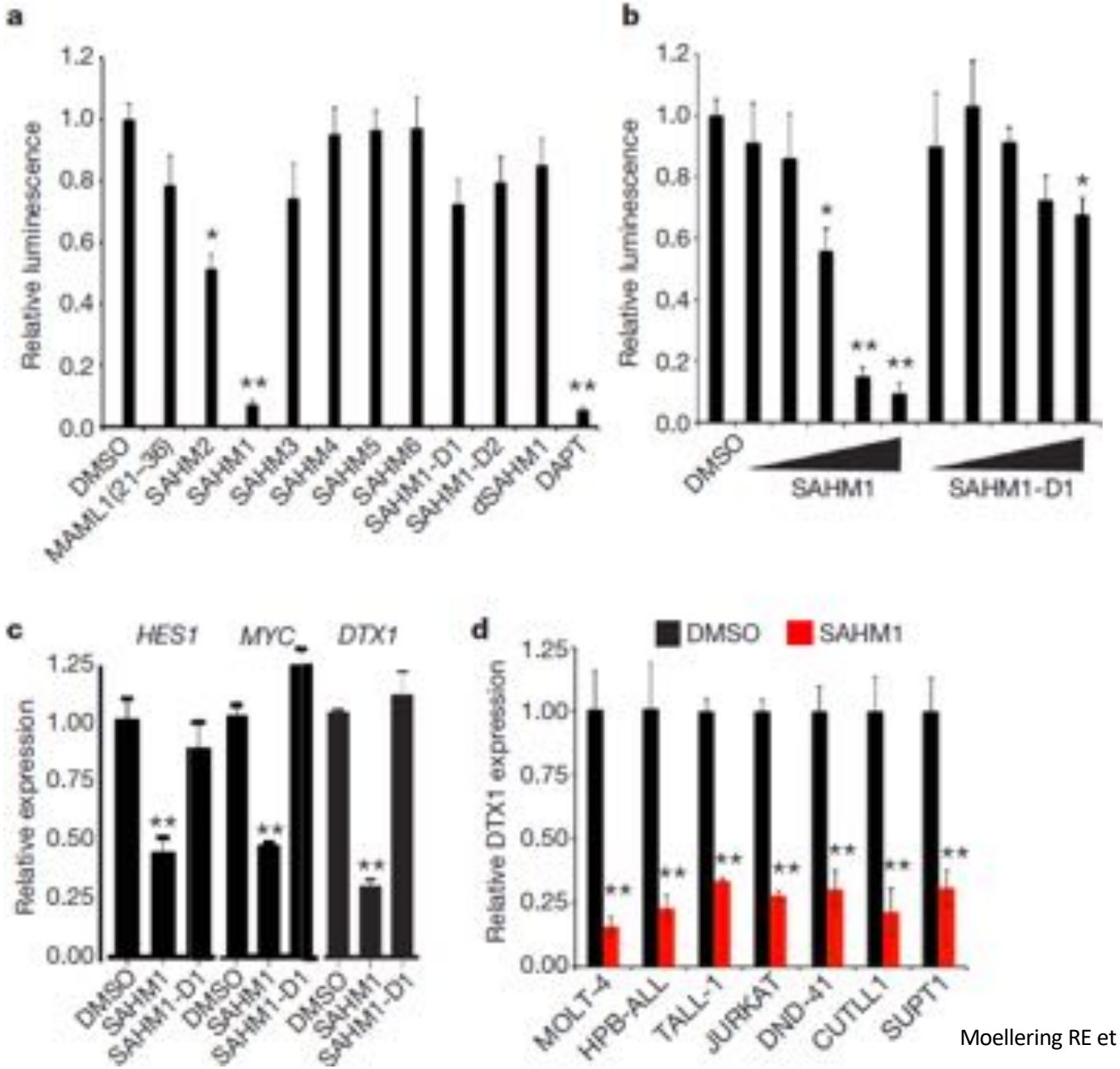
NATURE|Vol 462|12 November 2009



Compound	Sequence	Helicity
MAML1(21-36)	βAla-ERLRRRIELCRRHHST	23%
SAHM1-DN	βAla-ERLRRRI•LCR•HHST	40%
SAHM1	βAla-ERLRRRI•LCR•HEST	94%
SAHM2	βAla-SAYMERL•RRI•LCRRHH	73%
SAHM3	βAla-RIELCRRHH•TCE•RYEAV	59%
SAHM4	βAla-ERLELER•HTF•LHQR	67%
SAHM5	βAla-ELER•HTF•LHQR	72%
SAHM6	βAla-QHTFALH•RCI•AKAKR	56%
SAHM1-D1	βAla-RELRRRI•LCR•HHST	83%
SAHM1-D2	βAla-RELRRRI•LCE•HHST	78%
dSAHM1	βAla-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

Nucleic acid based medicines: a new therapeutic modality

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:

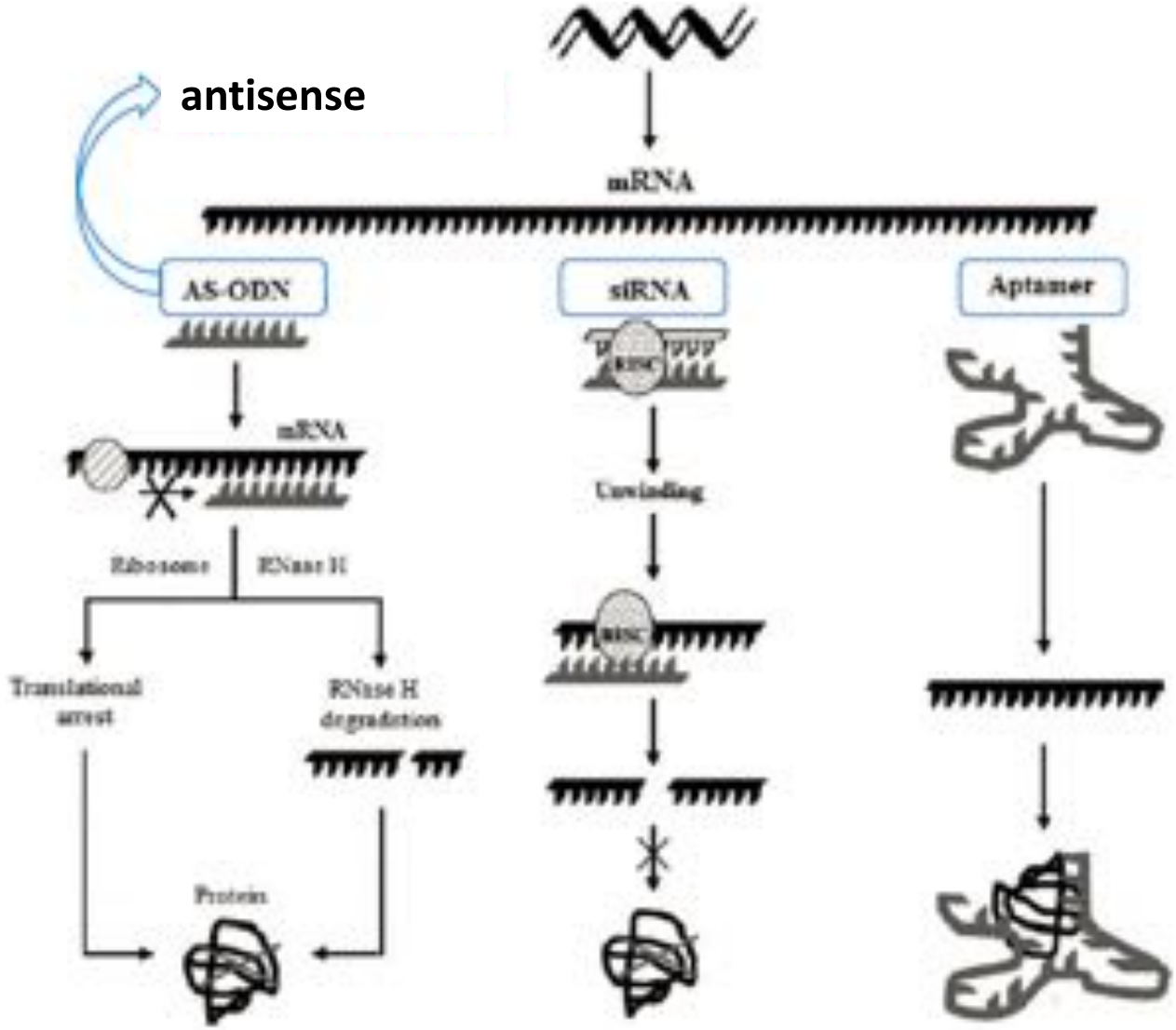
Human genome: 3 Billion Bases

- **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

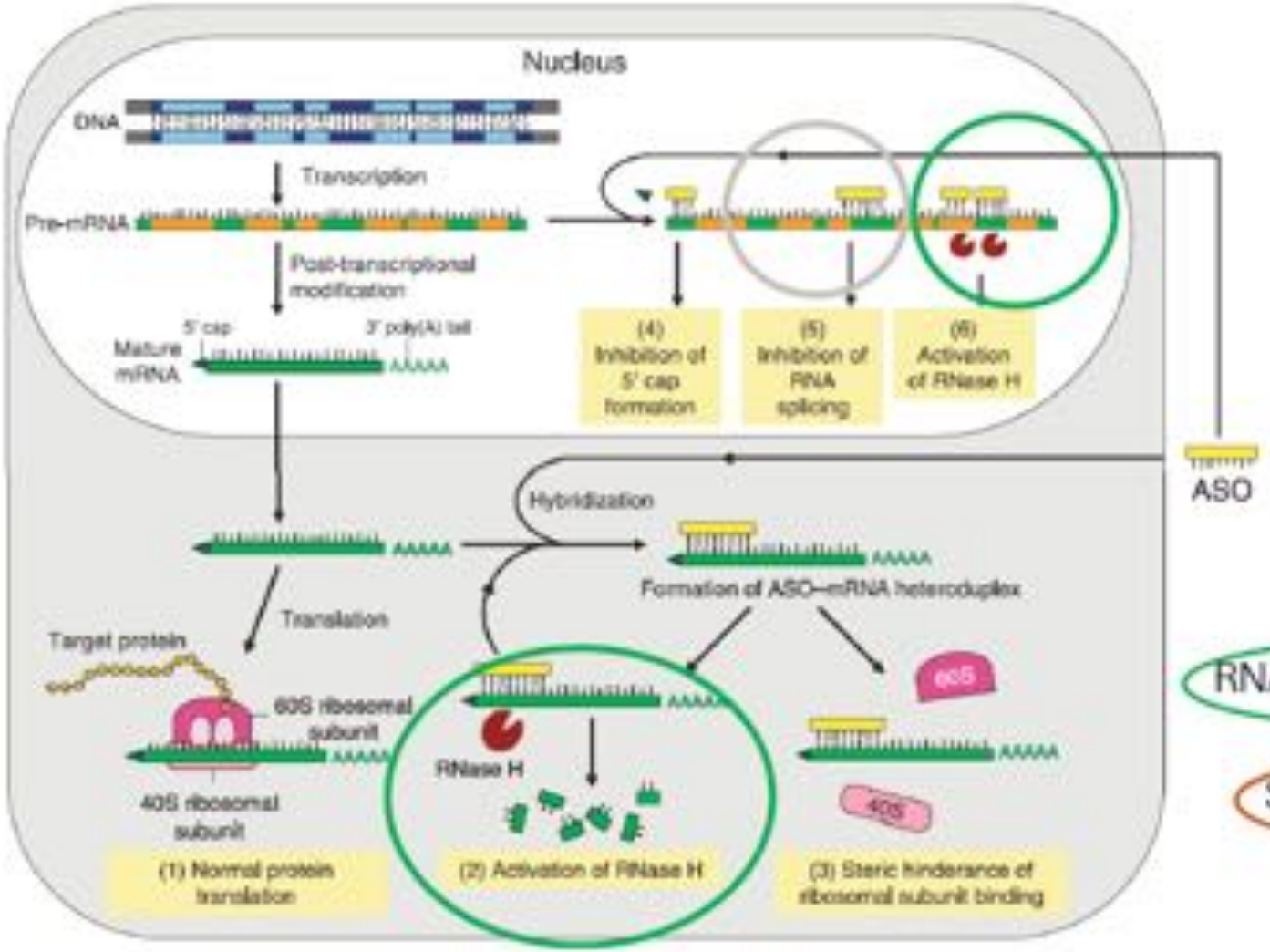


Basic concept:
Instead of binding to a mature protein and thereby alter its function

- **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:

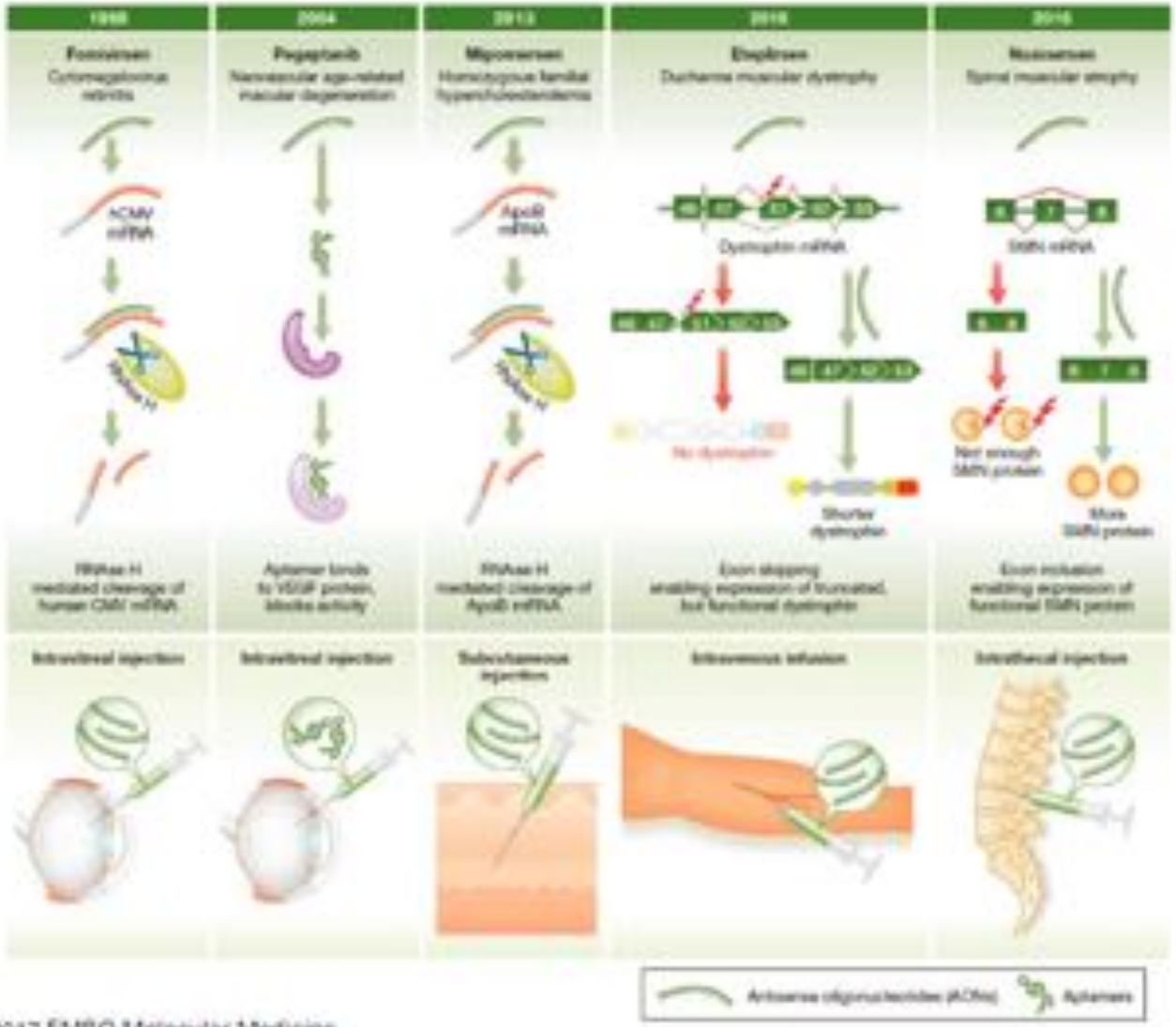
  'productive' pathway
- Exact mechanisms of escape poorly understood

RNAseH degradation

Splice switching

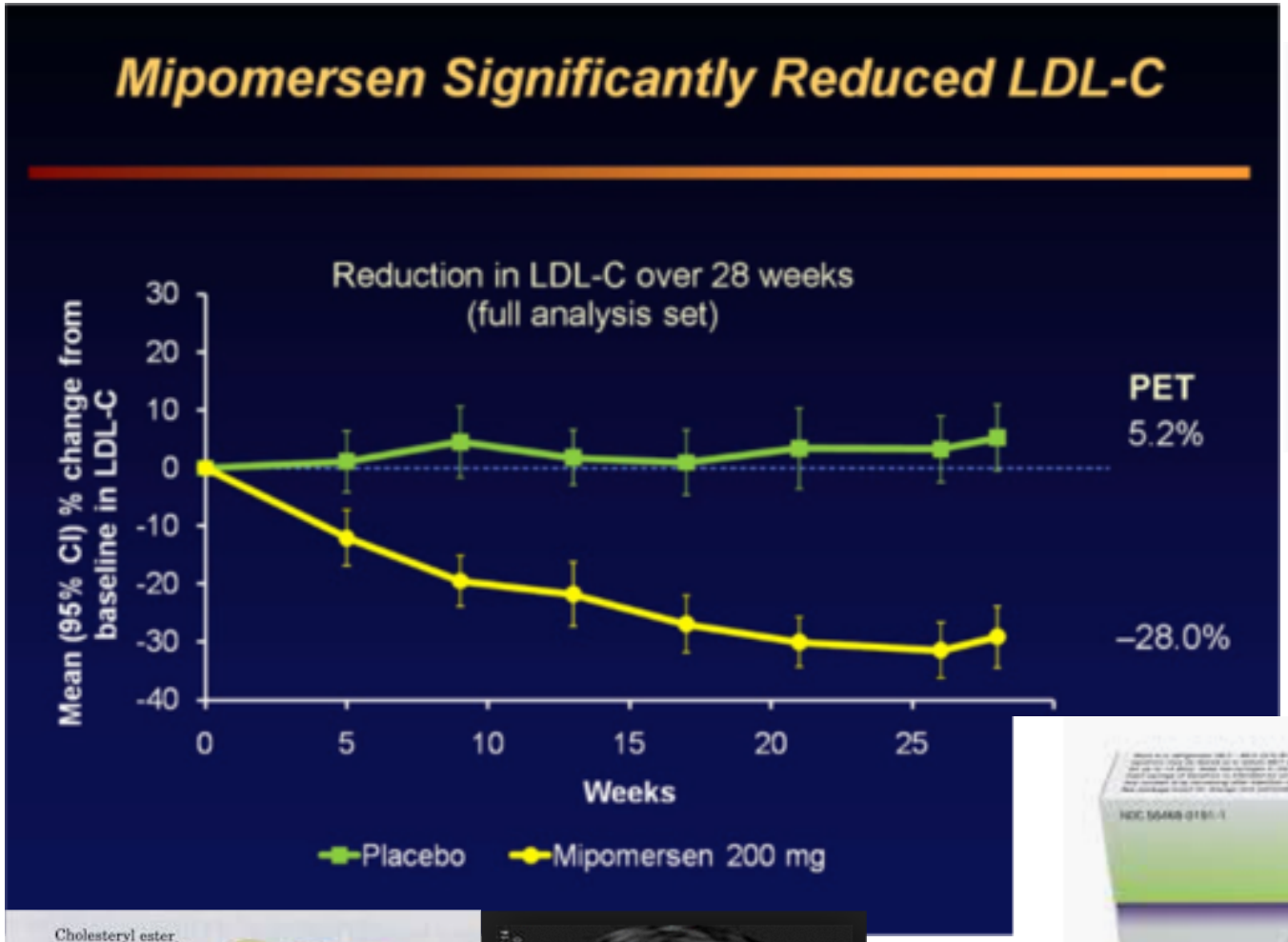
Antisense DNA oligonucleotides in the clinical setting

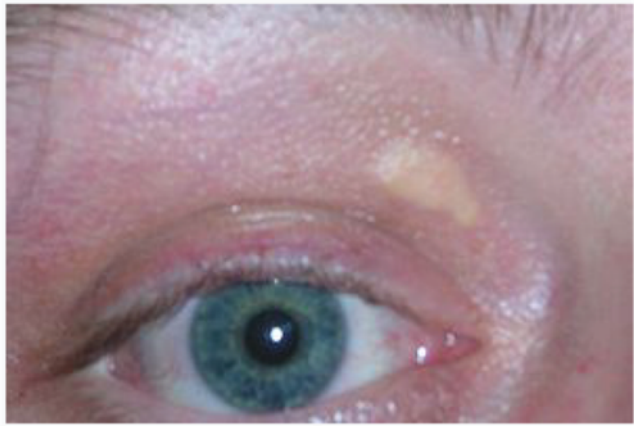
FDA Approved drugs:



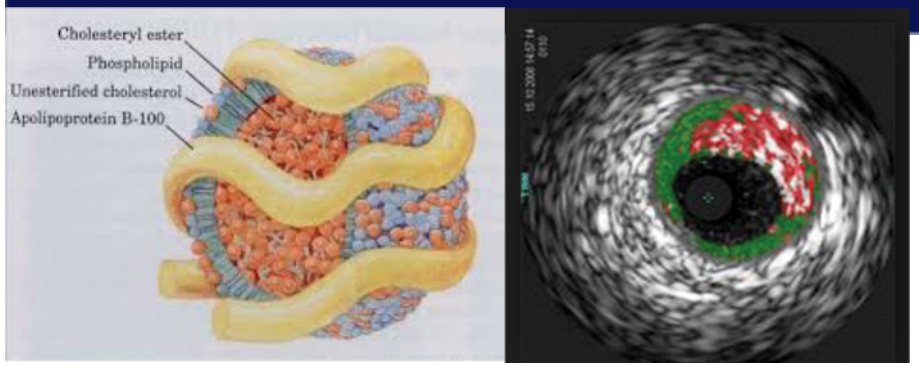
C. Godfrey et al. 2017 EMBO Molecular Medicine

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

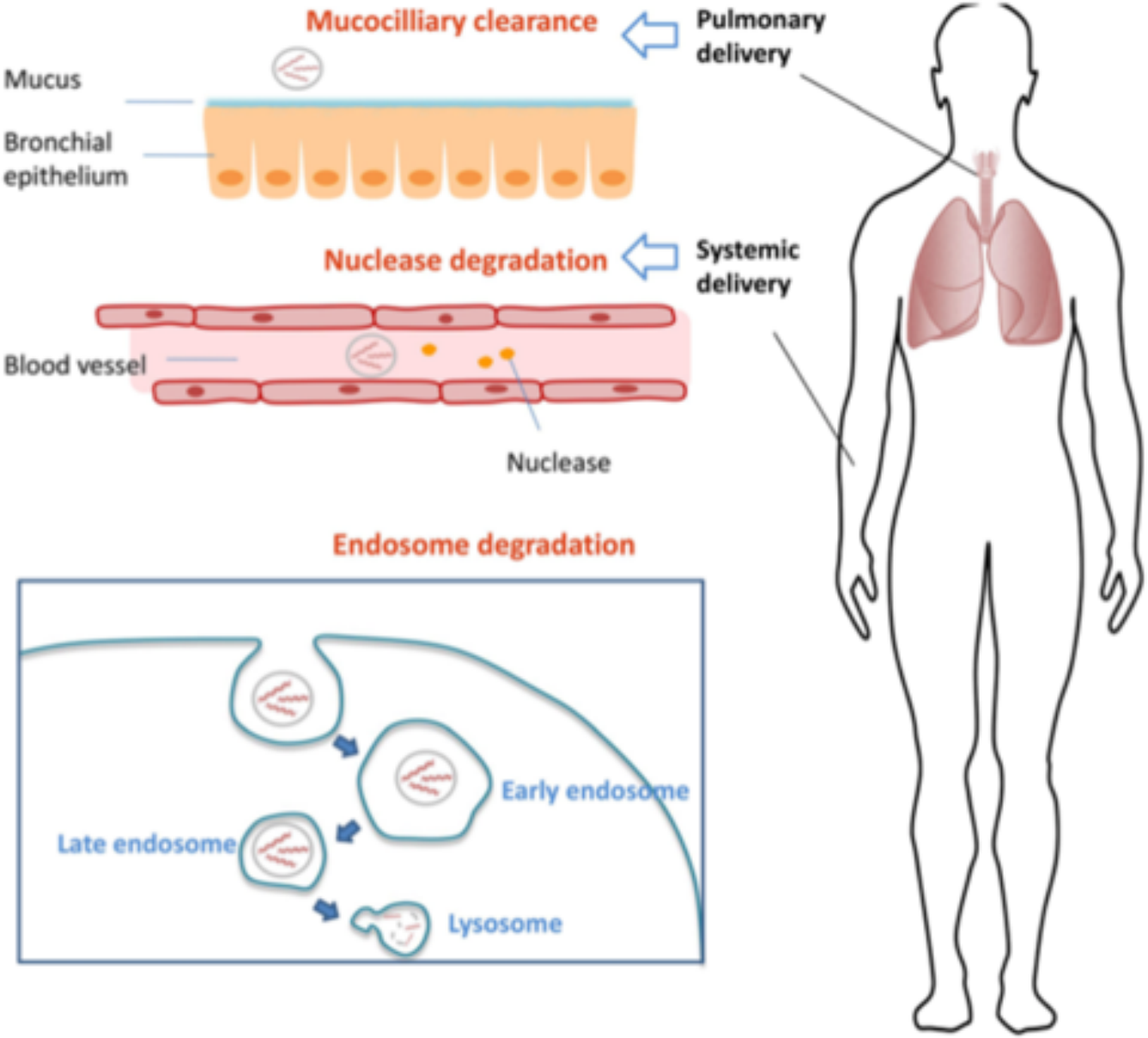
- GalNAc conjugation is the most mature effort so far: a method to reduce dose by a factor 20 or more for hepatocyte specific targets

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



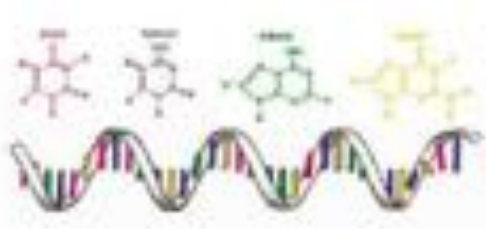
Antisense DNA oligonucleotides

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

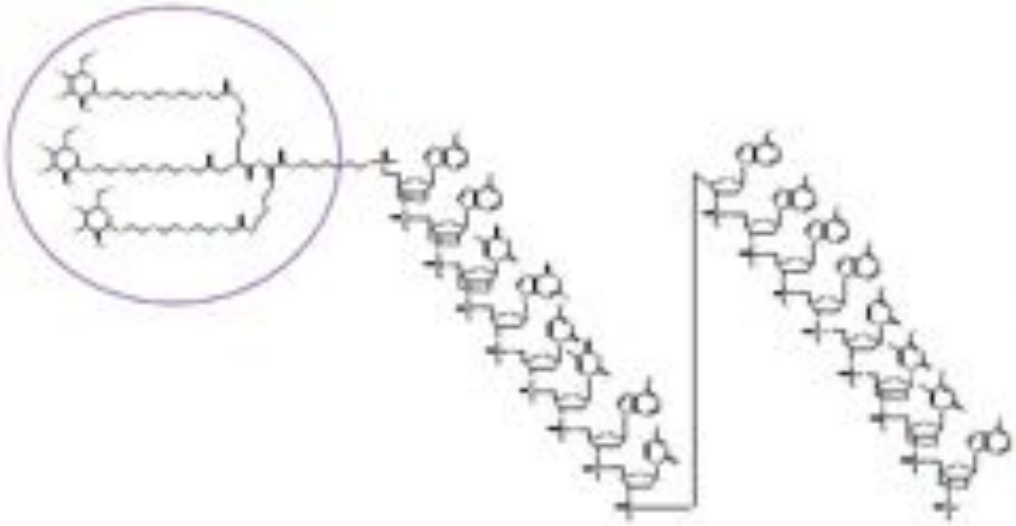
Crystal structure of a bacterial RNA

Antisense DNA oligonucleotides

SEARCHING FOR THE OPTIMAL SEQUENCE IN SILICO AND TESTING IN VITRO

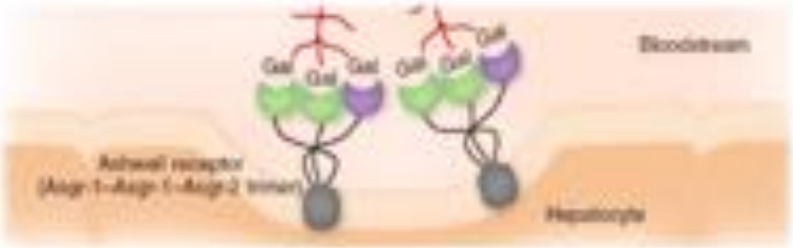
- GalNAc conjugation is the most mature effort so far: a method to reduce dose by a factor 20 or more for hepatocyte specific targets

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)



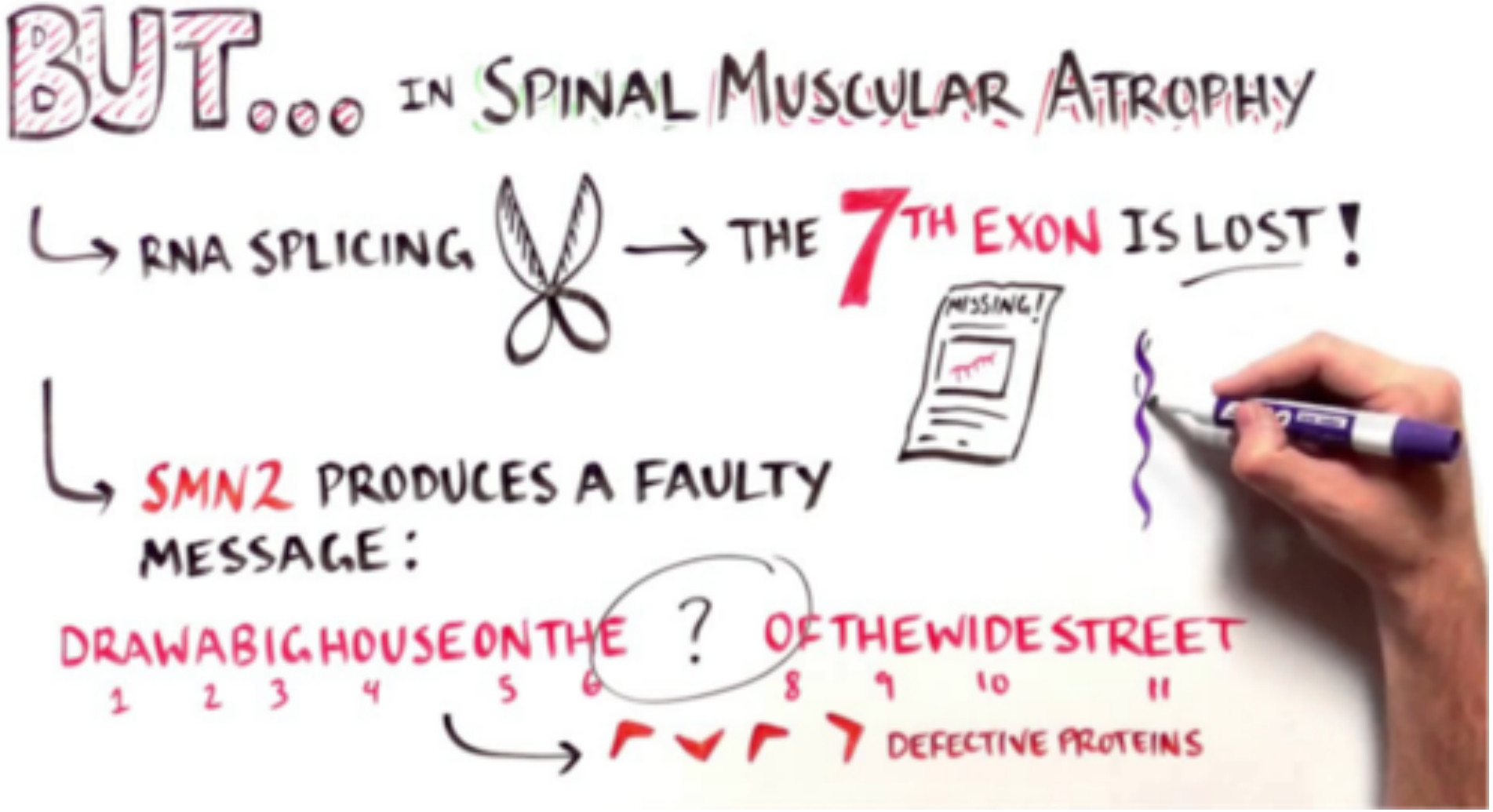
Antisense DNA oligonucleotides : spinal muscular atrophy : the nusinersen trials

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)

SMA : spinal muscular atrophy

SEARCHING FOR THE OPTIMAL SEQUENCE IN SILICO AND TESTING IN VITRO



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

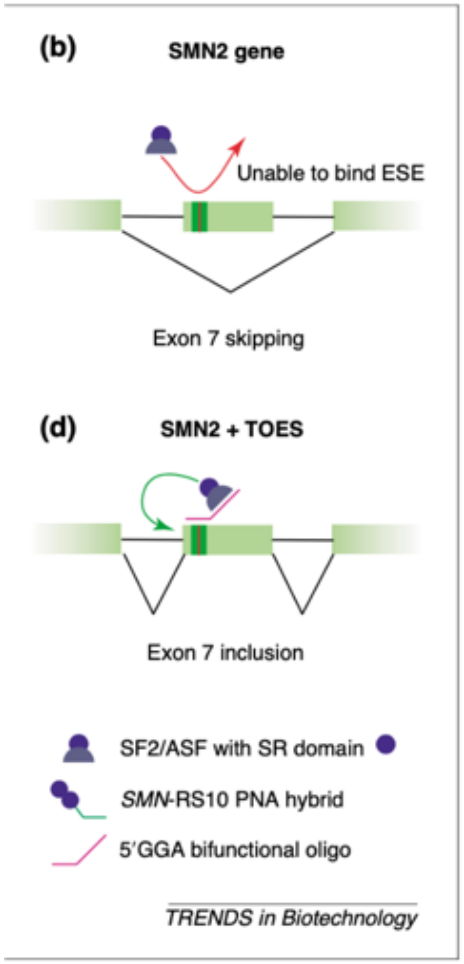
SEARCHING FOR THE OPTIMAL SEQUENCE IN SILICO AND TESTING IN VITRO

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

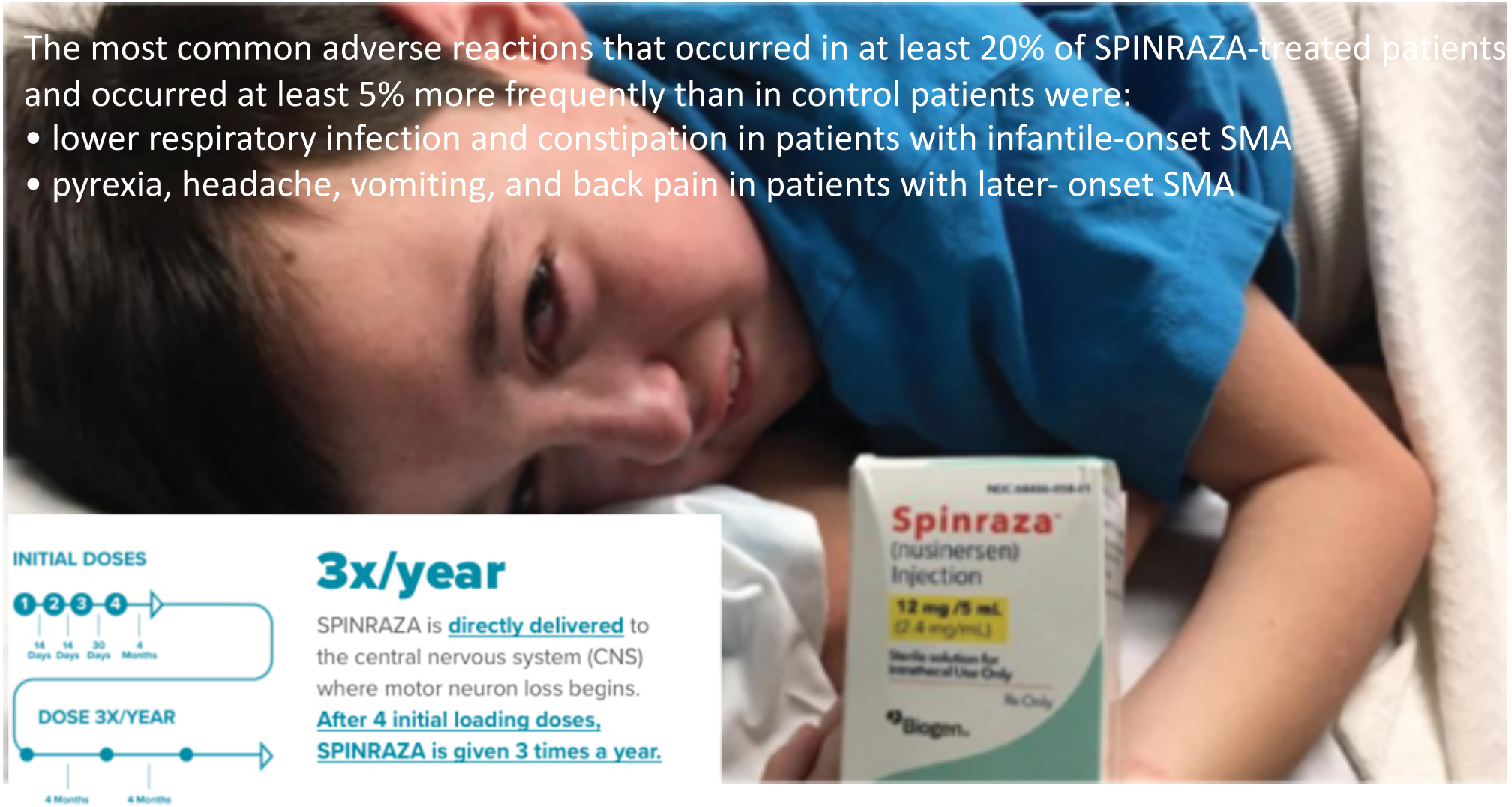
CSHL Professor Adrian Krainer, winner of the 2019 Breakthrough Prize in Life Science, in his laboratory.



« 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)



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

obal development Football Tech Business Environment Obituaries

$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 | 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.

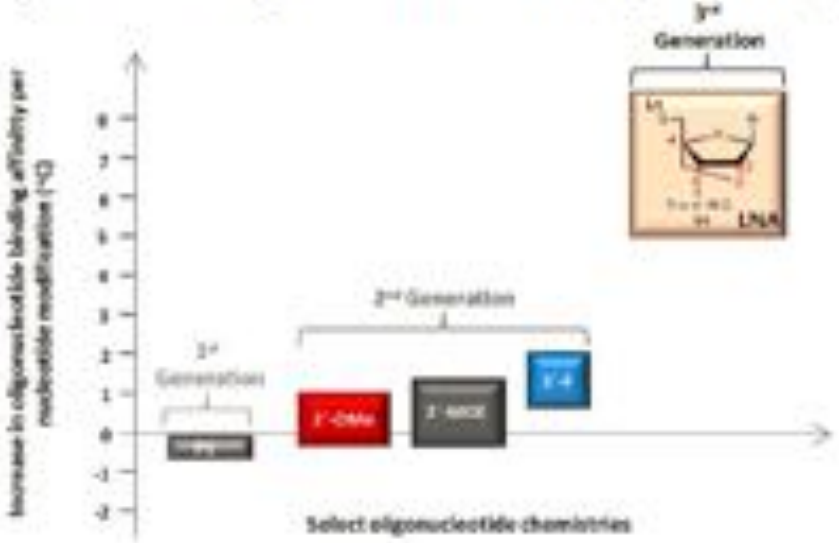
The company said it's working with the FDA to "identify any additional actions necessary to resume dosing" in the spinal-injection trial, dubbed Strong, and will continue to monitor safety events in patients.

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:

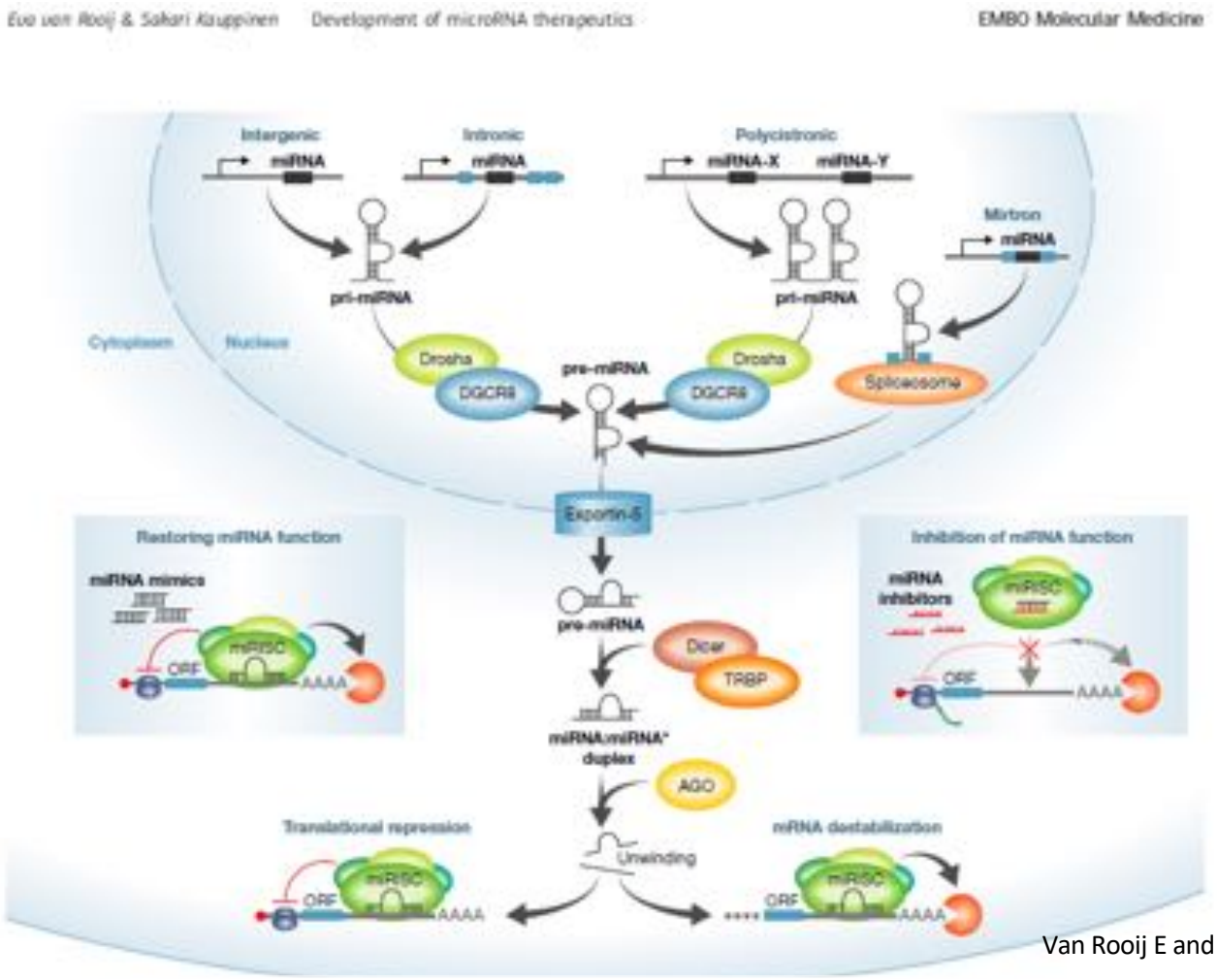
- Substantially improved potency and specificity
- Multiple *modes-of-action*
- Activity in multiple tissues
- Potential for good tolerability profiles
- Lower cost of goods

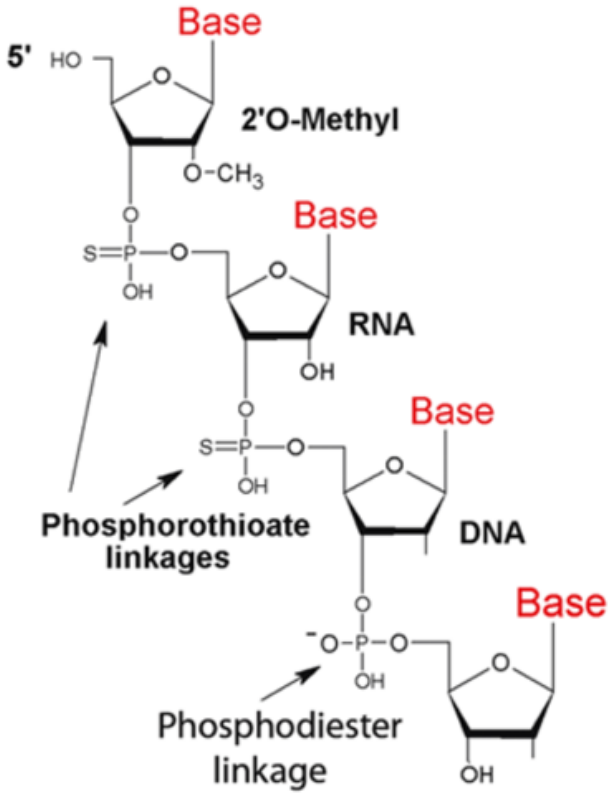
.. enables smaller and diverse designs

LNA gapmers

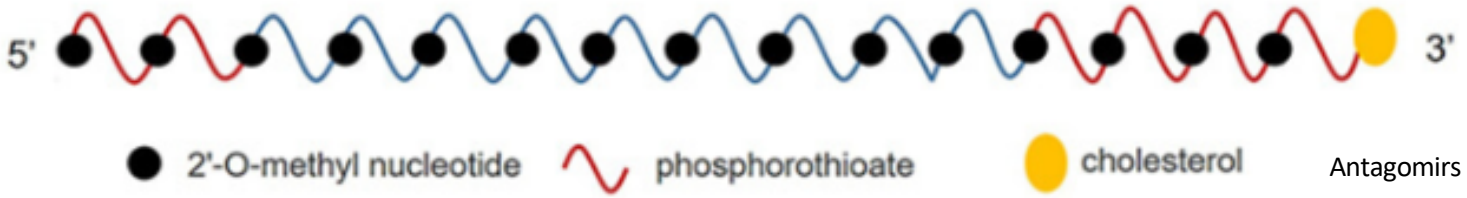
LNA mixmers /antimiRs

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

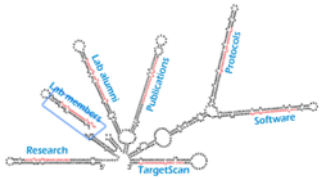




Van Rooij E and Kauppinen S. 2014 EMBO Mol Med J 6:851-864

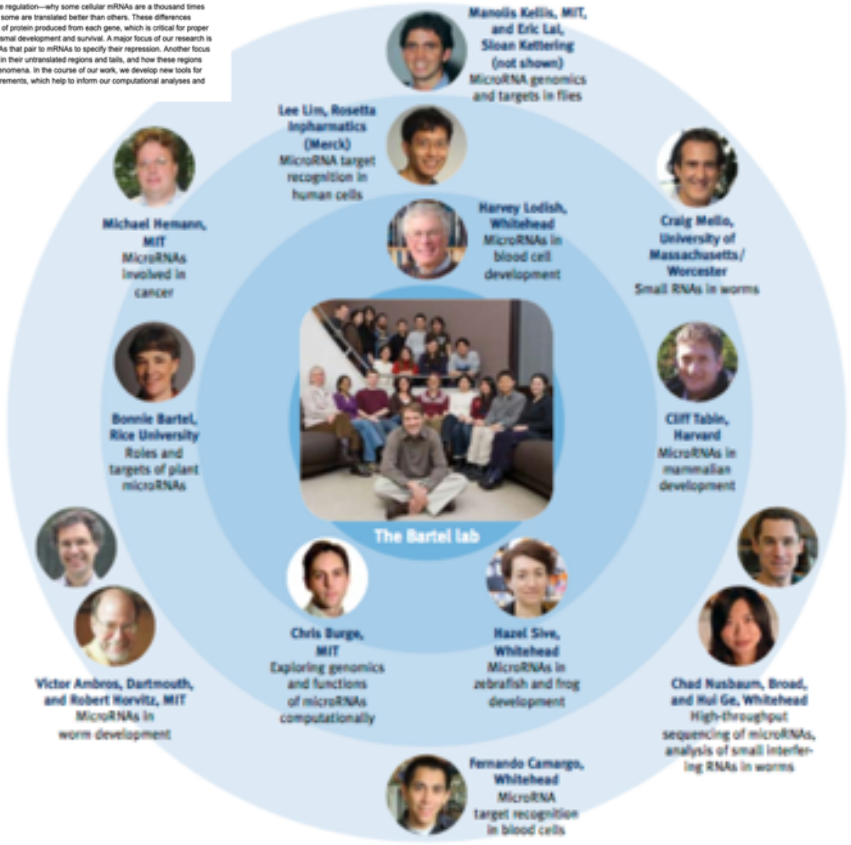


Antagomirs sequences



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

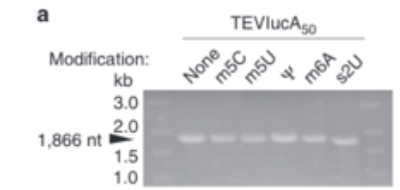
Katalin Karikó[1], Hiromi Muramatsu[1], Frank A Welsh[1], János Ludwig[2], Hiroki Kato[3], Shizuo Akira[3] and Drew Weissman[4]

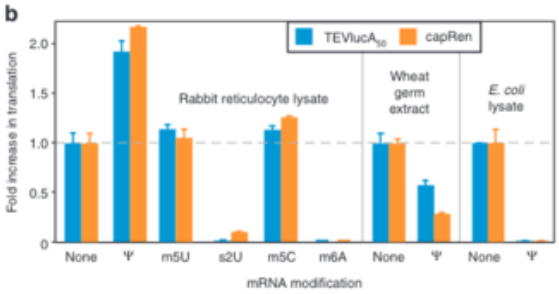
[1]Department of Neurosurgery, University of Pennsylvania, Philadelphia, Pennsylvania, USA; [2]Laboratory of RNA Molecular Biology, The Rockefeller University, New York, New York, USA; [3]Department of Host Defense, Research Institute for Microbial Diseases, Osaka University, Osaka, Japan; [4]Department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

BIONTECH

Katalin Karikó

Biographie





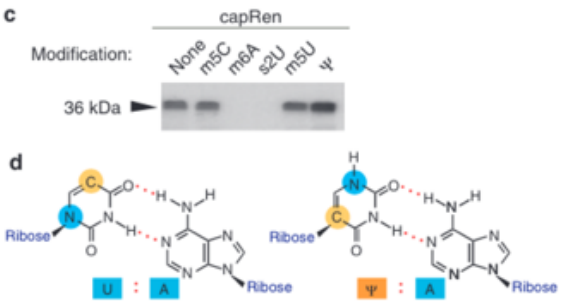


Figure 1 In vitro transcription and translation of nucleoside-modified mRNAs. (a) Aliquots of *in vitro*–transcribed TEVluc A$_{50}$ containing no

Antagomirs (anti miRs) therapeutics in drug development

Table 1. MicroRNA-based therapeutics in development

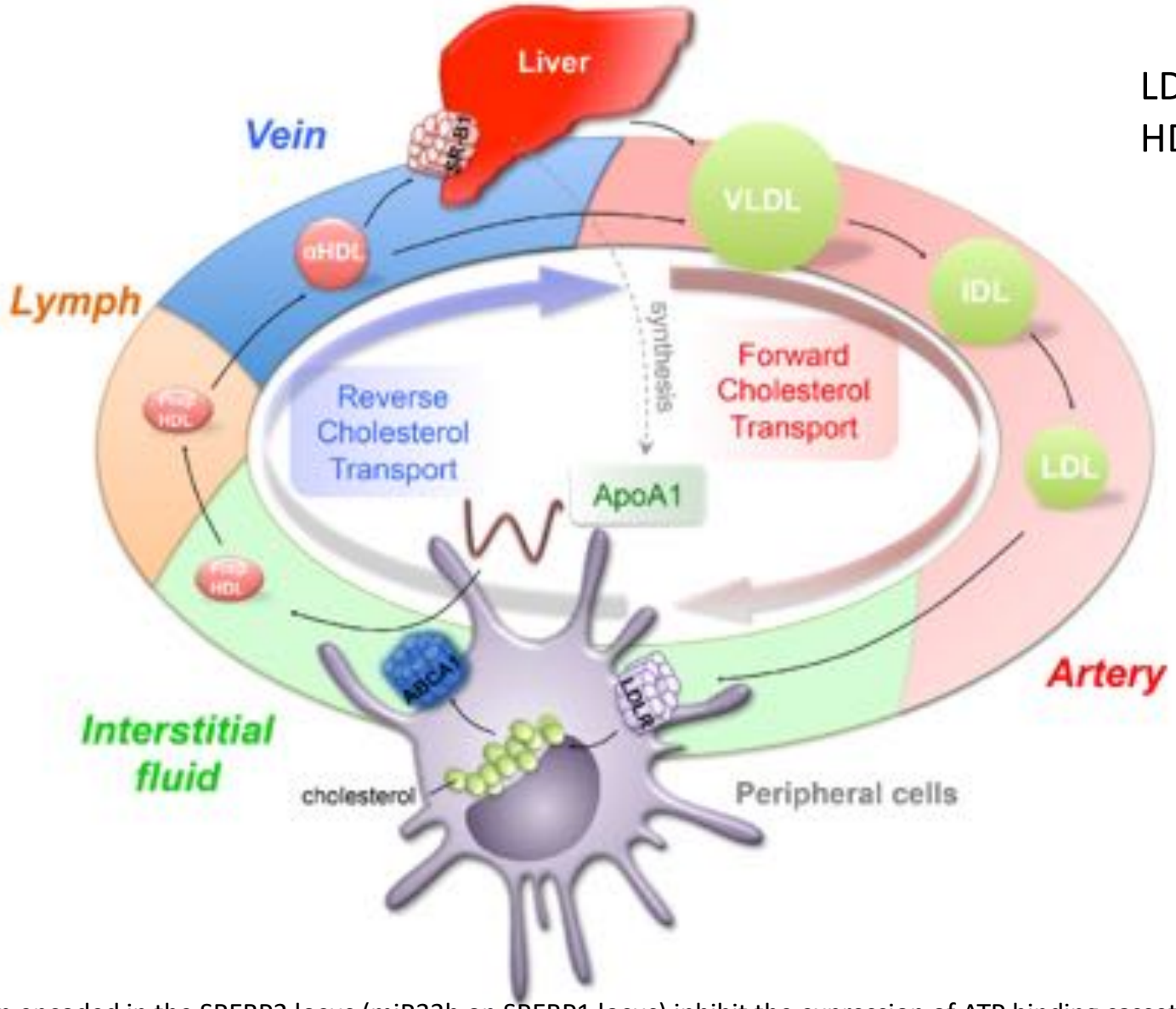
Company	miRNA target	Mode of action	Indication	Status
Santaris Pharma	miR-122	antimiR	HCV	Clinical Phase II
Mirna Therapeutics	miR-34	mimic	Unresectable primary liver cancer	Clinical Phase I
	let-7	mimic	Cancer	Preclinical
Regulus Therapeutics	miR-122	antimiR	HCV	Clinical Phase I
	miR-221	antimiR	Hepatocellular 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)

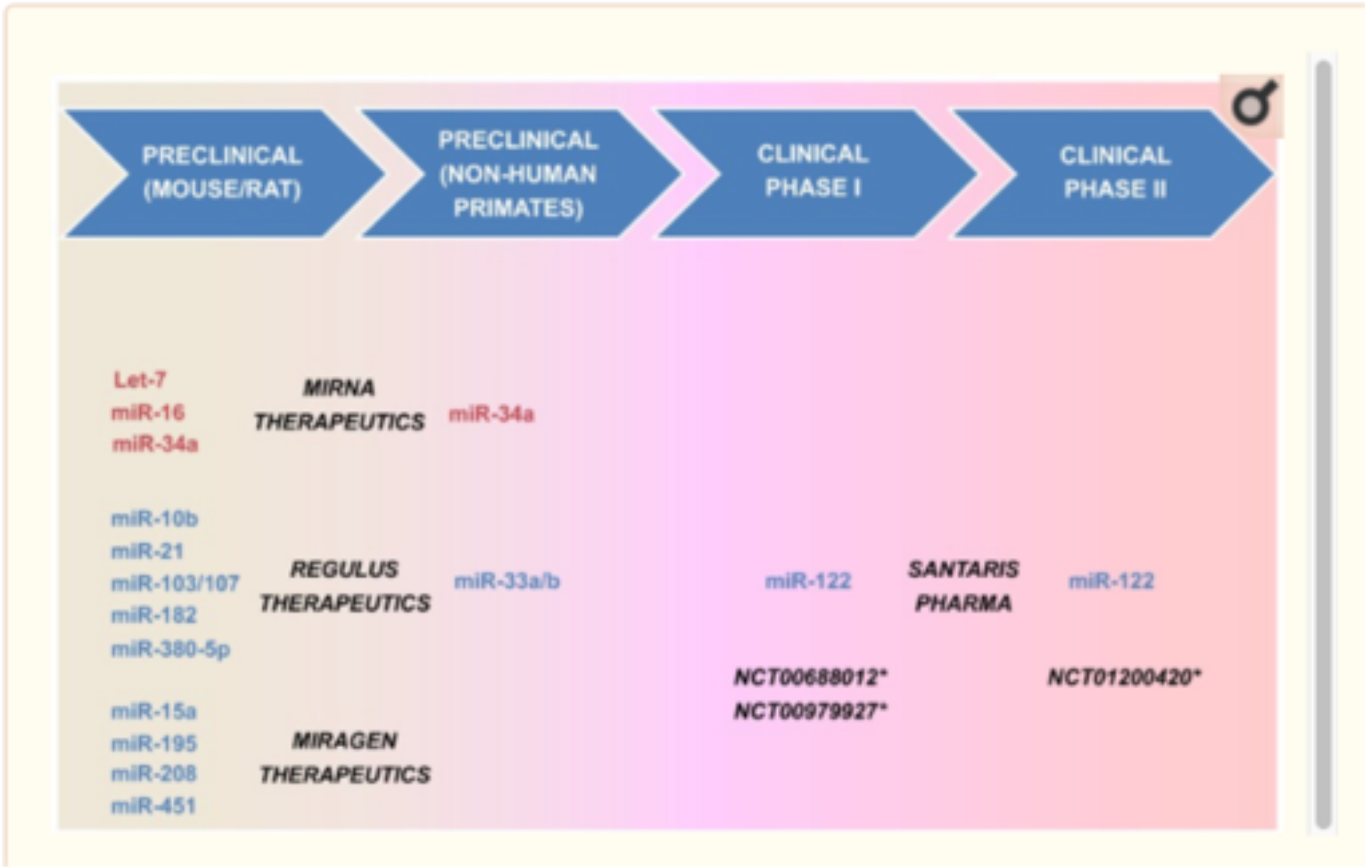
Antagomirs (anti miR33a) therapeutics in CVD drug development

LDL = mg/dl
HDL = mg/dl

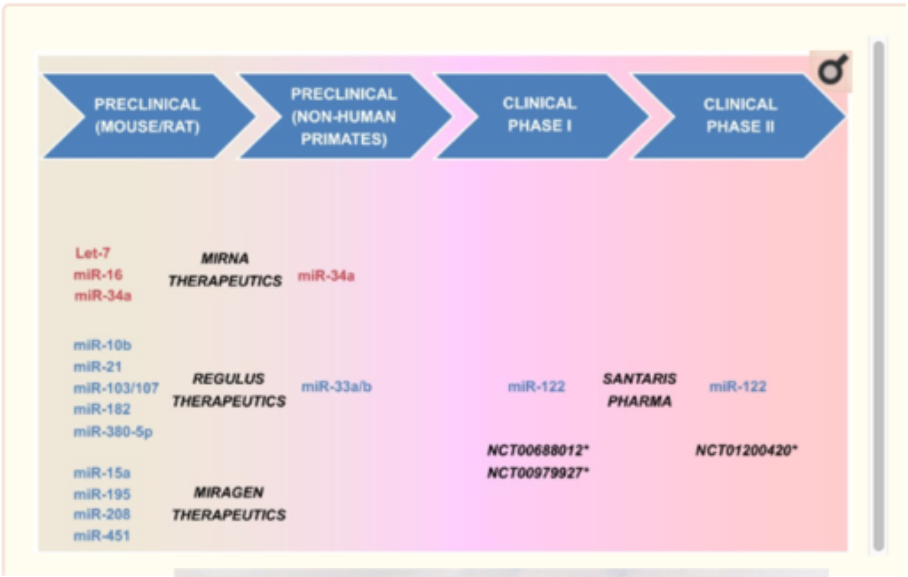


eg. miR33a intron encoded in the SREBP2 locus (miR33b on SREBP1 locus) inhibit the expression of ATP binding cassette transporter ABCA1, a cholesterol efflux pump essential in RCT (reverse cholesterol transport) (SREBP = sterol response binding protein)

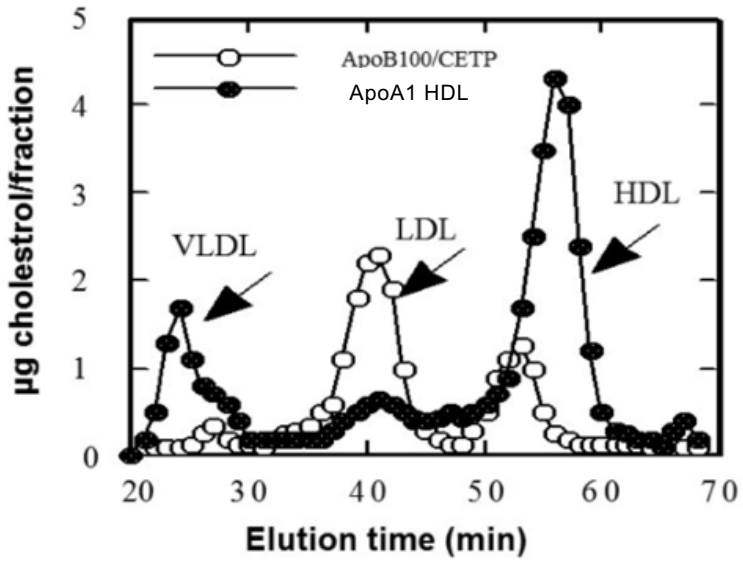
Development of microRNA therapeutics is coming of age



Development of miR33a antagomirs therapeutics in CVD



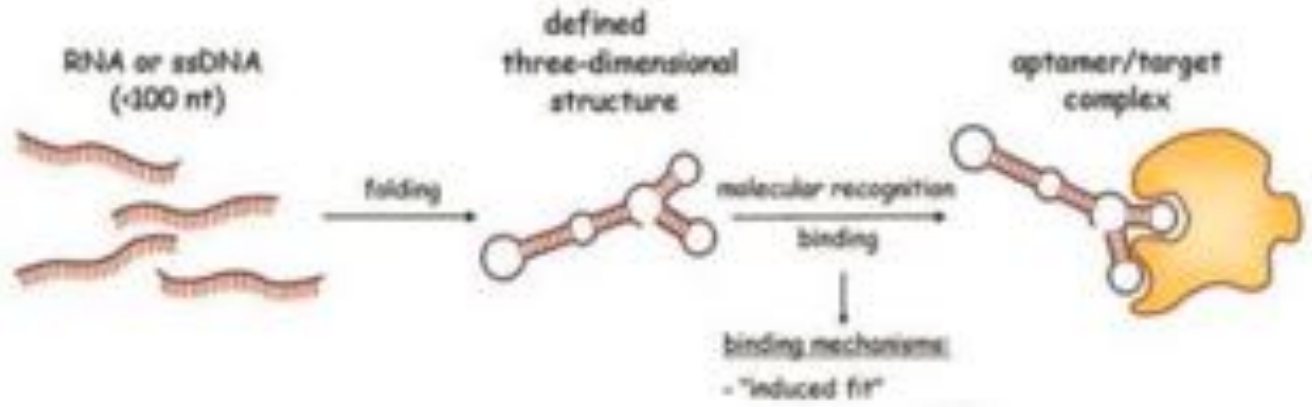
SUBSTANTIAL PRECLINICAL WORK HAS BEEN COMPLETED INCLUDING A NON- HUMAN PRIMATE STUDY OF INHIBITING miR-33A/B FOR THE TREATMENT OF ATHEROSCLEROSIS. BY TREATING AFRICAN GREEN MONKEYS SUB CUT WITH ANTI miR-33 , A DECREASE IN VLDL AND LDL AND AN INCREASE OF HDL WAS OBSERVED. REDUCED REPRESSION OF ABCA1 GENE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



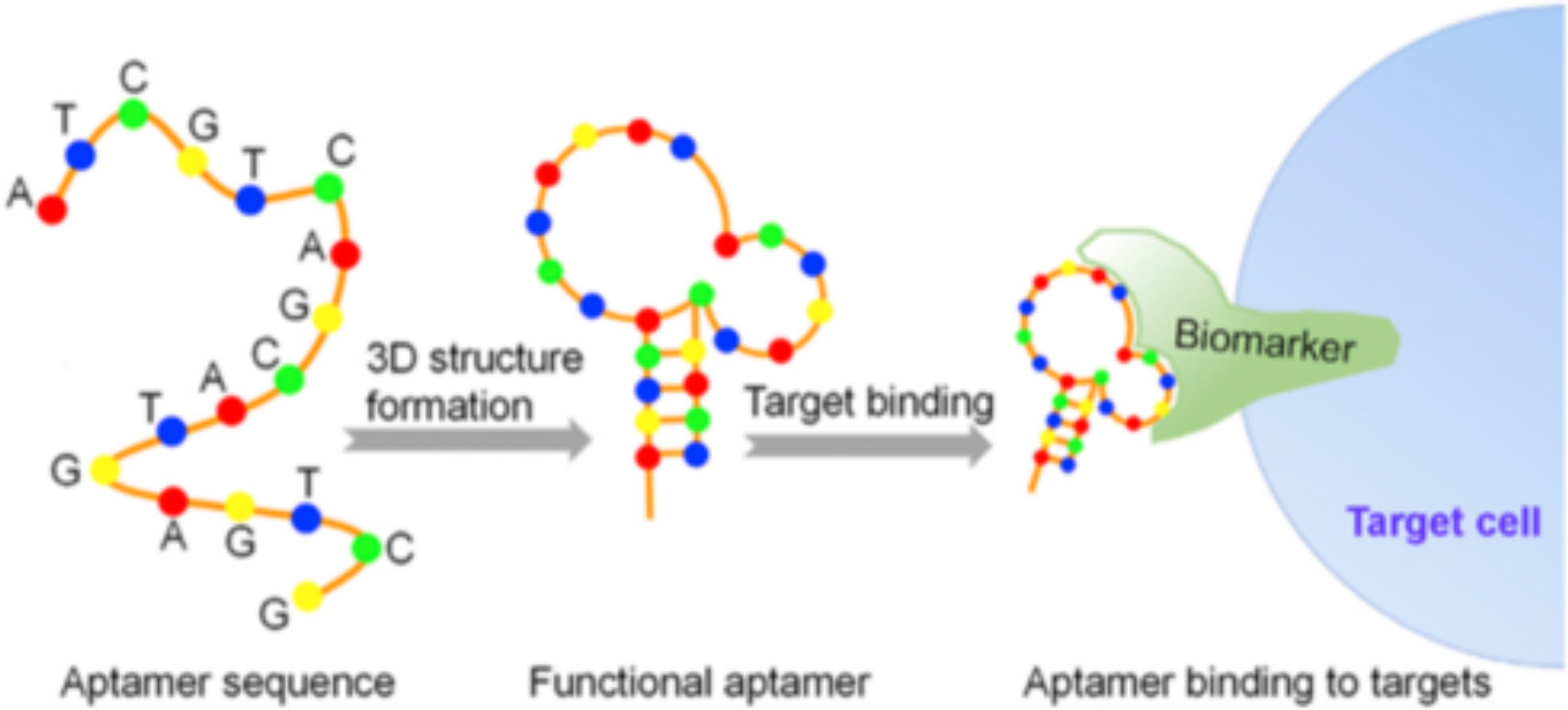
SELEX (systematic evolution of ligands by exponential enrichment) allows over several rounds of selection from a pool of sequences against the therapeutic target high affinity and specificity cdps to the target : similar affinities as ABs with low immunogenitcity however

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 3′-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/R9006 plus R9007 (Regado Biosciences)	2′-ribo purine/2′-fluoro pyrimidine (R9006)/40 kDa PEG plus 2′-O-methyl antidote (R9007)	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

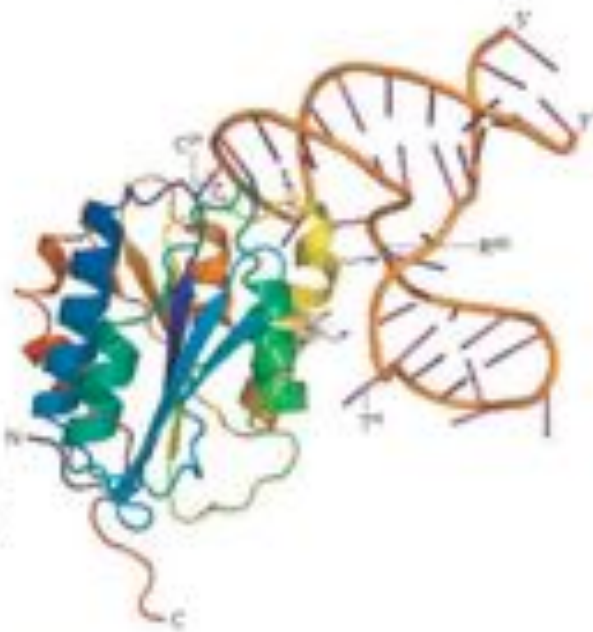


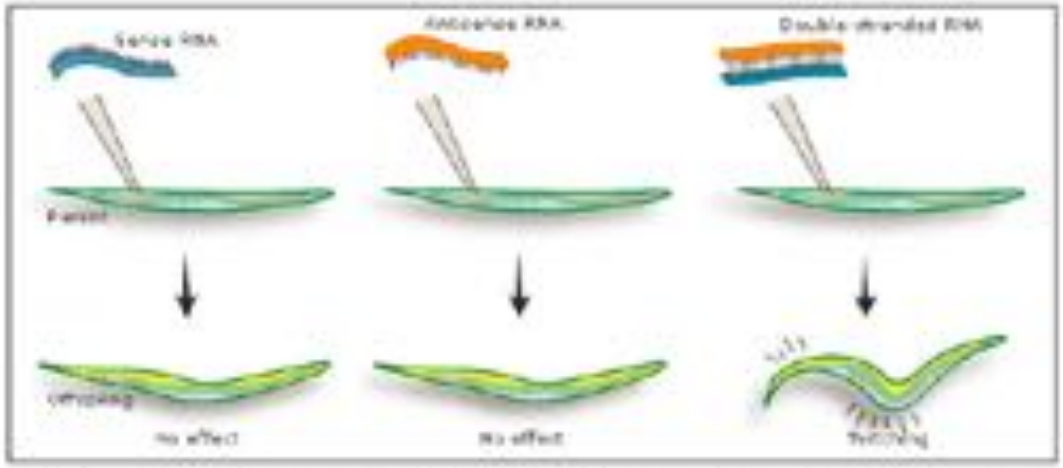
Figure 3 | **Crystal structure of the all-DNA parent of ARC1779 bound to the A1 domain of von Willebrand factor.***

*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*.



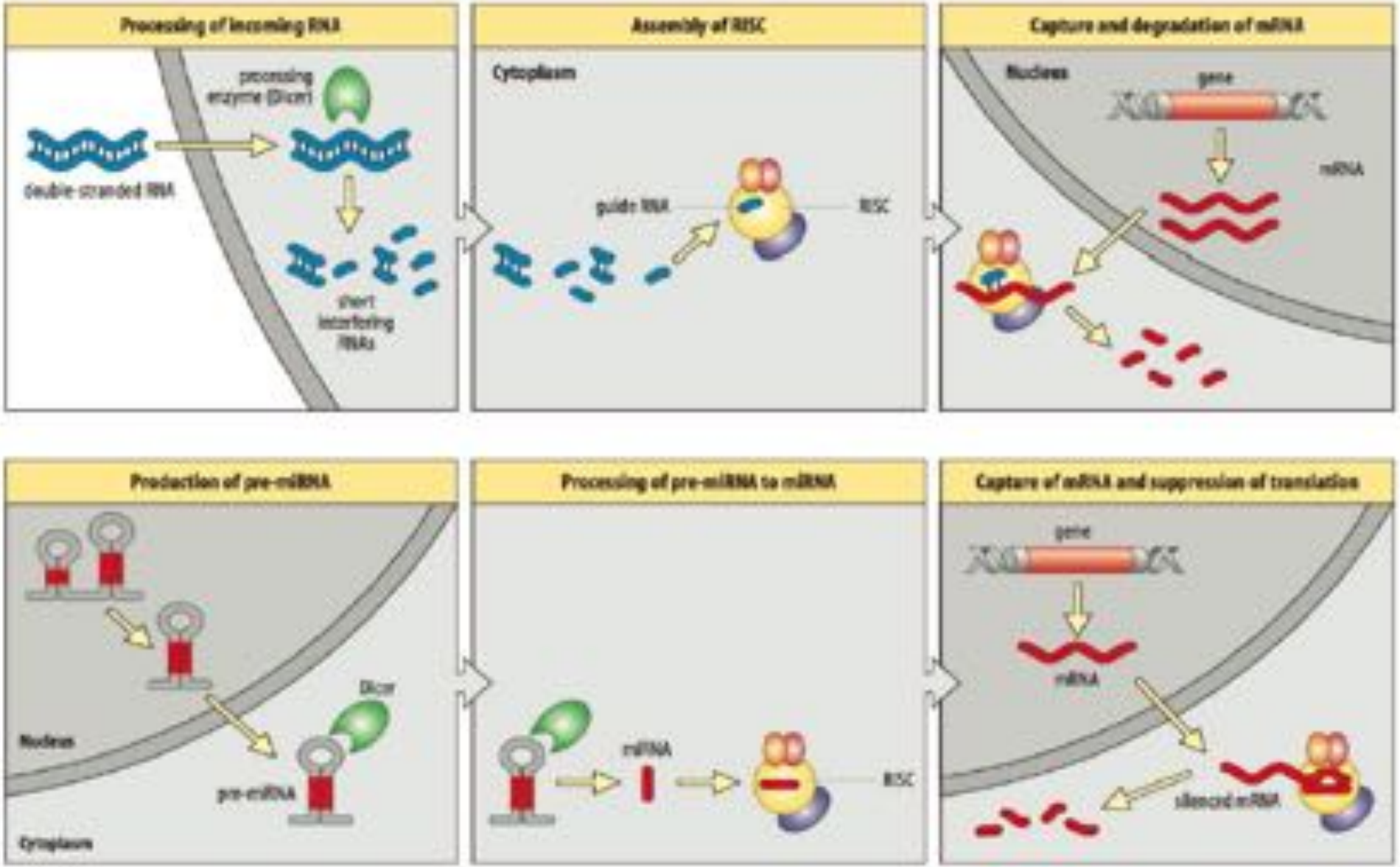
unc-22 encodes a myofilament protein. Decrease in unc-22 activity is known to produce severe twitching movements

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.

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 !



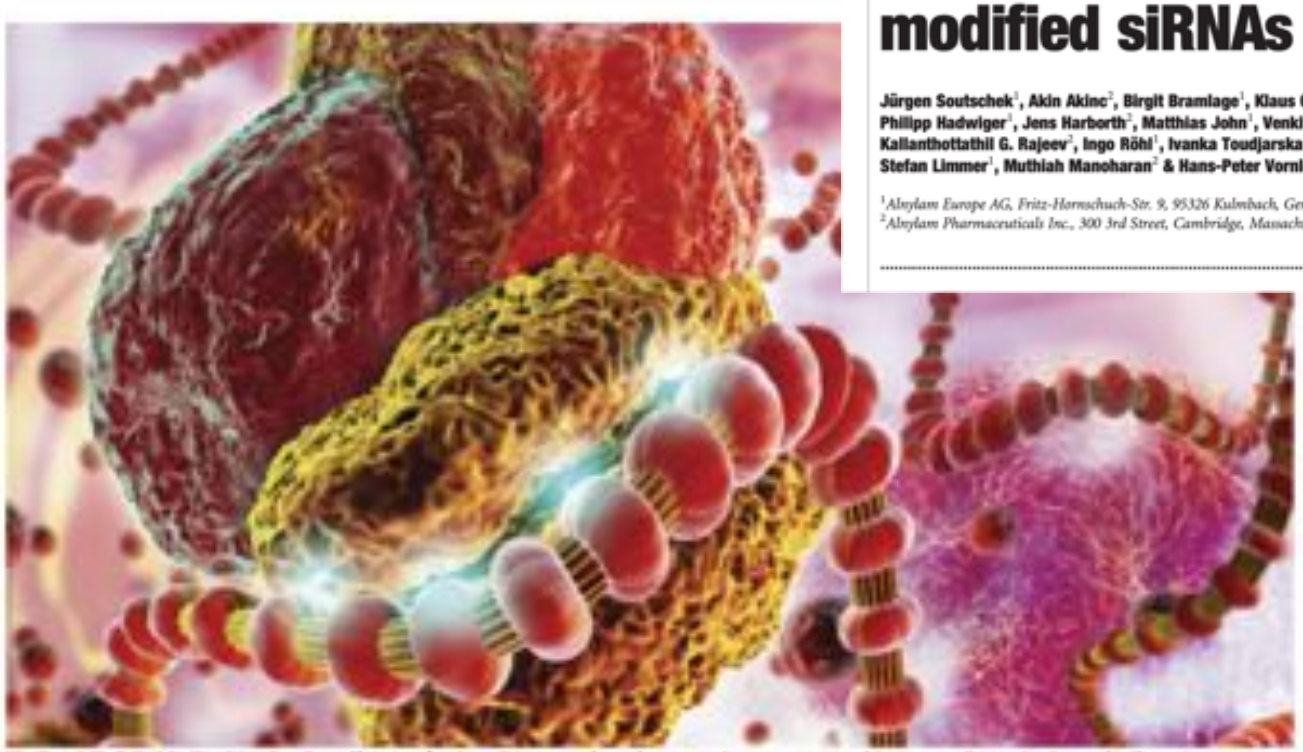
RNA interference: small RNAs as therapeutics eg in liver : nanoparticle, cholesterol moiety, systemic delivery improved ?!

The Scientist » September 2014 Issue » Features

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.

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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]

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PROTACs : linking therapeutic target to ubiquitin ligases for degradation

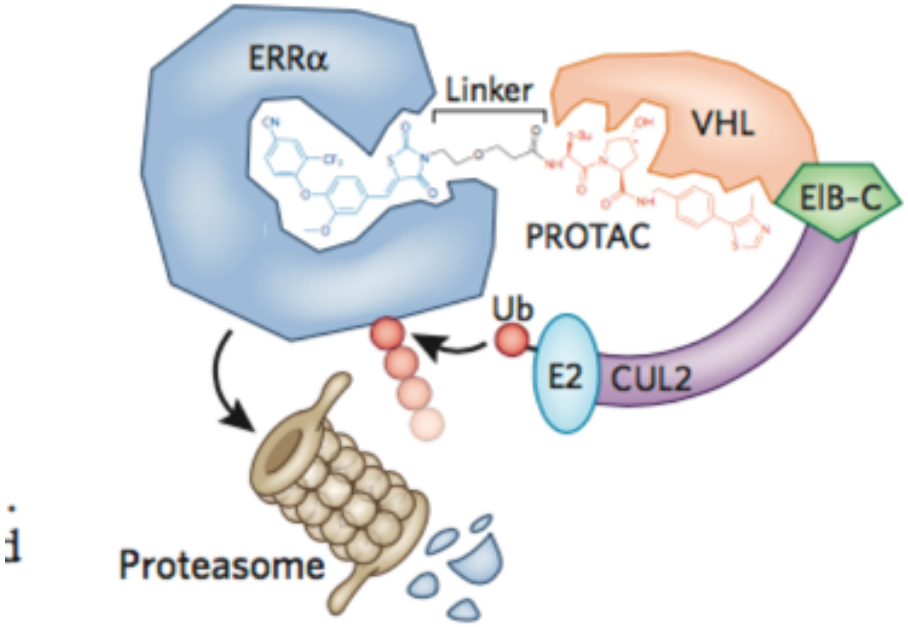

Figure 1 | PROTAC mechanism of action and modular structure. This PROTAC links ERRα to the $CRL2^{VHL}$ ubiquitin ligase. $CRL2^{VHL}$ recruits a ubiquitin-loaded ubiquitin-conjugating enzyme (E2), which discharges its cargo onto BRD4. Multiple rounds of ubiquitin (Ub) transfer build up a chain of ubiquitins on ERRα, which targets ERRα to the proteasome for degradation. CUL2, cullin 2; EIB–C, elongin B–elongin C heterodimer.

Katie Vicari/Nature Publishing Group

M. Scudellari (2019) The protein slayer. Nature 367:298-300

"We're breaking the rules of what we thought would be druggable."

THANK YOU.....

DO YOU HAVE ANY QUESTIONS ?