What kind of drugs should we develop

Mathematical and Computational Biology in Drug Discovery (MCBDD) Module III

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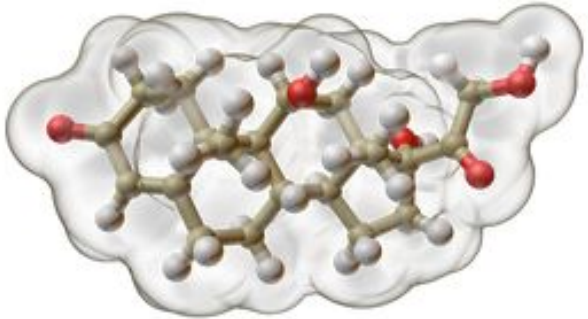
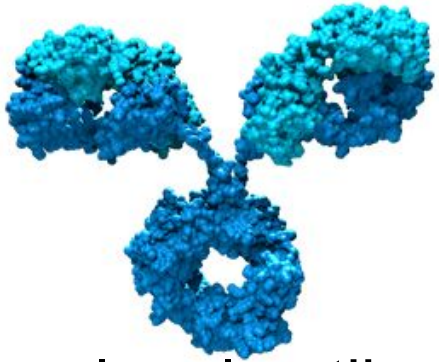
Overview

- Essentials of modalities
  - Small molecules: classical, protein degrader, RNA modulator
  - Large molecules: classical, DUTA-Fabs, protein design
  - Antisense oligonucleotides: siRNA, shRNA, ASO
  - Gene and cell therapy
- Three case studies:
  - Success stories:
    - [Small molecules] SMA (Evrysdi/Risdiplam and Nusinersen)
    - [Antisense] patisiran (KEGG DRUG) and givosiran (DrugBank, structure available at EMA)
    - [Offline read] mRNA vaccine (MIT Technology Review)
    - Turning failure into successes: [Multispecific drugs] Thalidomide, PROTAC, degraders
    - [Antibody] Cancer immunotherapy (CTLA4, PD1)
    - [Gene and Cell therapy] CAR-T
  - Challenges
    - [Antisense] HTT (Tominersen)
    - Difference between genetic and enzymatic inhibition

A zoo of modalities

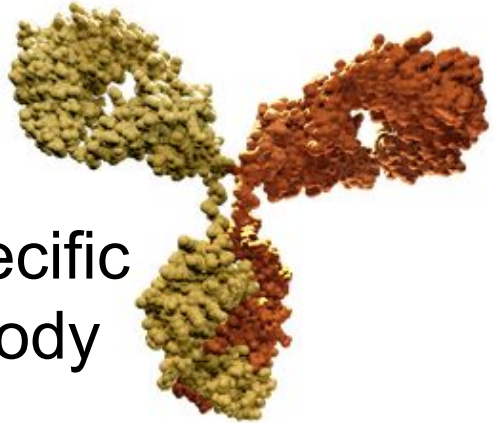


Small molecule

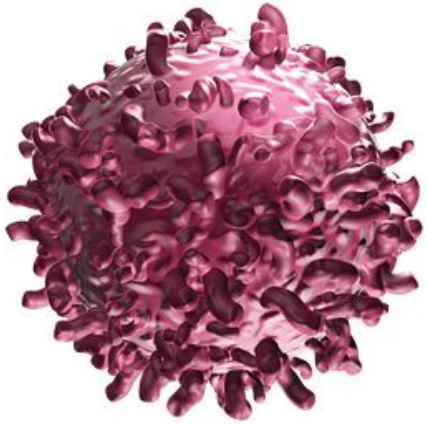


Monoclonal antibody

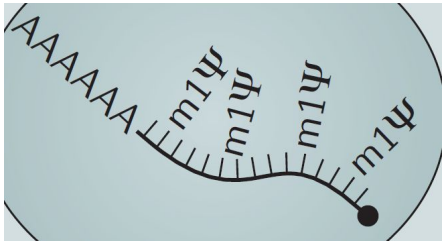
Oligonucleotides



Bispecific antibody



Chimeric Antigen Receptor (CAR) T-cells

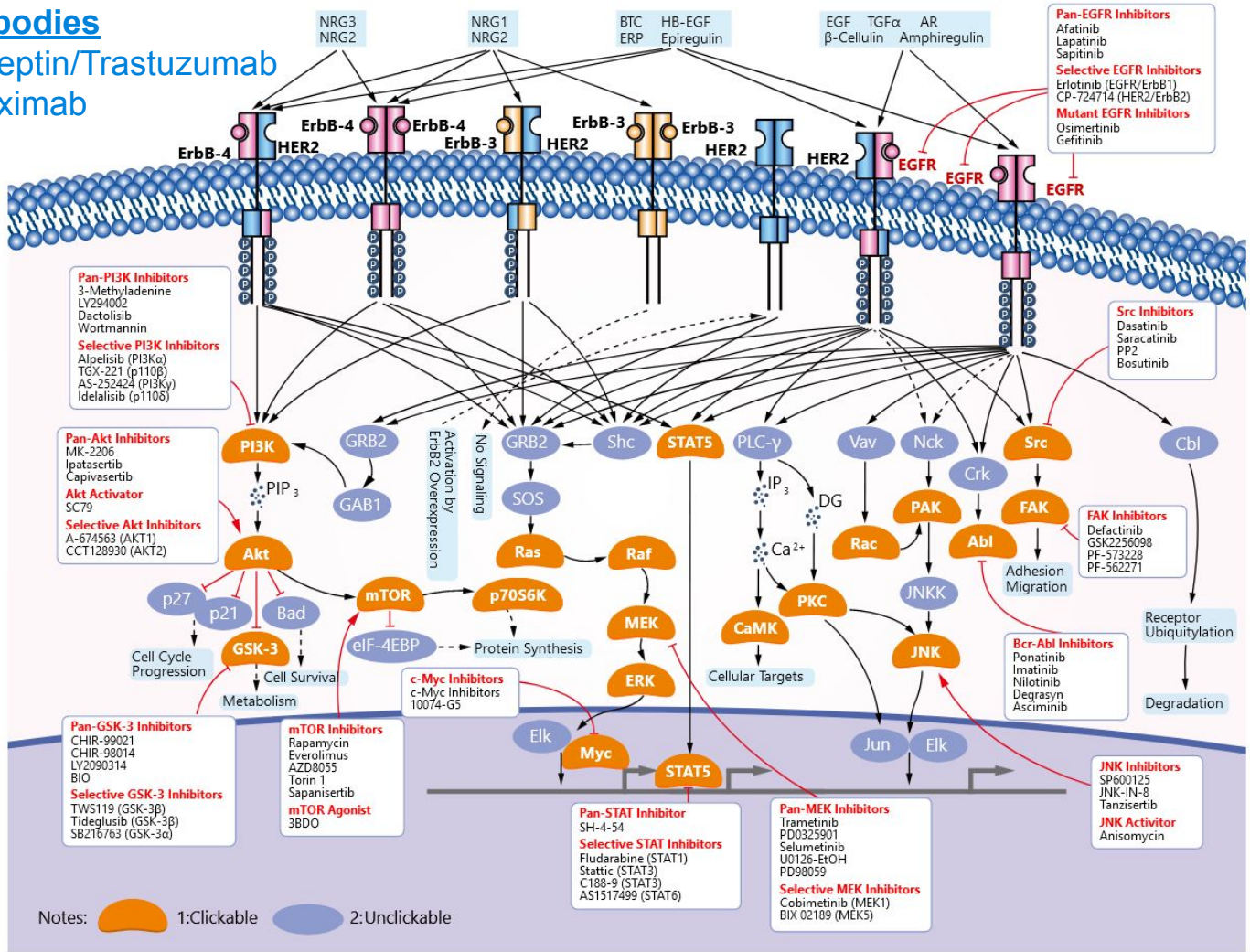


mRNA vaccines

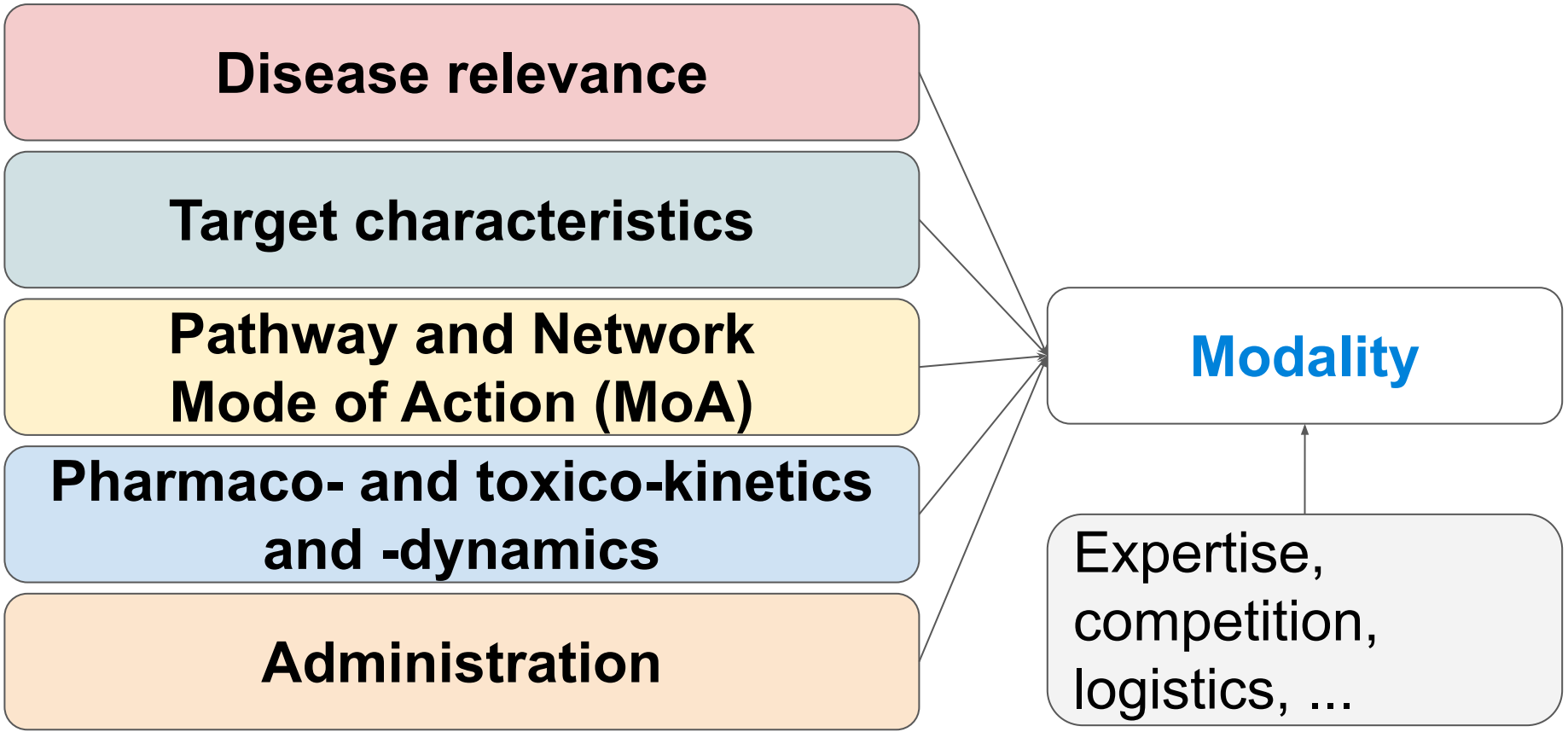
Multiple modalities can target the same biological process

An example: the epidermal growth factor receptor (EGFR) pathway

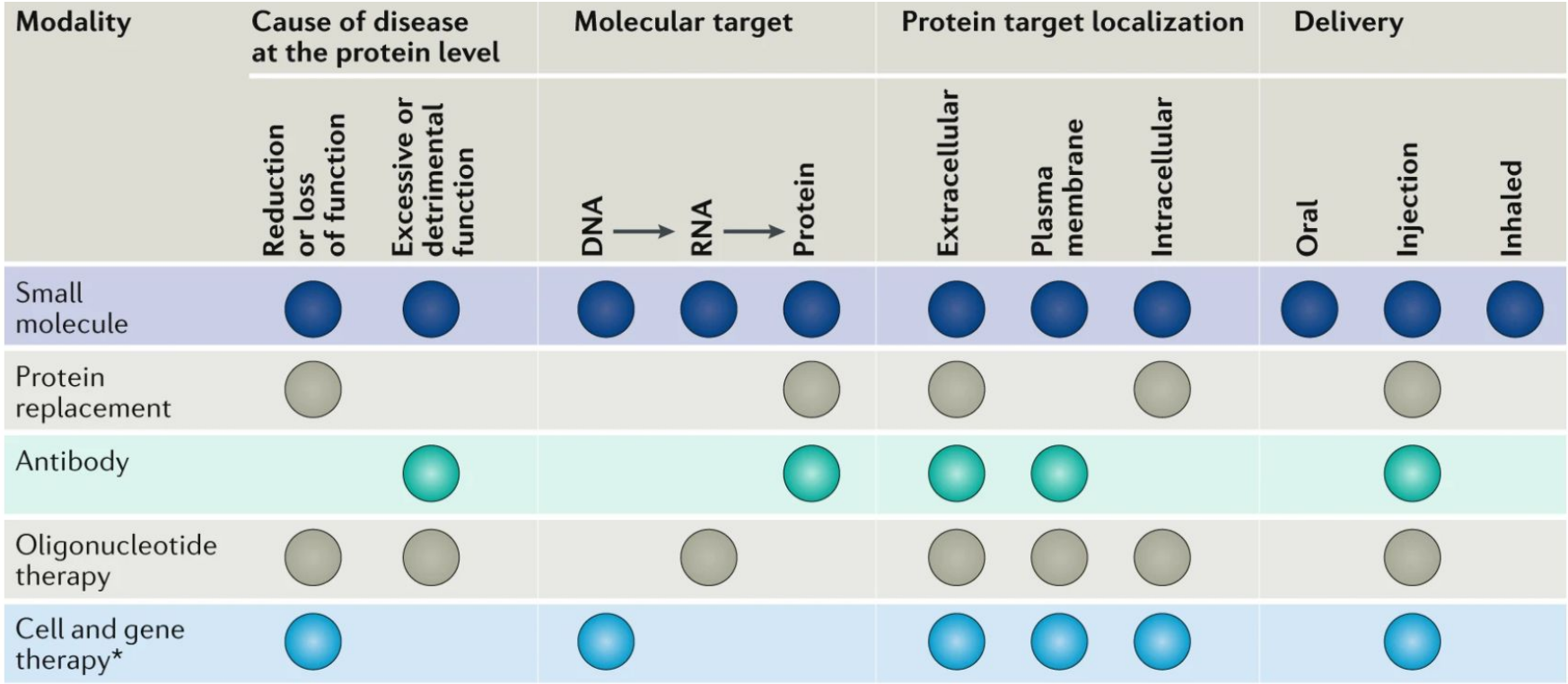
Antibodies
Herceptin/Trastuzumab
Cetuximab



Criteria to choose a modality

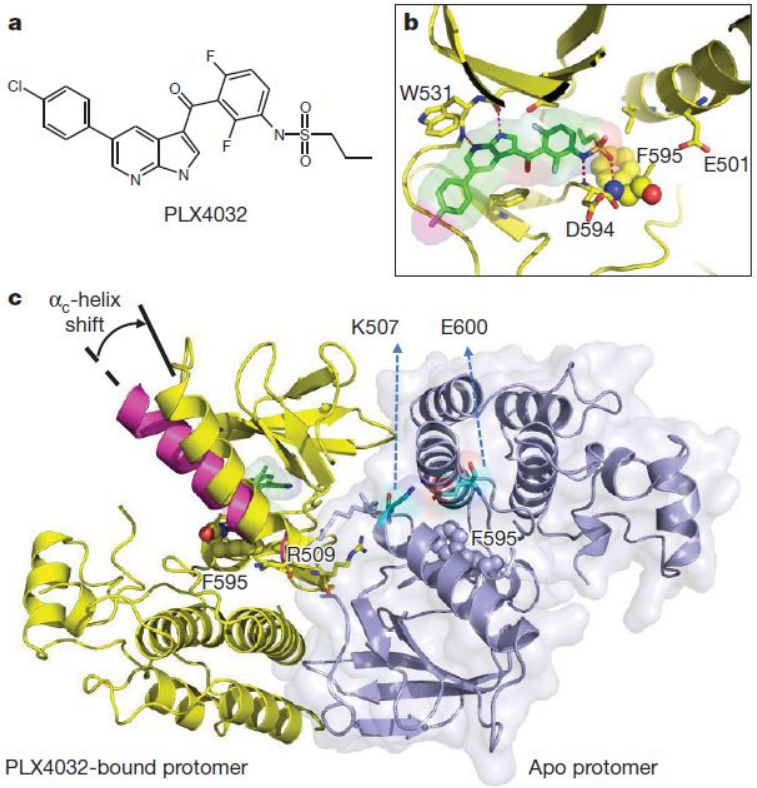


Characteristics of therapeutic modalities



Classical small molecules: an example from AMIDD

- Vemurafenib (Zelboraf, PLX4032)
  V600E mutated BRAF inhibition
- Lock and key: an oversimplified yet powerful metaphor, first proposed by Emil Fischer

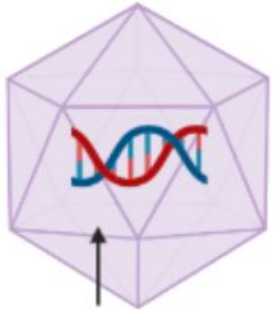


Facts about Spinal Muscular Atrophy (SMA)

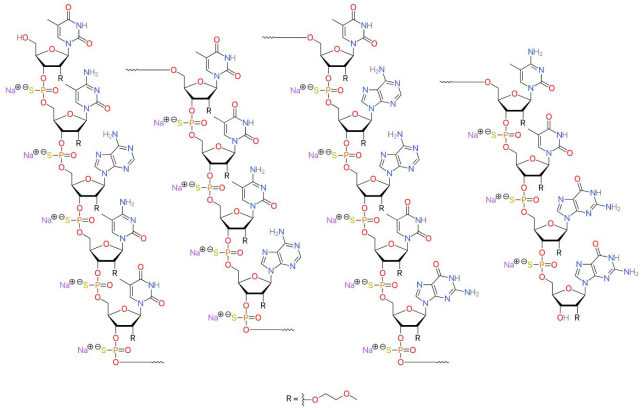
- SMA is caused by a defect in a gene called *SMN1*. People with SMA have reduced levels of the SMN protein.
- When SMN protein levels are reduced, motor neurons are unable to send signals to the muscles, causing them to become smaller and weaker over time.
- Depending on the severity, or type of SMA, people with the disease will have difficulties moving, eating, and in some cases breathing, making them increasingly dependent on parents and caregivers.
- A short movie: https://www.nejm.org/doi/full/10.1056/NEJMoa2009965

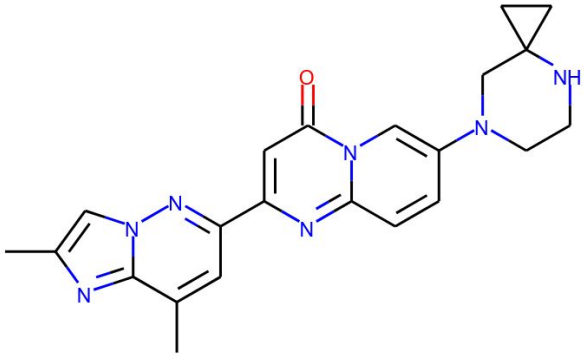
One Disease, Three Drugs

AAV9 capsid



SMN1 gene



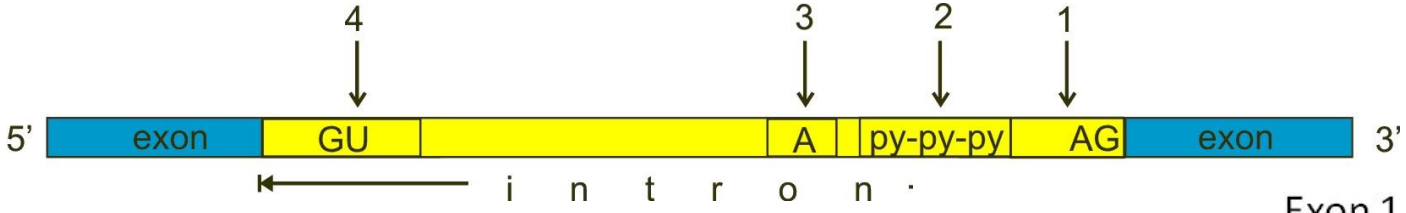


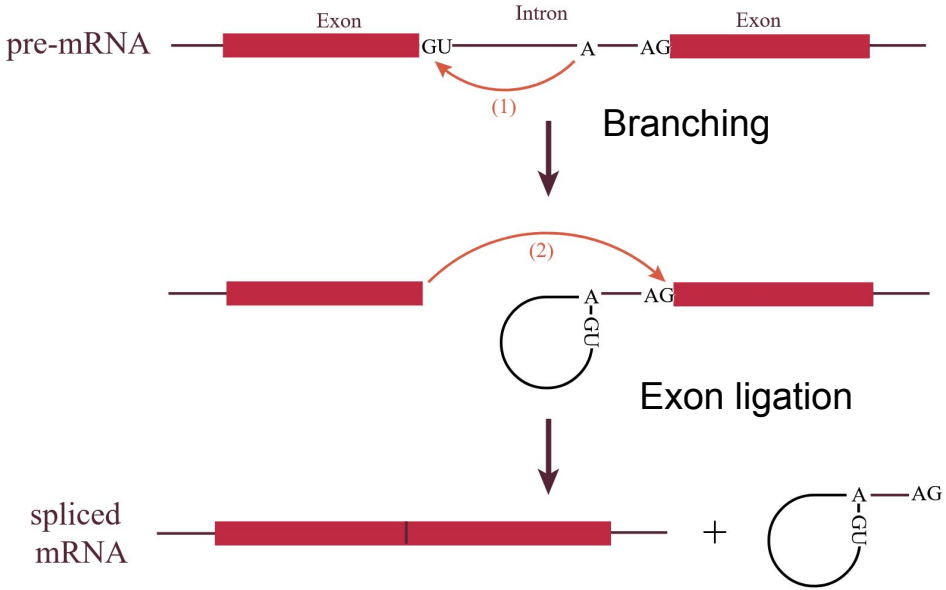
Onasemnogene Abeparvovec/ Zolgensma

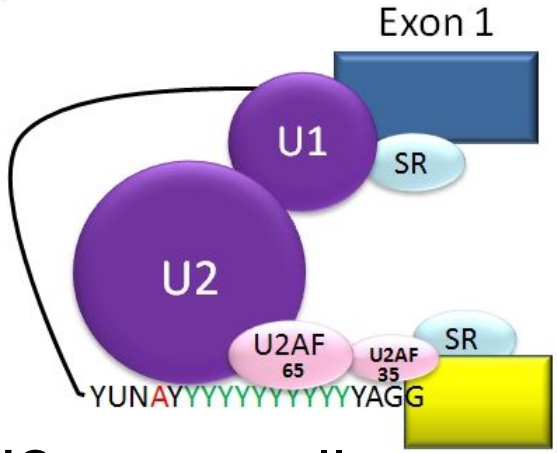
Nusinersen sodium/ Spinraza (CHEMBL3833342)

Risdiplam/ *Evrysdi* (CHEMBL4297528)

Spliceosome: the splicing machinery

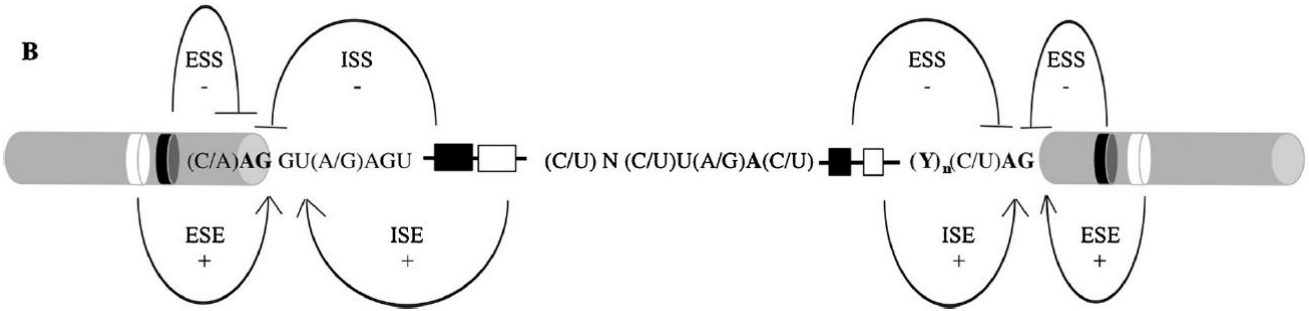






U1/U2 are **s**mall **n**uclear **r**ibo**n**ucleo**p**roteins (snRNPs = RNA+proteins)

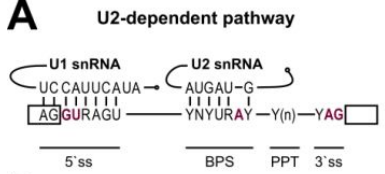
Splicing in action and under regulation

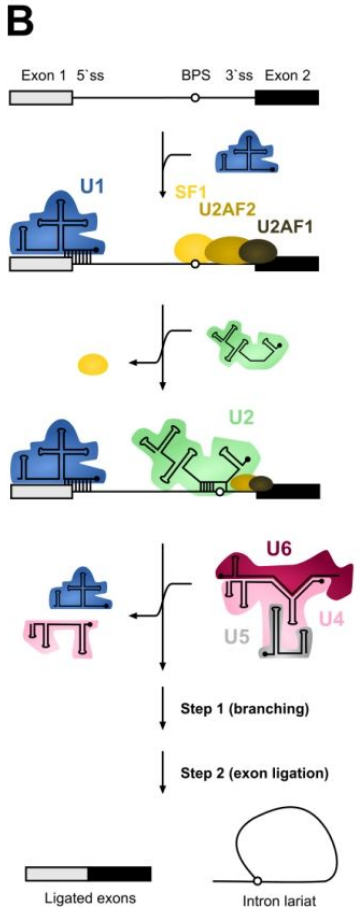


ESS=exon splicing silencer; ESE=exon splicing enhancer;

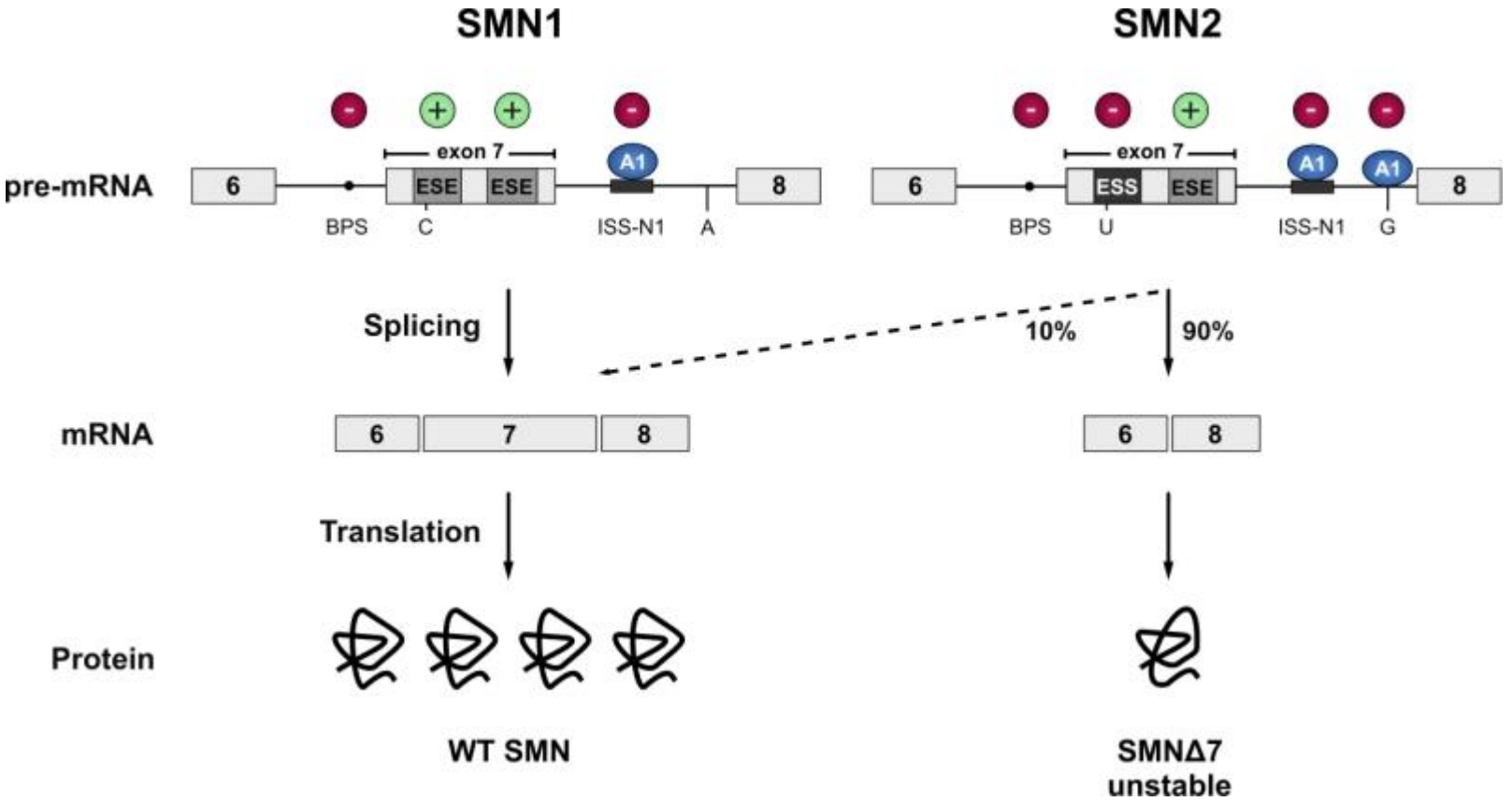
ISS=intron splicing silencer; ISE=intron splicing enhancer.

BPS=branch point sequence; PPT=polypyrimidine tract (C/U);

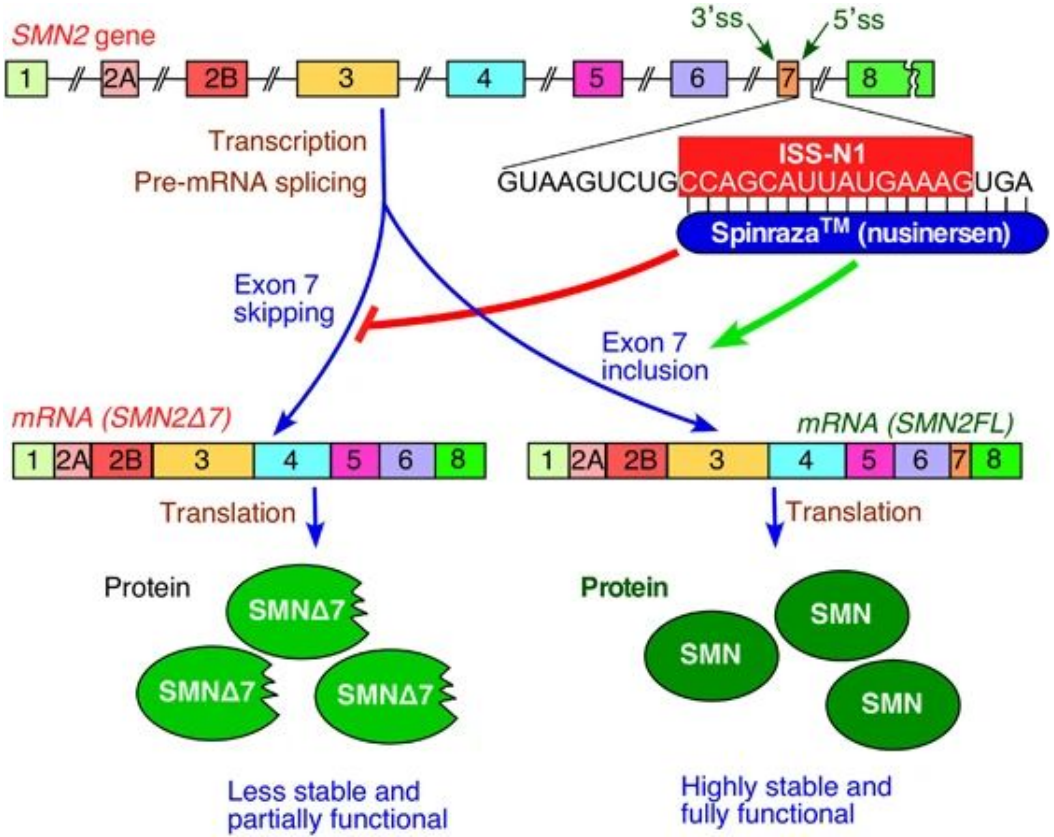




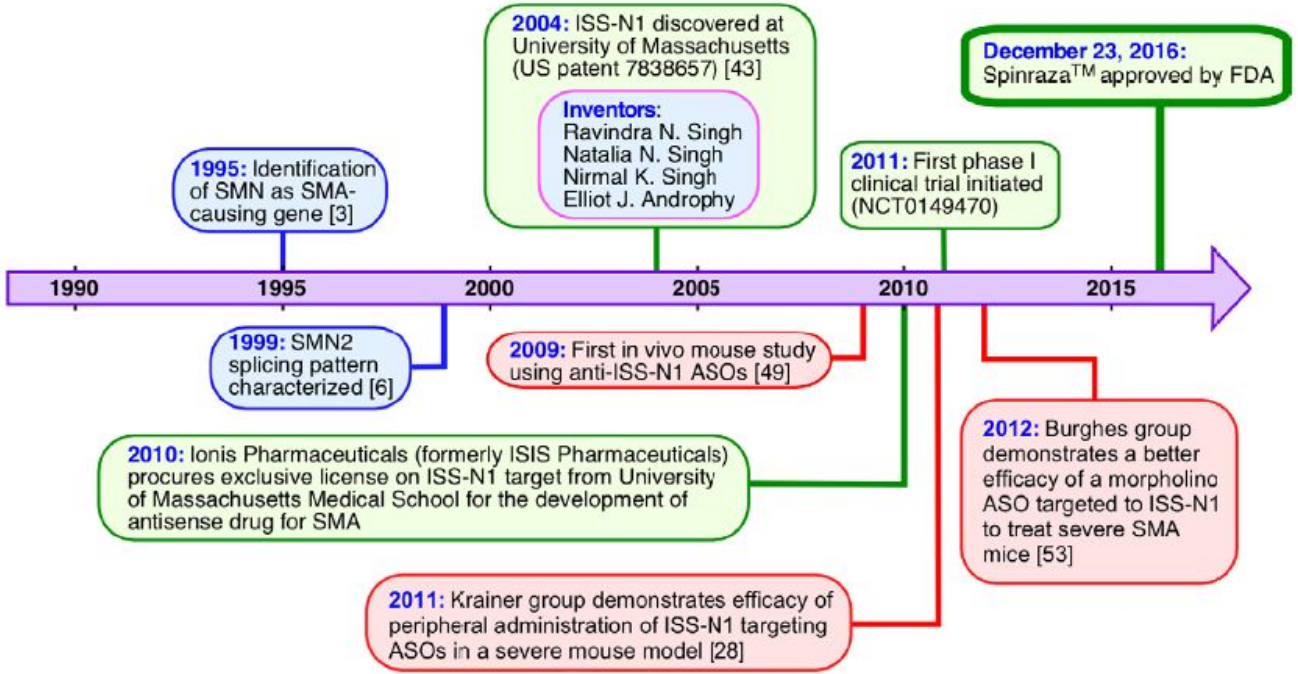
Different splicing of SMN1 and SMN2



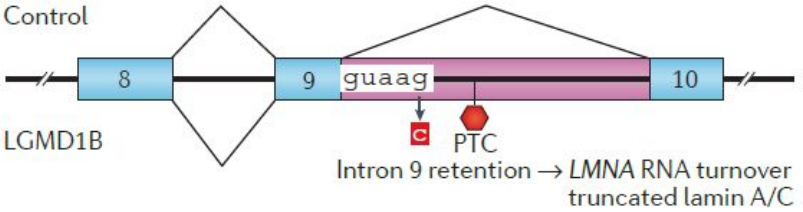
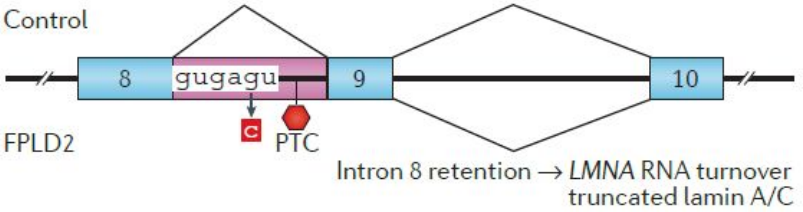
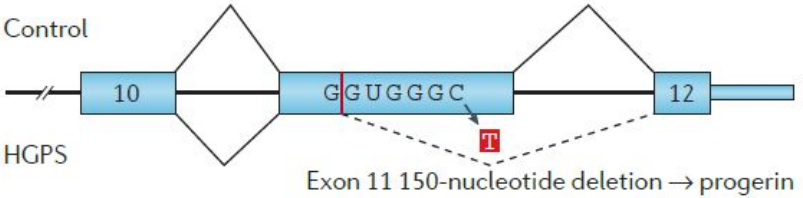
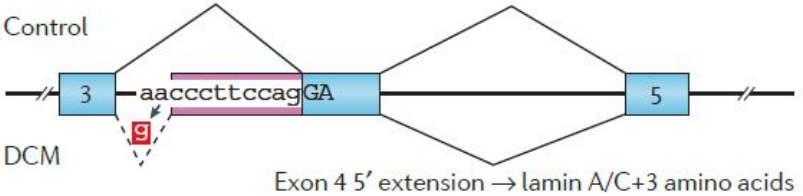
How Spinraza (nusinersen) works



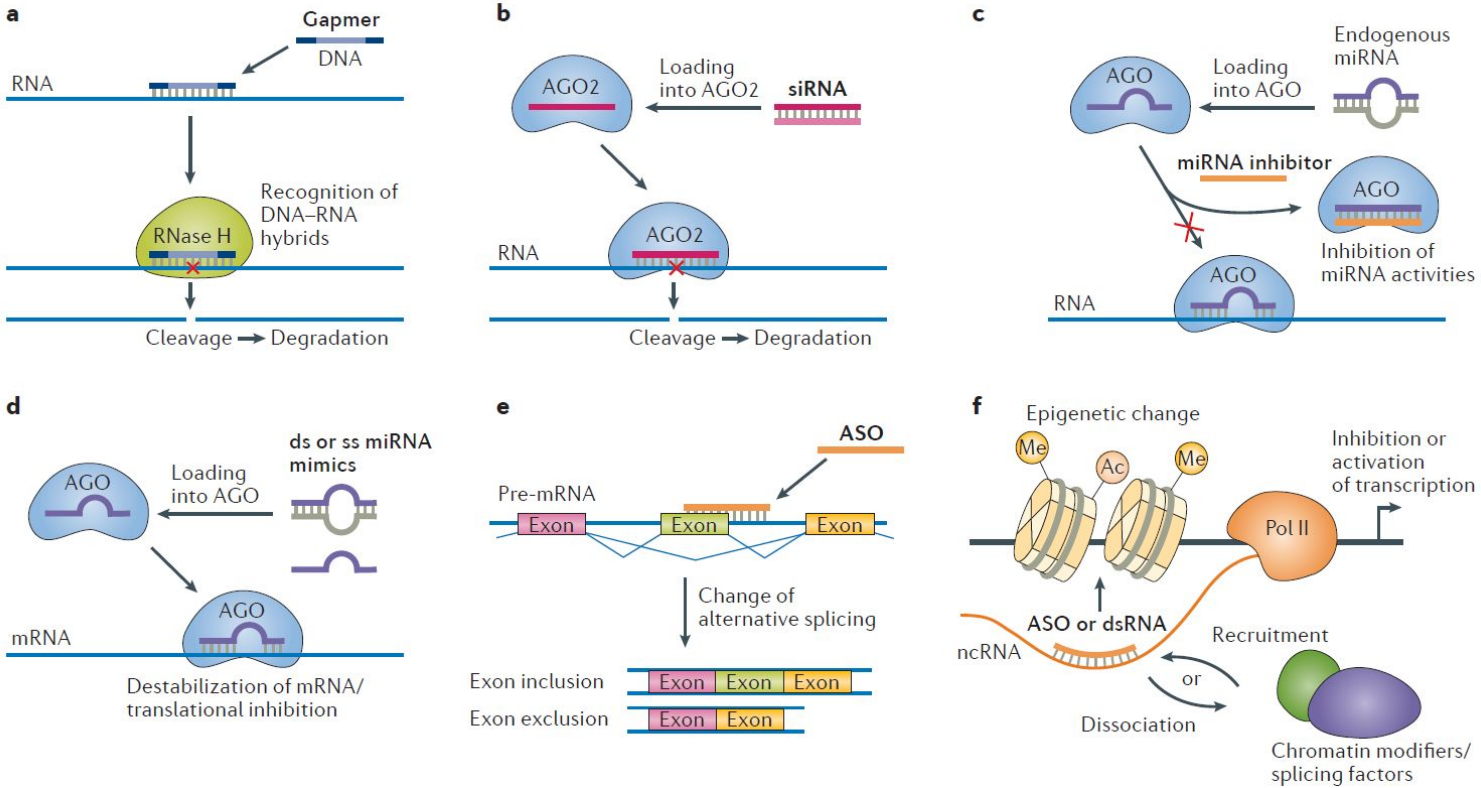
It takes 21 years to go from a molecular model to a population model



Splicing modifying oligo-nucleotide and other RNA therapeutics have strong disease relevances

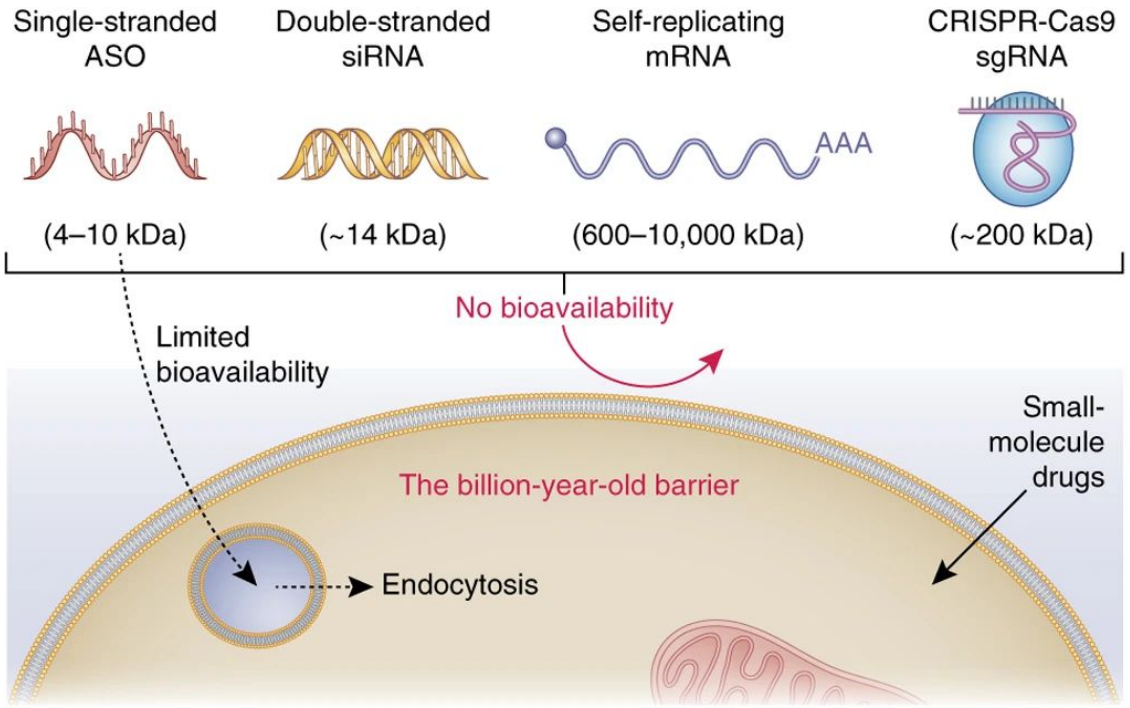
Disease	*LMNA* mutation	Mis-splicing
Limb girdle muscular dystrophy 1B (LGMD1B)	Mutant 5′ss (c. 1608+5G>C)	
Familial partial lipodystrophy type 2 (FPLD2)	Mutant 5′ss (c. 1488+5G>C)	
Hutchinson-Gilford progeria syndrome (HGPS)	Alternative 5′ss (c. 1824C>T)	
Dilated cardiomyopathy (DCM)	Alternative 3′ss (c. 640-10A>G)	

Regulating RNA levels or splicing with ASOs and duplex RNAs

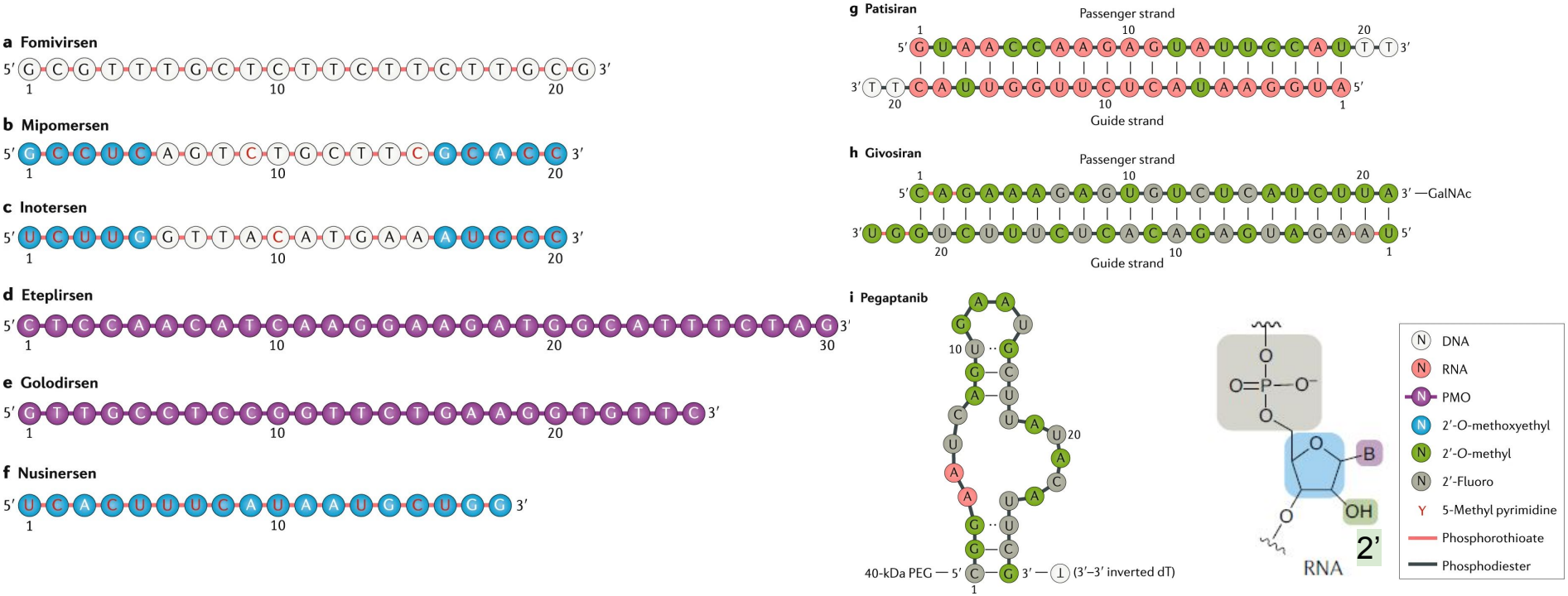


The four-billion-year-old barrier to RNA therapeutic

- Too large and charged to pass lipid bilayers
- Degradable by RNases
- Rapid clearance from liver and kidney
- Immunogenicity
- Endocytosis
- Delivery into organs other than liver and eye

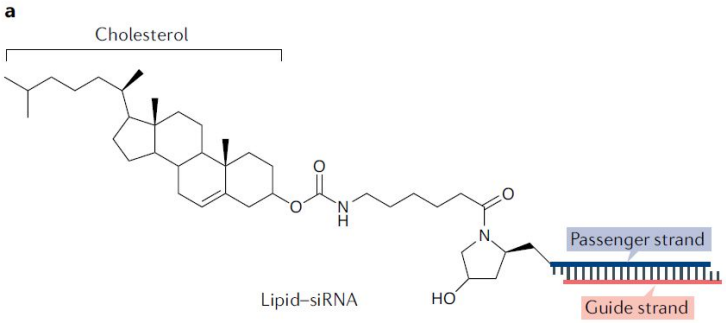


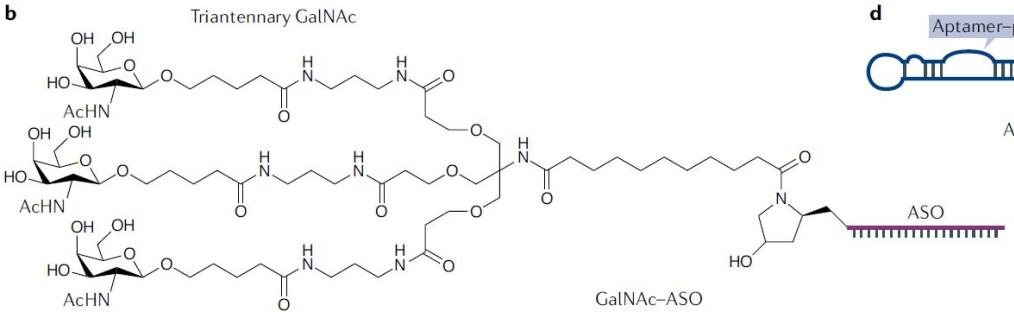
Chemistry of oligonucleotides evolves with time

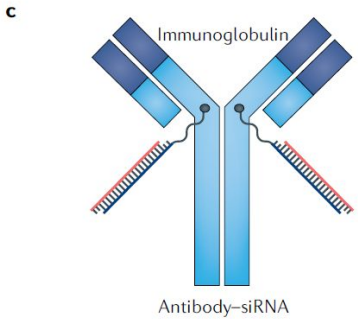


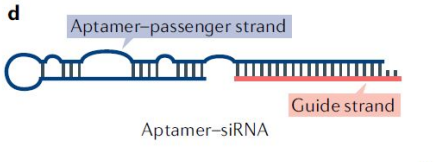
PMO=phosphorodiamidate morpholino oligomer

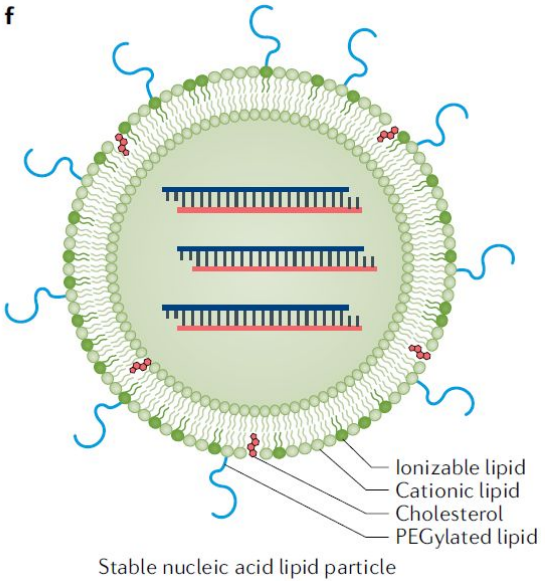
Delivery systems of antisense oligonucleotides





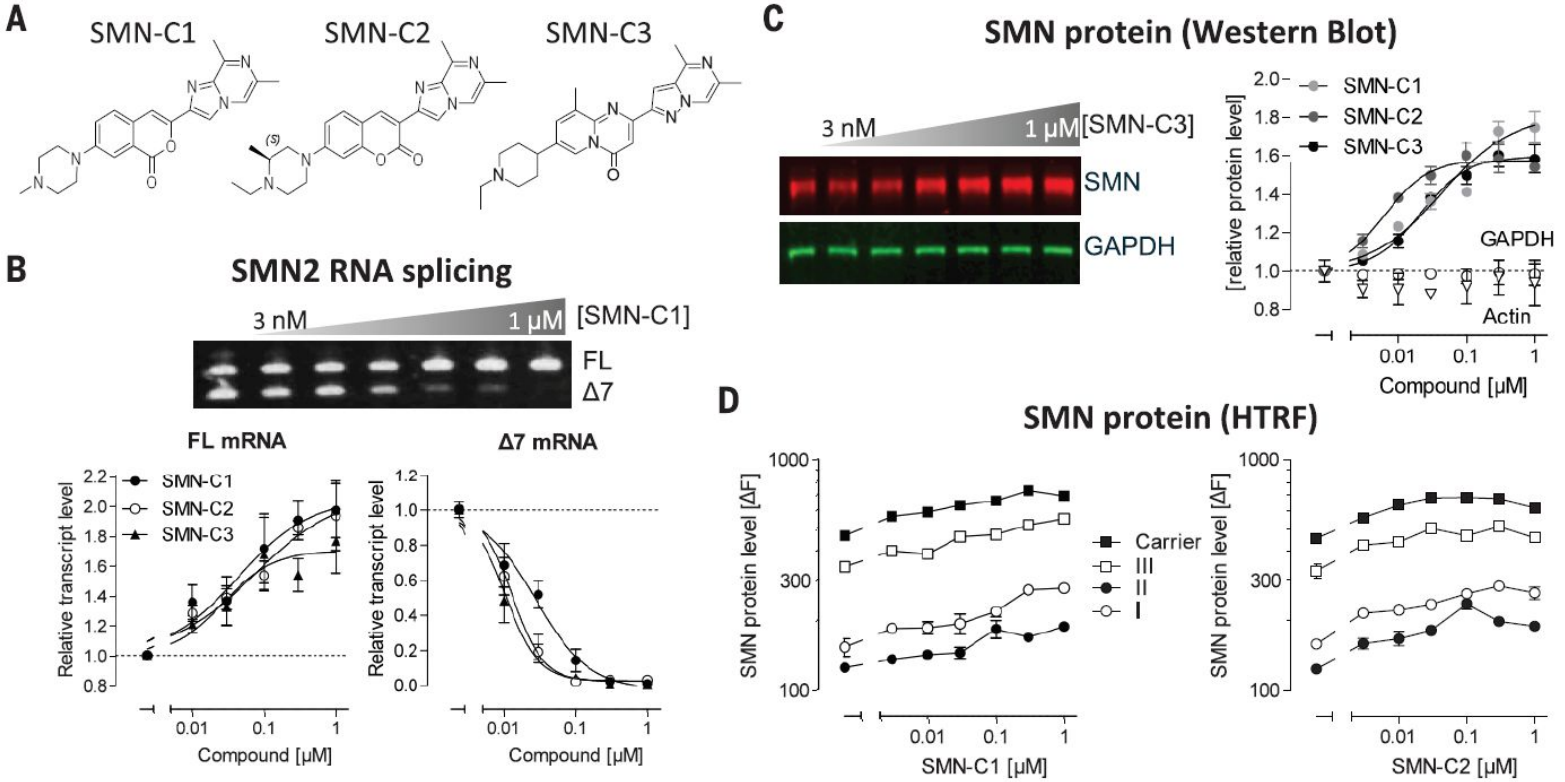




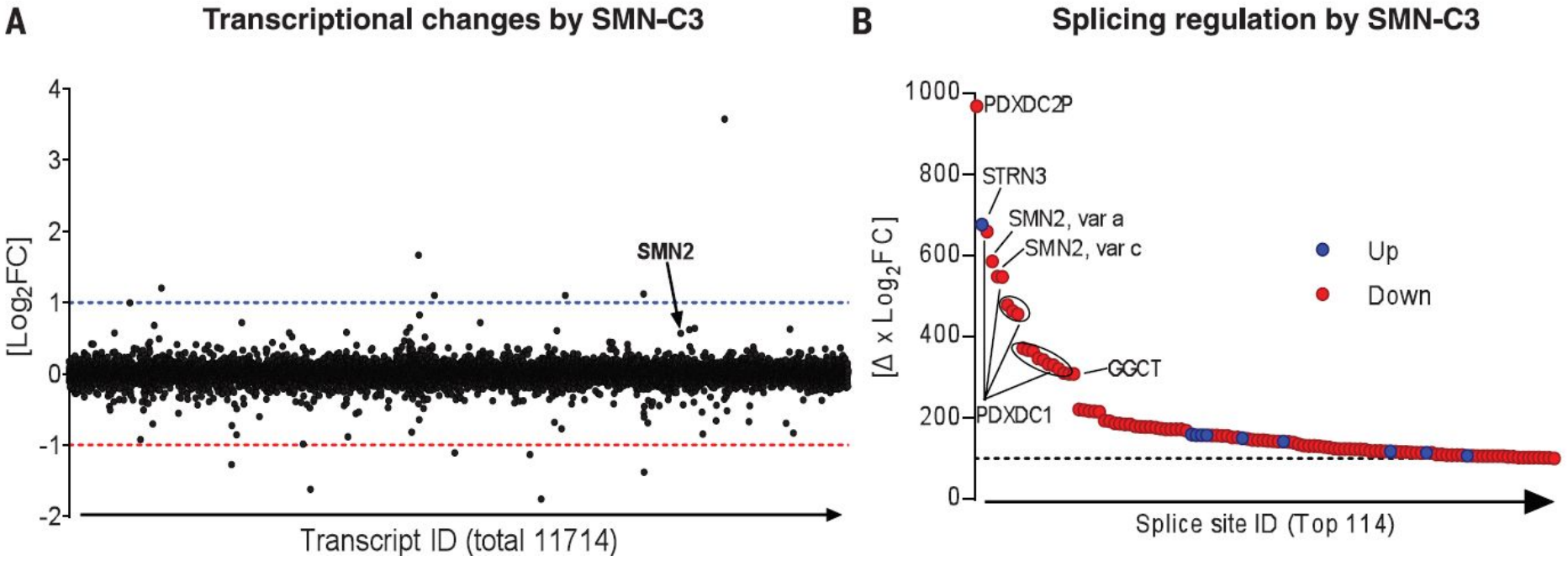


lipid nanoparticles

Small molecules as RNA splicing modifiers

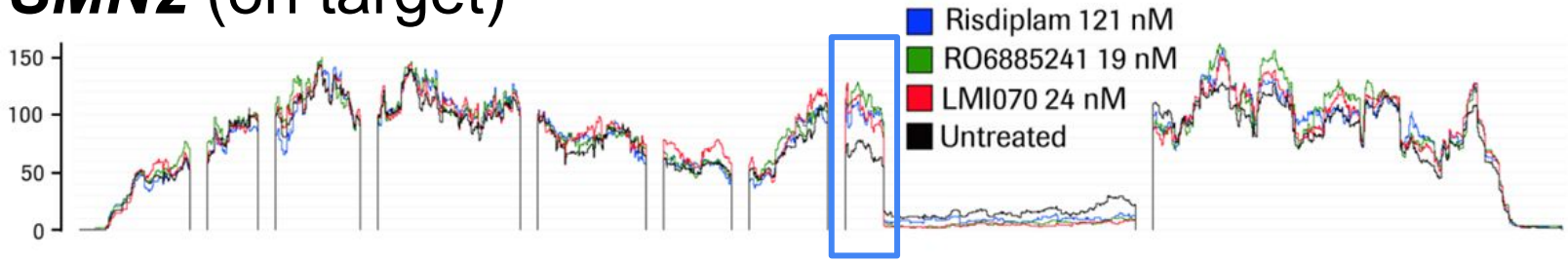


RNA sequencing confirms the specificity of SMN-C3

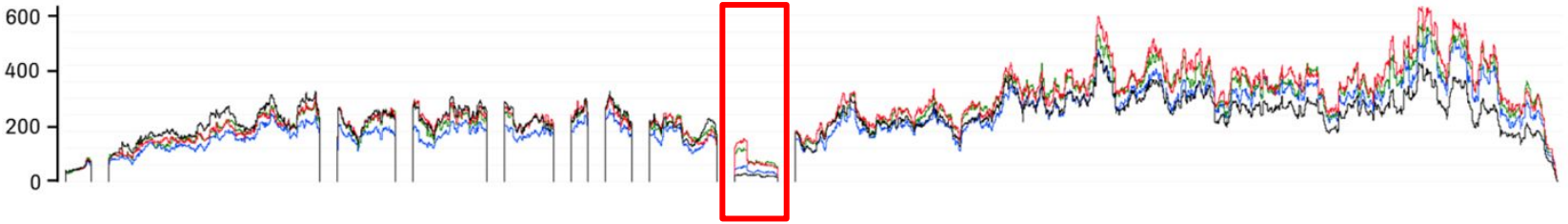


RNA sequencing confirms the specificity of SMN-C3

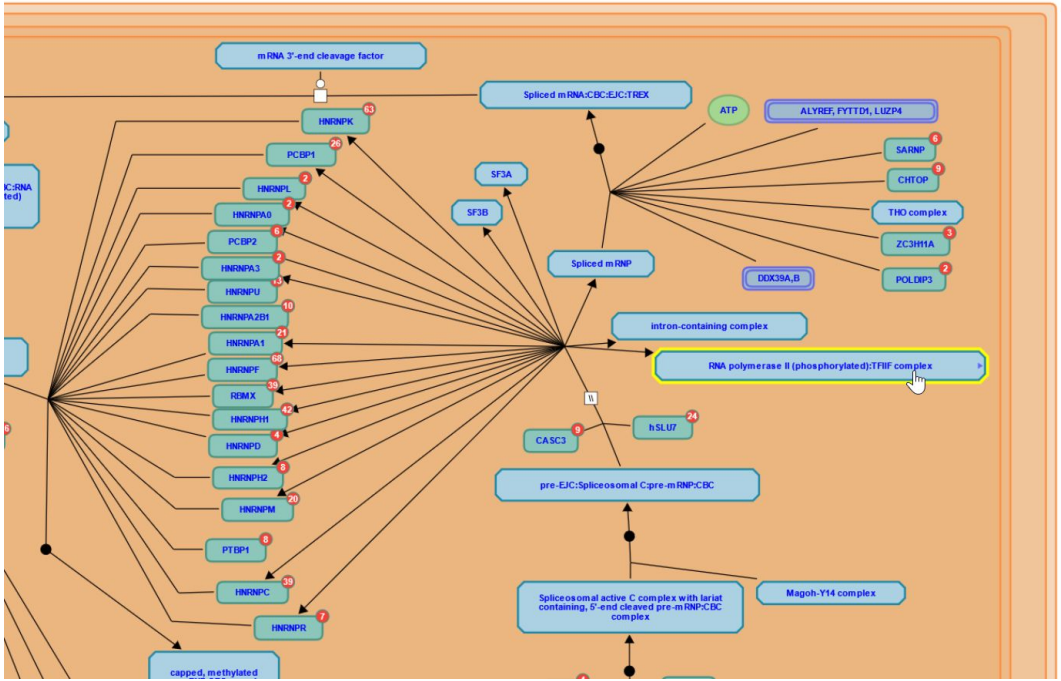
SMN2 (on target)

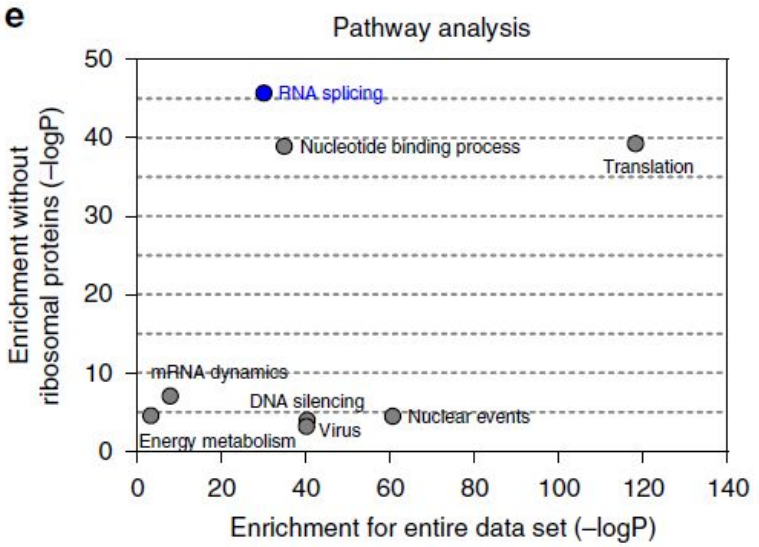


FOXM2 (off target)



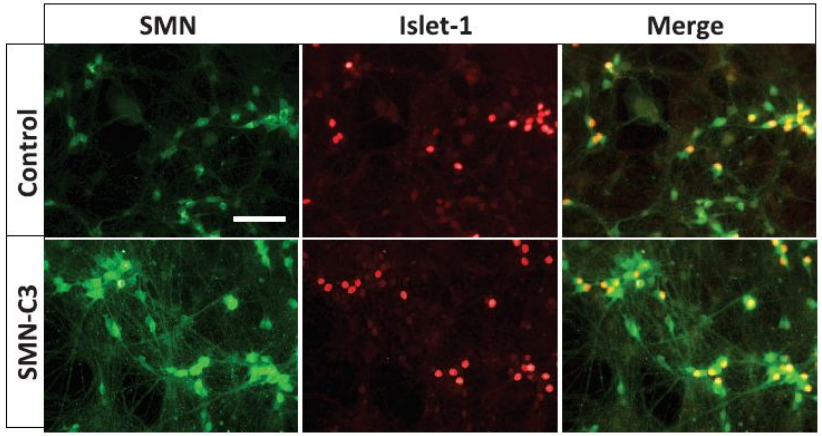
Gene-enrichment analysis confirms specific regulation of RNA splicing

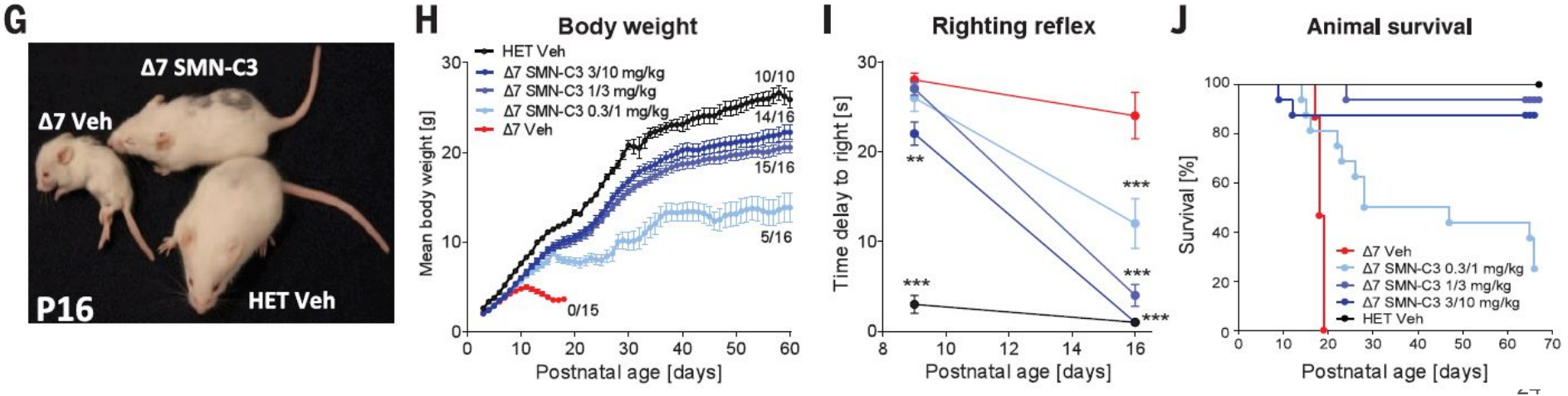




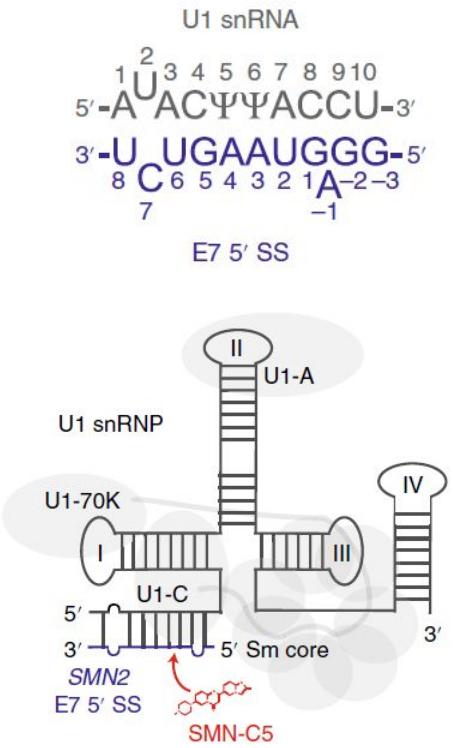
[Part of the mRNA splicing pathway in Reacome]

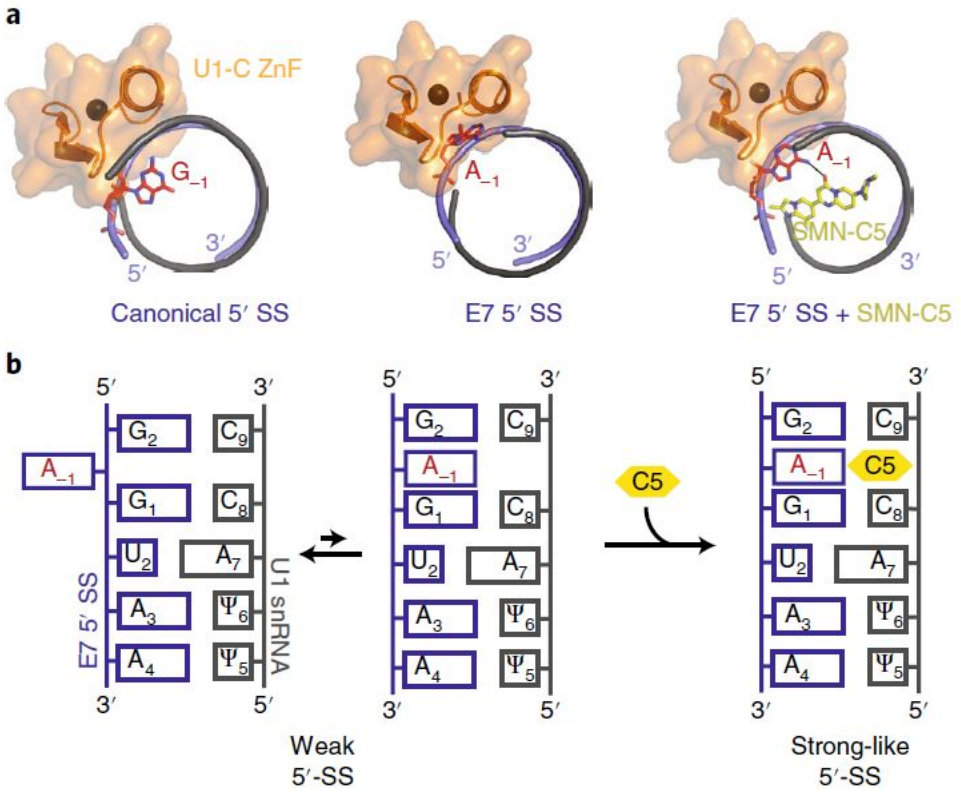
Experiments *in vitro* and *in vivo* support efficacy profiles of SMN-C3





Structural basis of specific splicing correction





Clinical trial (FIREFISH Part 1) Results

Table 1. Demographic and Clinical Characteristics of the Patients at Baseline.*

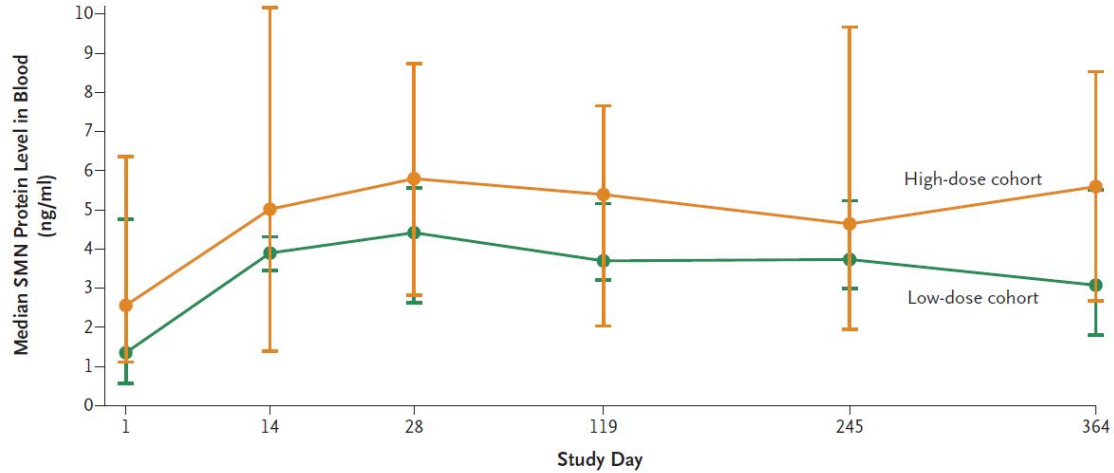
Characteristic	Low-Dose Cohort (N=4)	High-Dose Cohort (N=17)	All Infants (N=21)
Sex — no. (%)			
Female	4 (100)	11 (65)	15 (71)
Male	0	6 (35)	6 (29)
Median age (range) — mo			
At onset of symptoms	2.7 (2.0–3.0)	1.5 (0.9–3.0)	2.0 (0.9–3.0)
At diagnosis	3.3 (2.5–5.1)	3.0 (0.9–5.4)	3.0 (0.9–5.4)
At enrollment	6.9 (6.7–6.9)	6.3 (3.3–6.9)	6.7 (3.3–6.9)
Motor measures†			
Median CHOP-INTEND score (range)	23.5 (10–25)	24 (16–34)	24 (10–34)
Median HINE-2 score (range)	1 (0–3)	1 (0–2)	1 (0–3)
Respiratory support — no. (%)	0	5 (29)‡	5 (24)‡

Note: Table 2 is not complete

Table 2. Adverse Events.*

Event	Infants (N=21)
Total no. of adverse events	202
≥1 Adverse event — no. (%)	21 (100)
Total no. of serious adverse events	24
≥1 Serious adverse event — no. (%)	10 (48)
≥1 Adverse event of grade 3–5 — no. (%)	9 (43)
Serious adverse event with fatal outcome — no. (%)†	3 (14)
Most common adverse events — no. (%)‡	
Pyrexia	11 (52)
Upper respiratory tract infection	9 (43)
Diarrhea	6 (29)
Cough	5 (24)

Clinical trial (FIREFISH Part 1) Results



No. of Patients						
High-dose cohort	16	13	17	15	15	15
Low-dose cohort	4	3	3	3	3	3

Median SMN Protein Level in Blood (range) — ng/ml						
High-dose cohort	2.54 (1.10–6.40)	5.01 (1.42–10.22)	5.87 (2.84–8.76)	5.44 (2.03–7.71)	4.64 (1.95–9.67)	5.66 (2.66–8.60)
Low-dose cohort	1.31 (0.58–4.82)	3.87 (3.45–4.37)	4.49 (2.61–5.55)	3.67 (3.24–5.21)	3.71 (2.97–5.24)	3.05 (1.75–5.51)

Figure 1. SMN Protein Concentration in Whole Blood.

Blood was mixed with lysis buffer in a 1:1 ratio. I bars indicate the range. The data-cutoff date was February 27, 2019. SMN denotes survival of motor neuron.

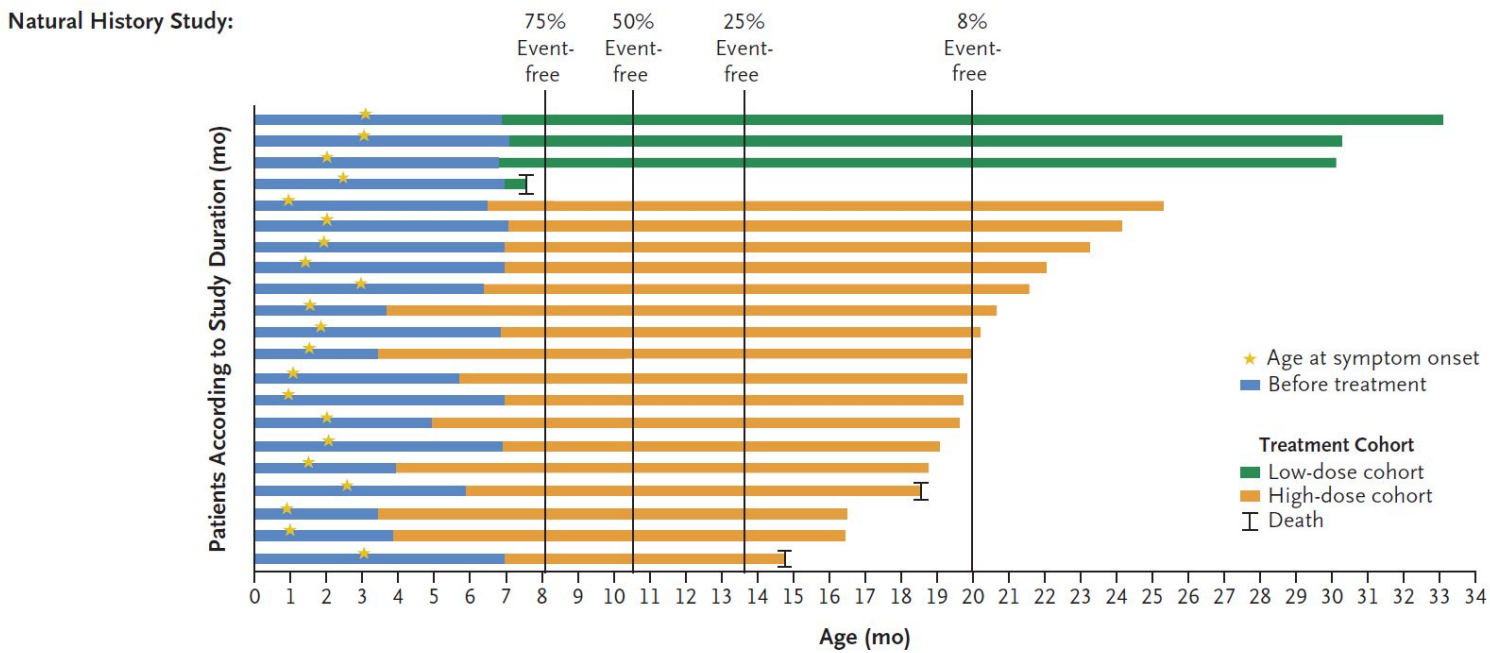


Figure 2. Event-free Survival.

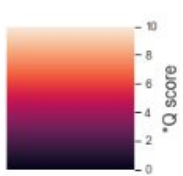
Event-free survival was defined as being alive and not receiving permanent ventilation (tracheostomy or ventilation [bilevel positive airway pressure] for ≥16 hours per day continuously for >3 weeks or continuous intubation for >3 weeks, in the absence of, or after the resolution of, an acute reversible event). The percentages of patients who were event-free in a previous natural history study of spinal muscular atropy[7] are shown at the top of the graph for comparison. The median age at the combined outcome among patients in the previous study who had two copies of *SMN2* was 10.5 months (interquartile range, 8.1 to 13.6); event-free survival in that study was defined as being alive and not receiving noninvasive ventilation for 16 hours or more per day continuously for 2 or more weeks. The duration of our study was measured from the date of enrollment to the data-cutoff date. As of the data-cutoff date, three infants (one in the low-dose cohort and two in the high-dose cohort) had died; one additional infant in the high-dose cohort died after that date (Table S5).

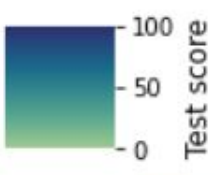
A fallible fairy tale about intelligence

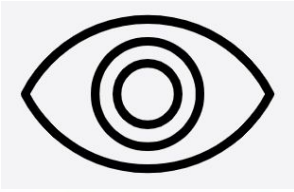
IQ Intellectual intelligence

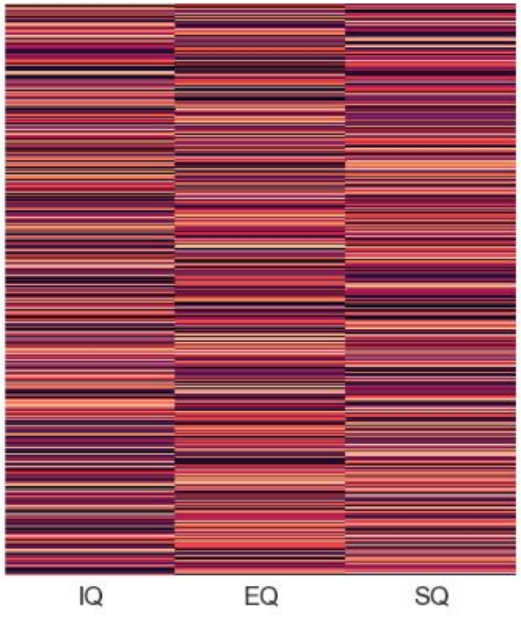
EQ Emotional intelligence

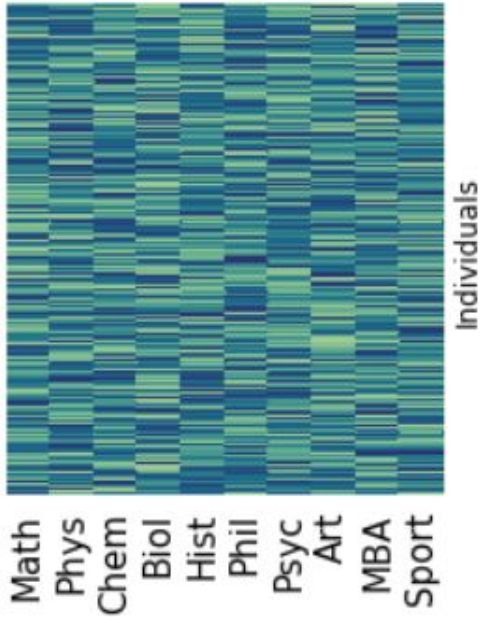
SQ Spiritual intelligence



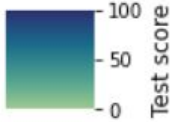


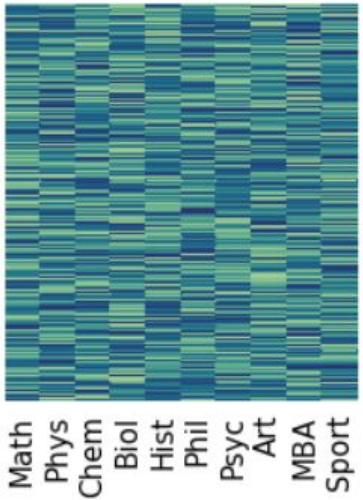






We may infer *Q if *Qs are independent and test scores are largely determined by individual *Qs





Math = 8 × IQ + 2 × EQ + 2 × SQ + ε

Philosophy = 6 × IQ + 2 × EQ + 8 × SQ + ε

…

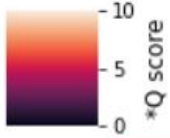
MBA = 4 × IQ + 7 × EQ + 5 × SQ + ε

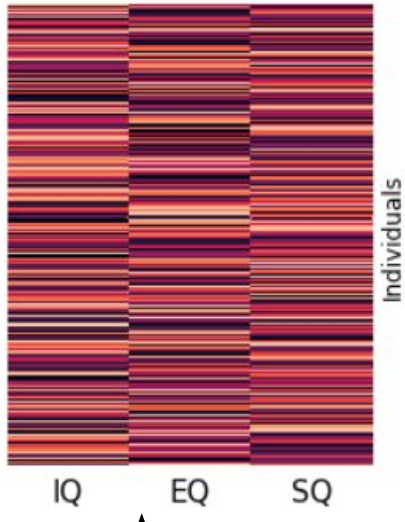
Sport = 5 × IQ + 6 × EQ + 5 × SQ + ε

Numbers in blue: loadings

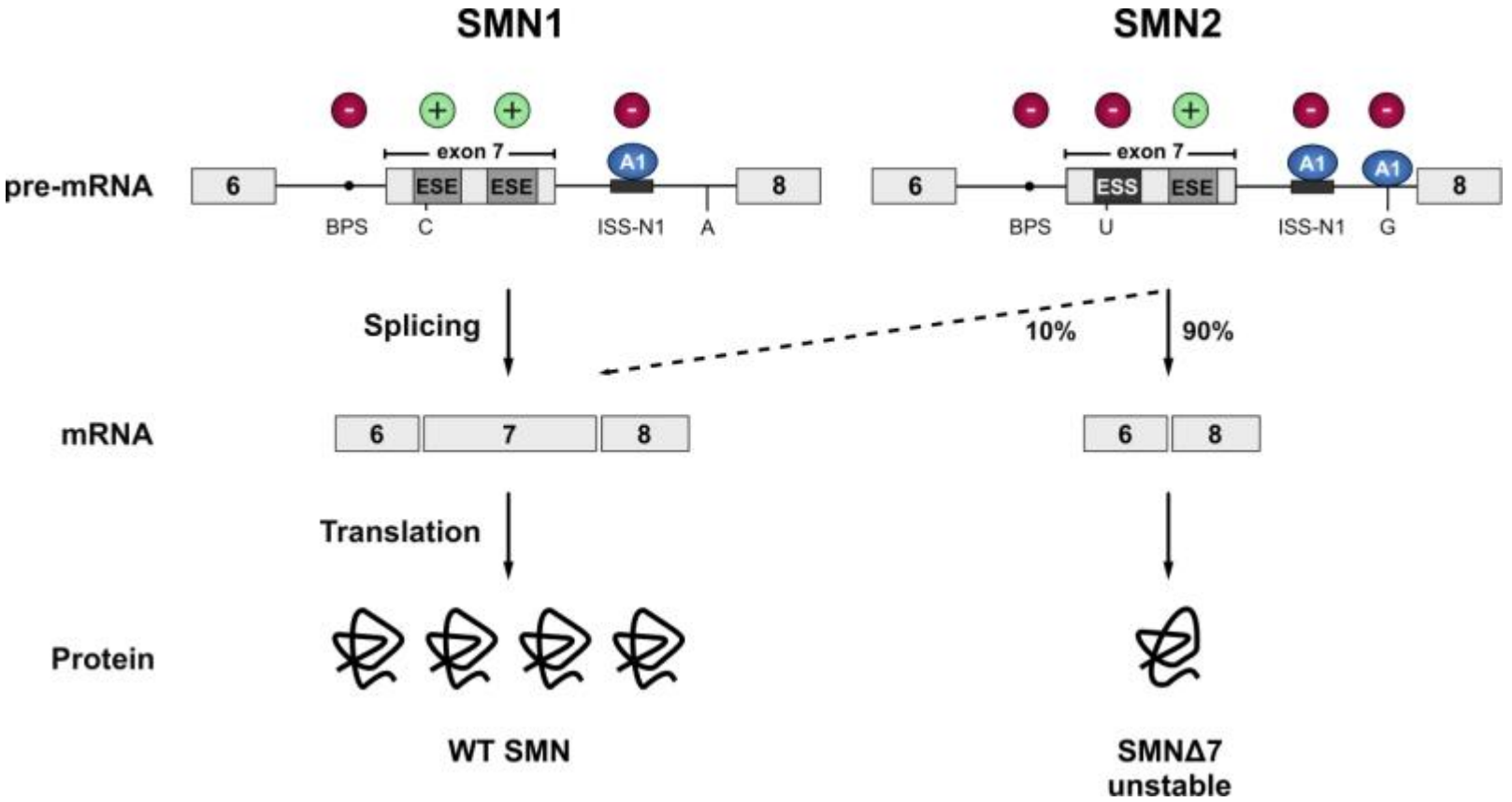
IQ/EQ/SQ: factors

Factor analysis



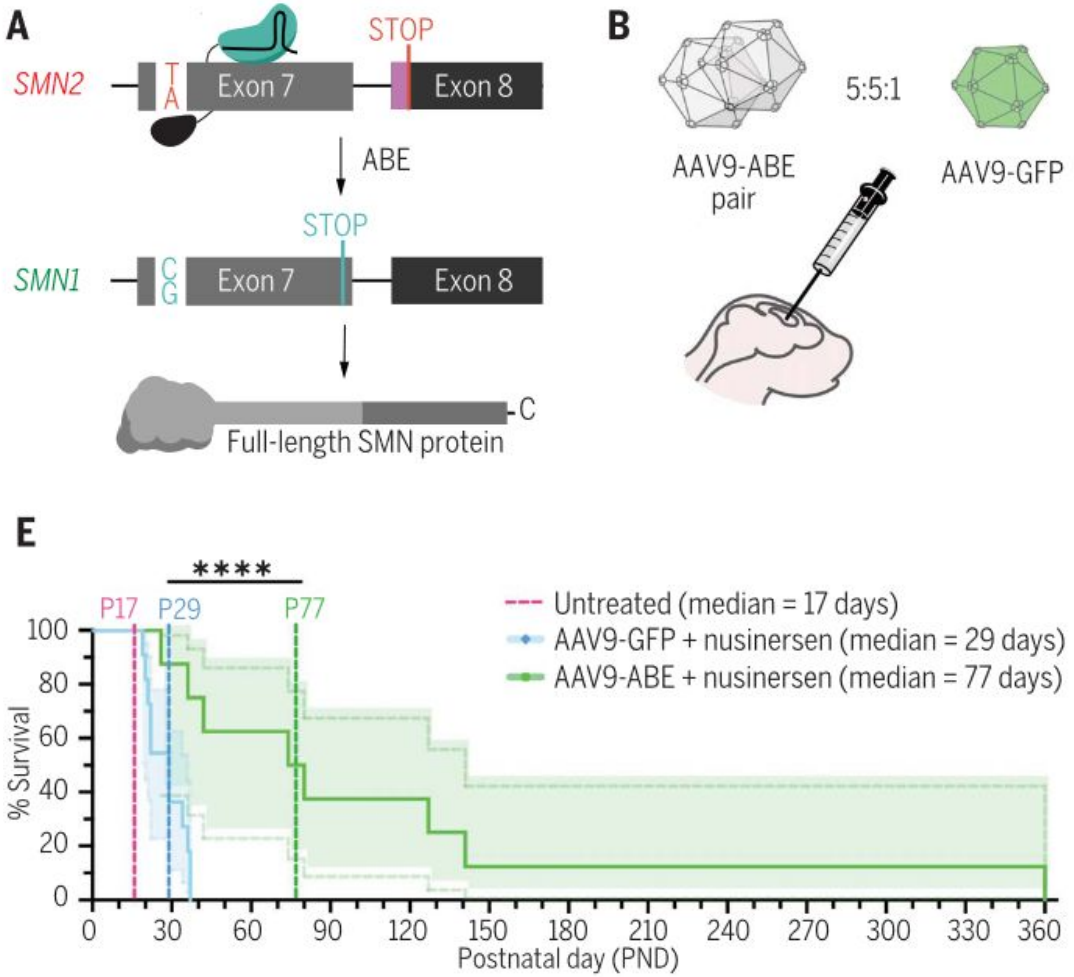


Different splicing of SMN1 and SMN2



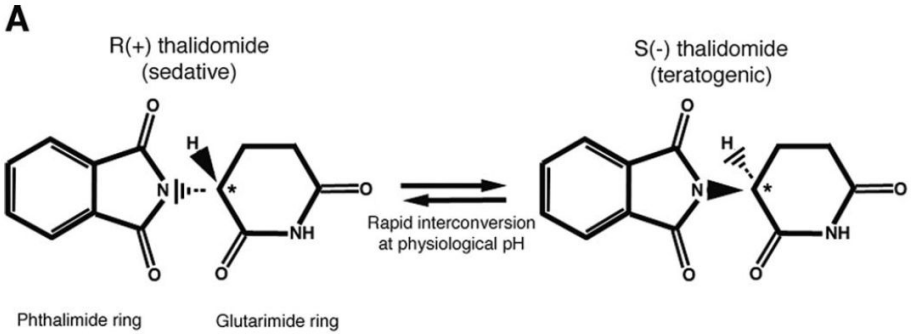
Base editing rescue of spinal muscular atrophy in cells and in mice

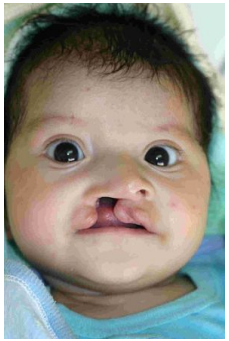
Arbab *et al.*, Science, April 2023

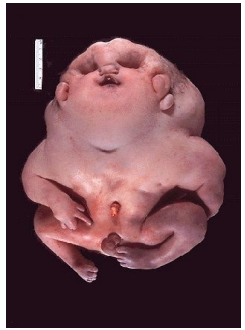


End of lecture on April 11th, 2025

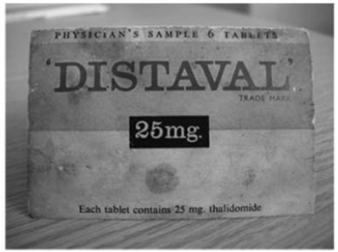
The Tragedy of teratogenic S(-) thalidomide in 1950s



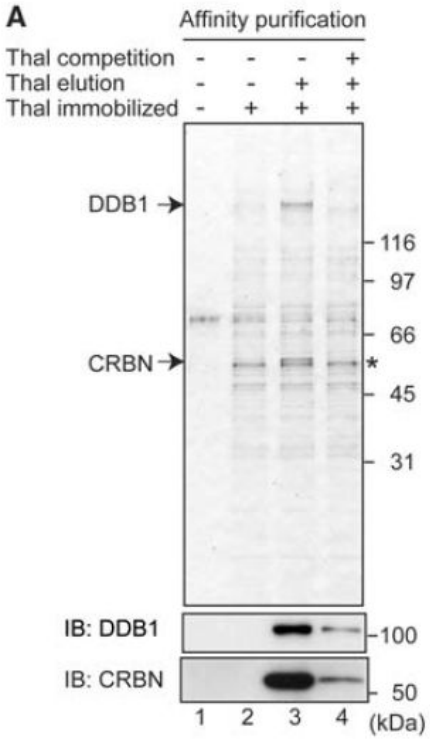


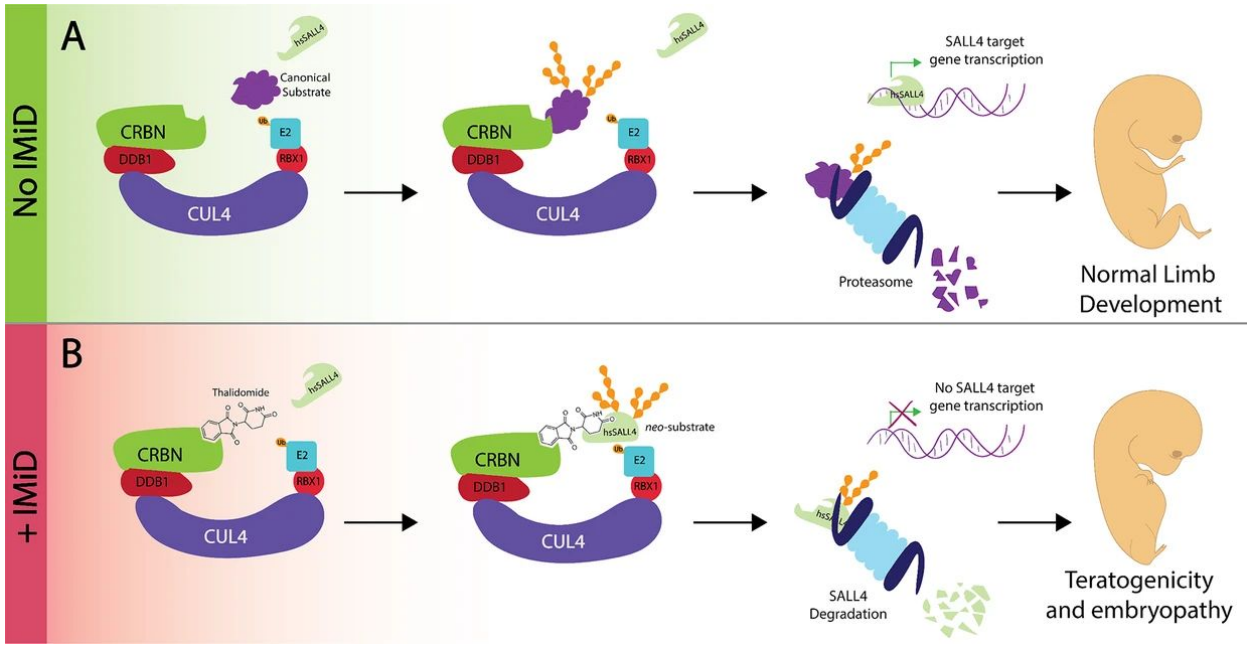


B



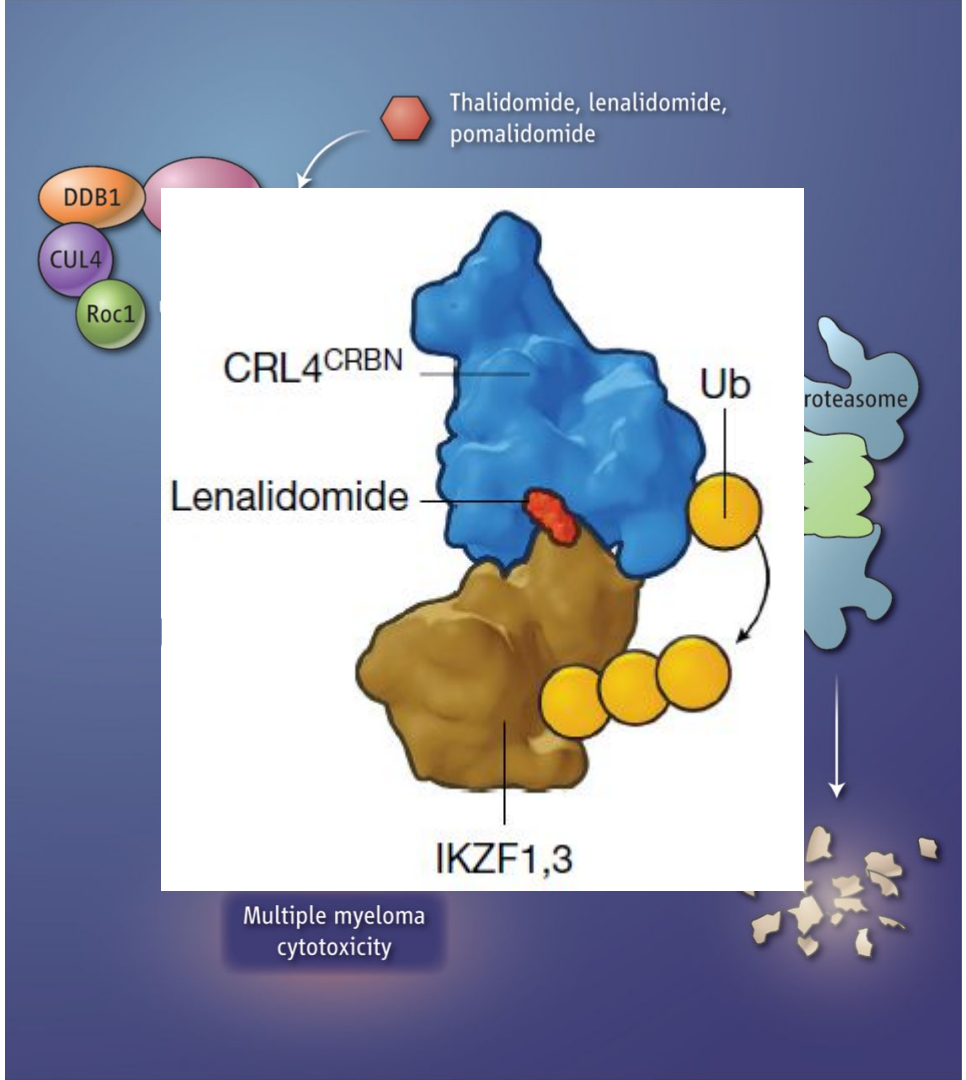
Molecular basis of the teratogenicity of thalidomide reported in 2010





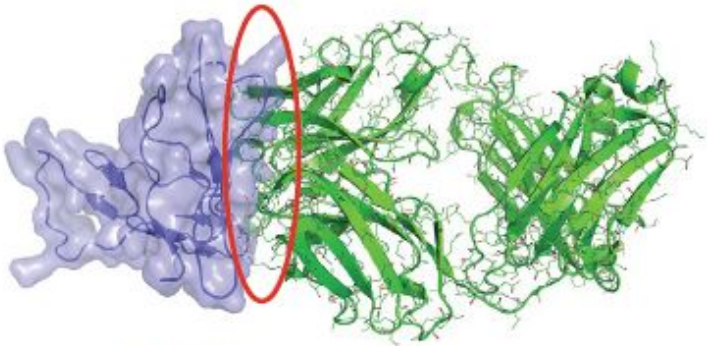
The same mechanism is responsible for efficacy against blood cancers

Thalidomide and derivatives bring proteins IKZF1 and IKZF3 close to E3 ubiquitin ligase, leading them to be degraded.



Multispecific Drug Use or Target Interactions

b Conventional drug:
- Forms 1 drug–target interface
- Can act throughout body
- Only works if its binding to target alters function of target

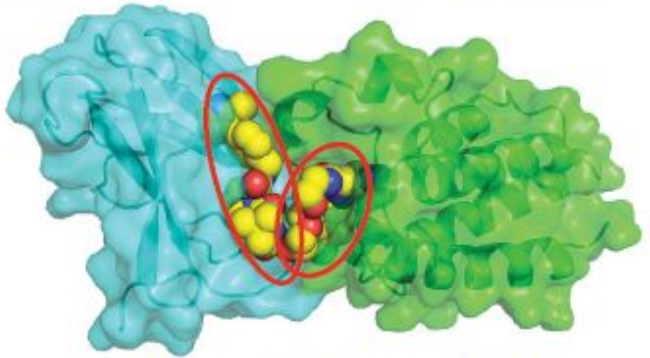


IL-2Rα Basiliximab

c Obligate multispecific drug:
- Forms 2 or more drug–target interfaces

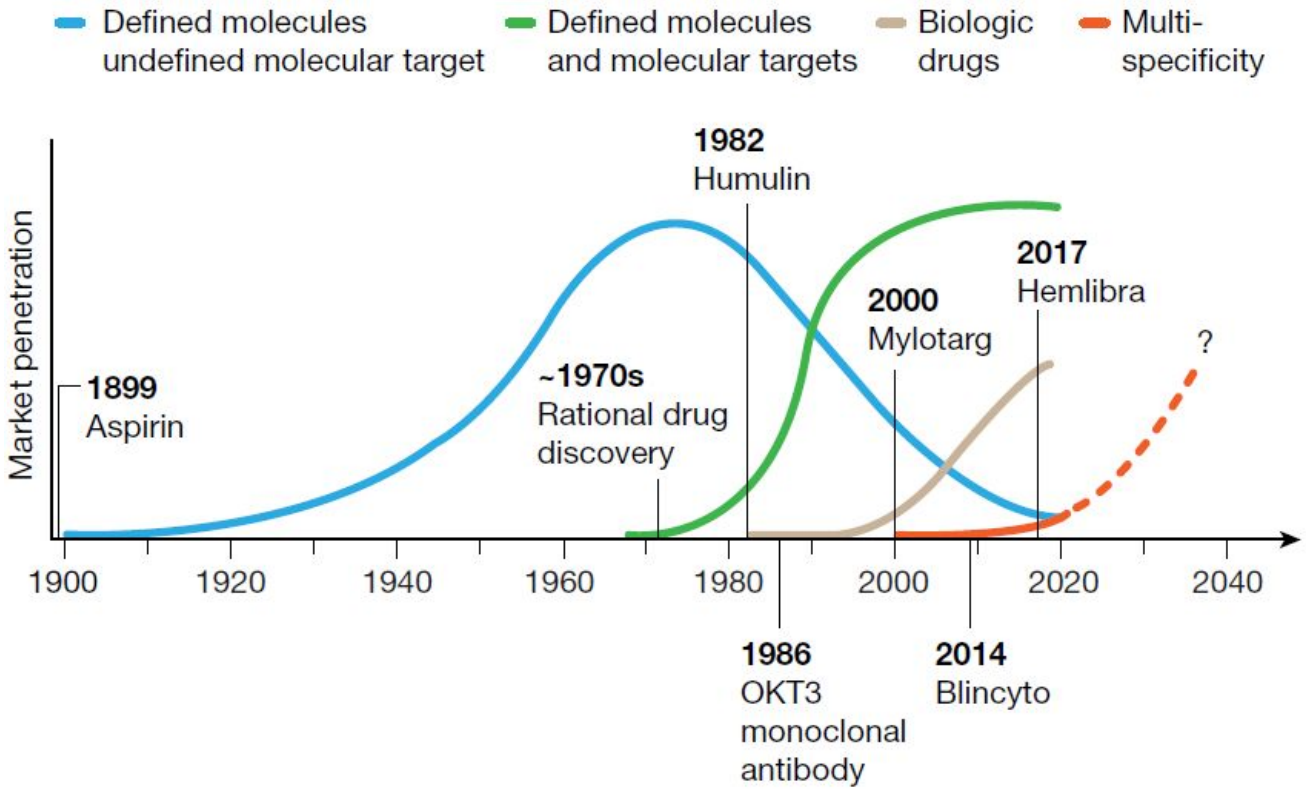
Class 1 'tetherbodies'
- Enrich drug at relevant site of action

Class 2 'matchmakers'
- Link drug to a biological effector

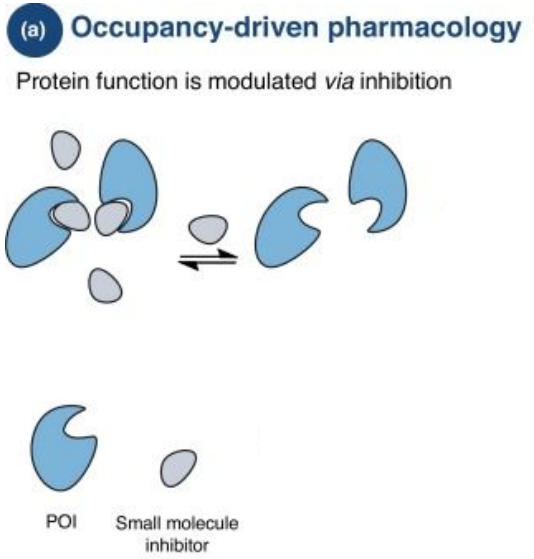


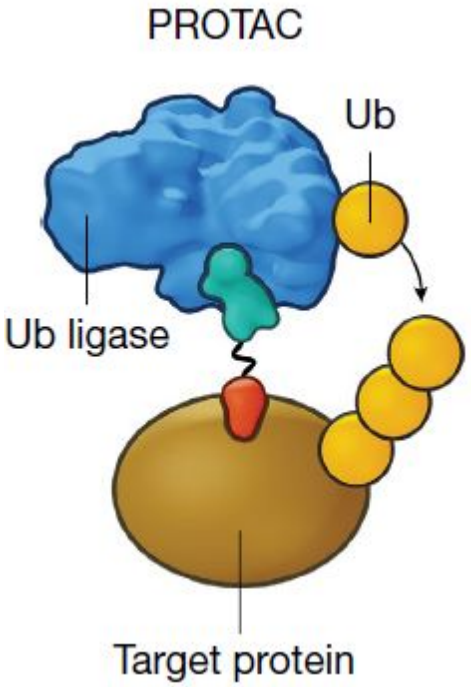
VHL MZ1 $BRD4^{BD2}$

Paradigm shifts and paradigm expansion



PROteolysis TArgeting Chimera (PROTAC)

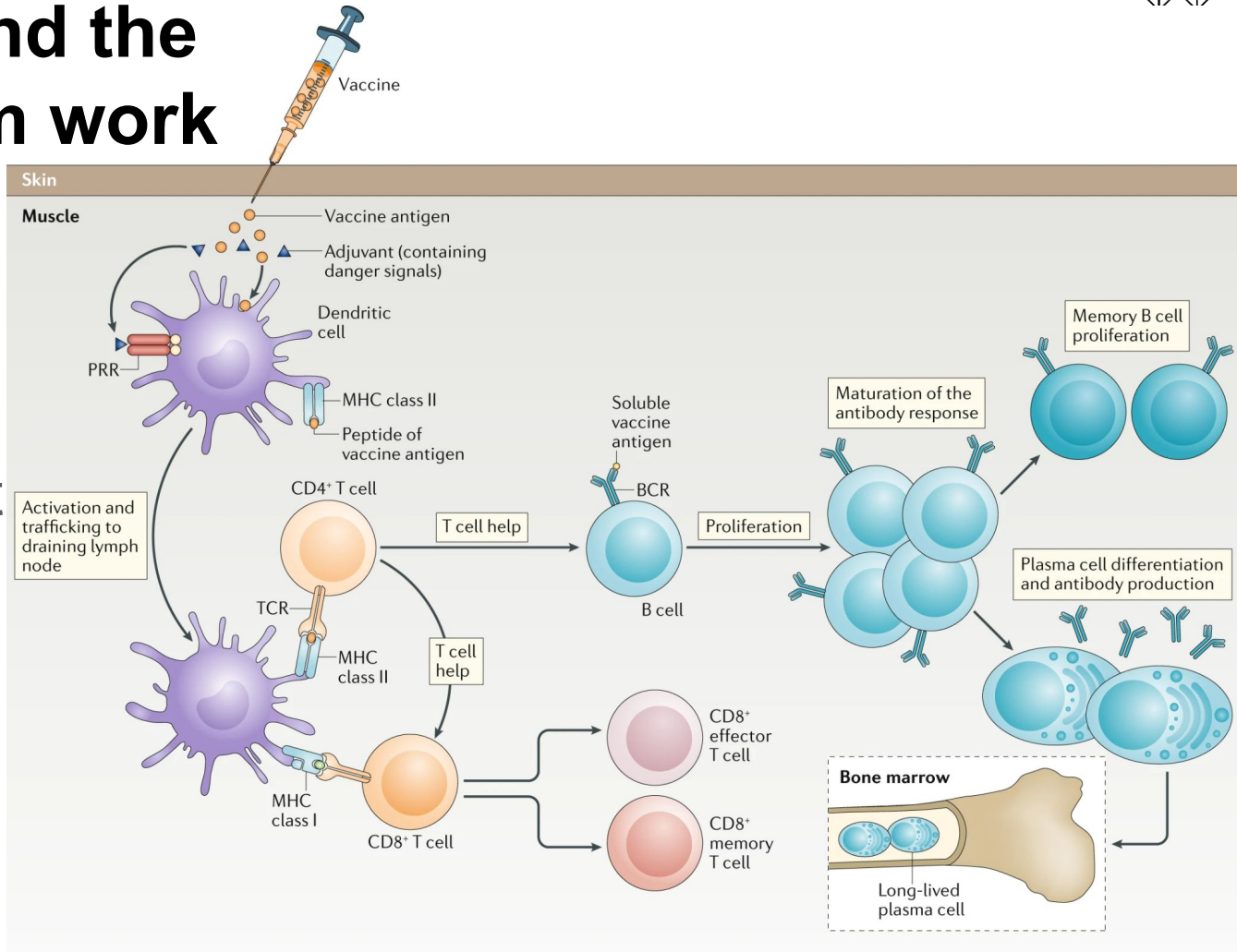




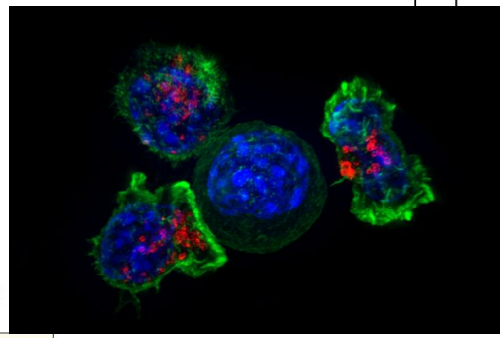
How vaccine and the immune system work

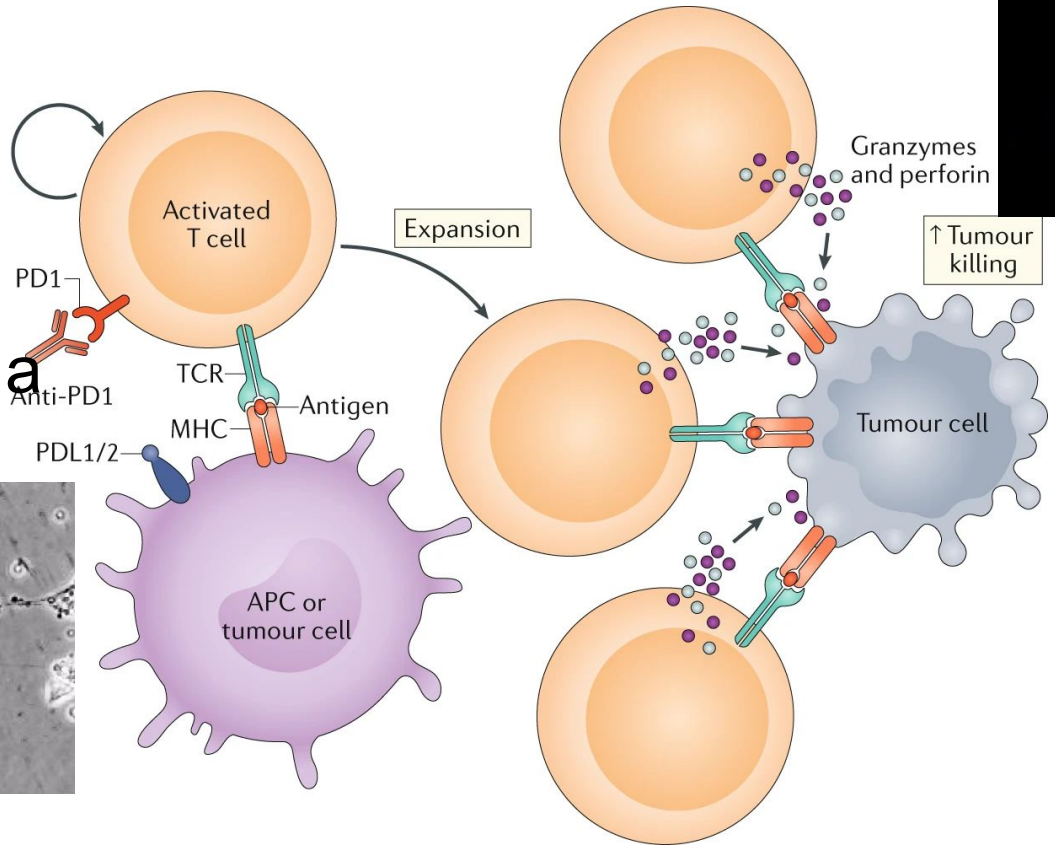
Key players:

1. Antigen-presenting cells (e.g. dendritic cells)
2. T cells
3. B cells



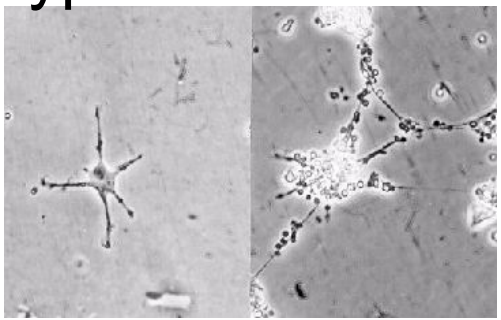
Antigen-presenting cells (APC) and T cells work together to kill tumour cells



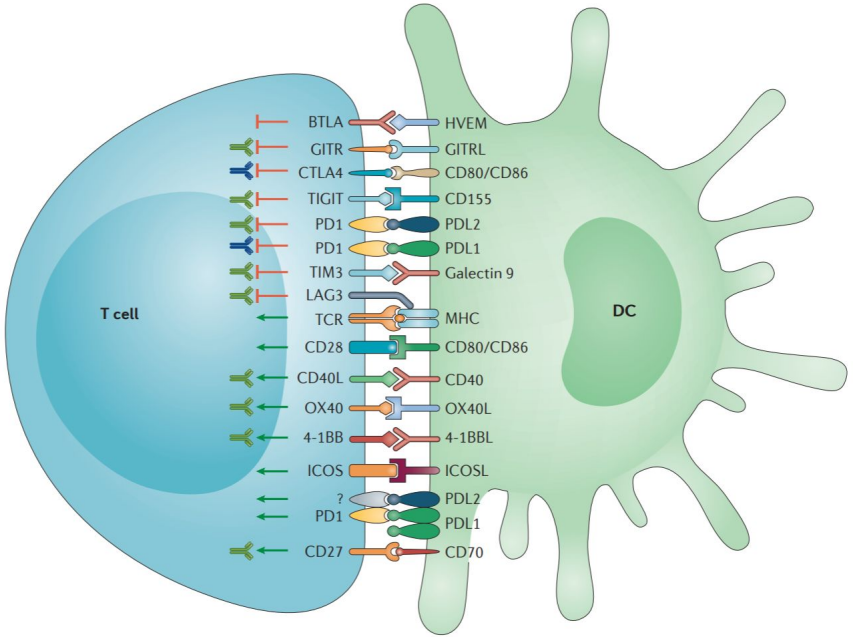


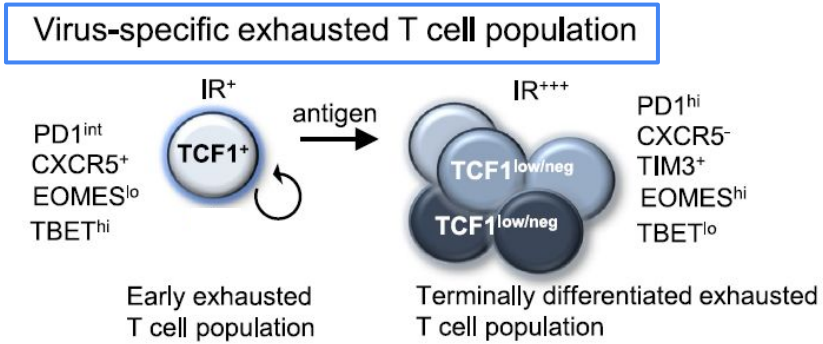
Killer T cells attacking a cancer cell

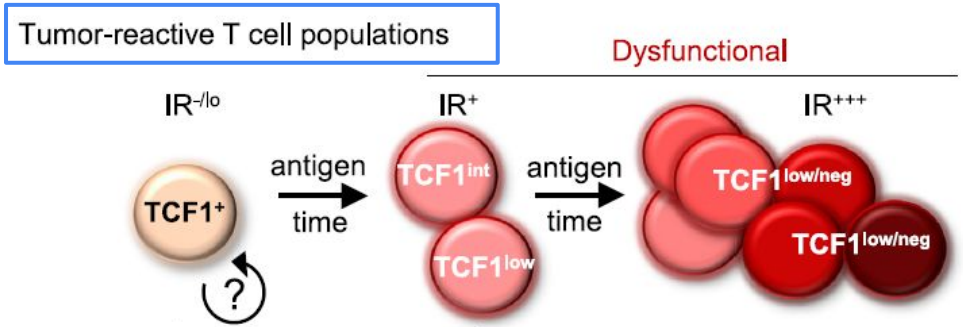
Dendritic cell, a type of APC



Exhausted T cells reduces immune system's capacity to clear pathogenic cells

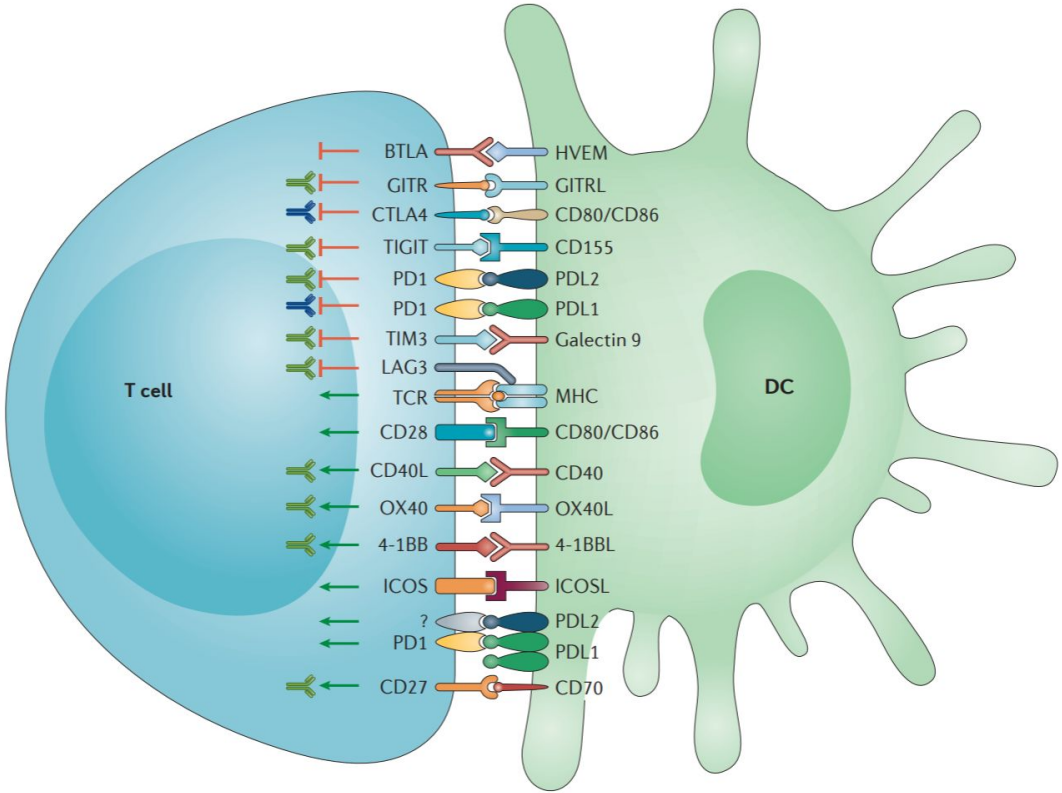


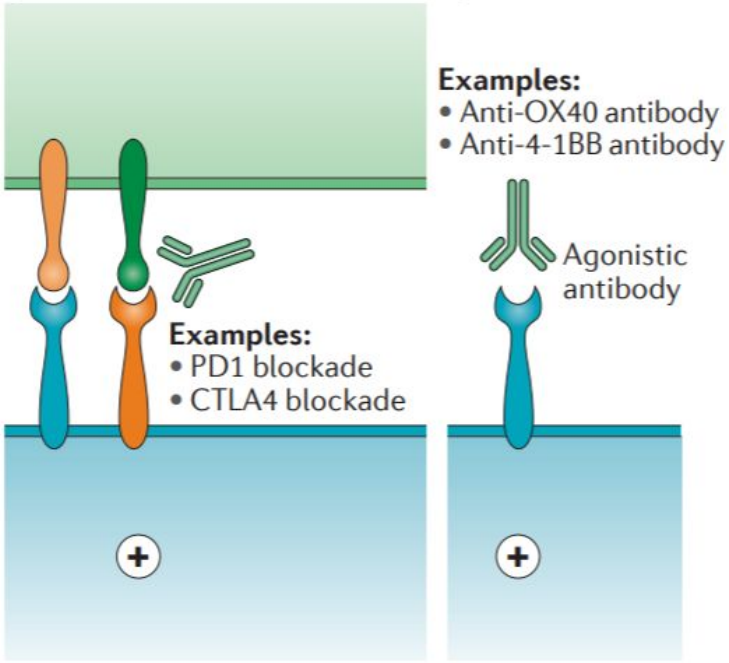




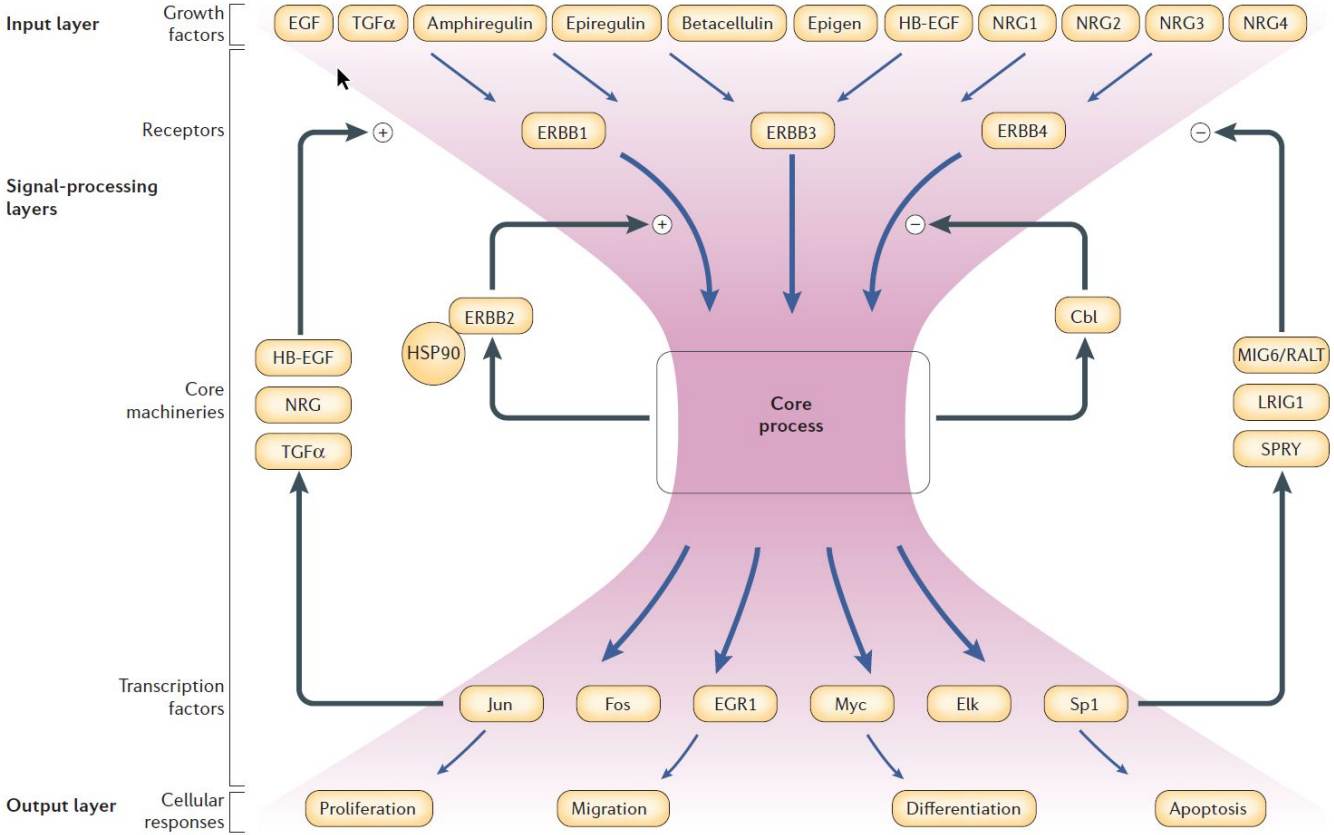
IR=inhibitory receptors (left panel). They are like 'breaks' controlled by dendritic cells.

Cancer Immunotherapy with immune checkpoints as drug targets





Why antibodies work like a wonder? The Bow-Tie model of signaling transduction



Extracellular

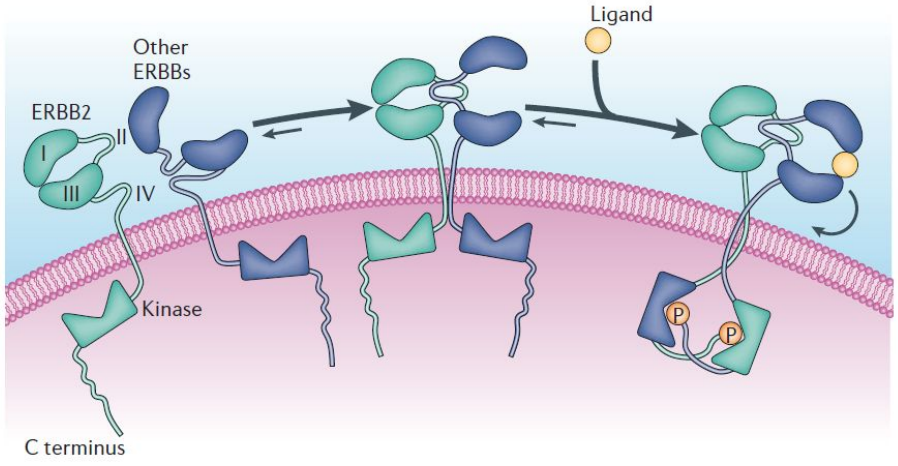
Cell Membrane

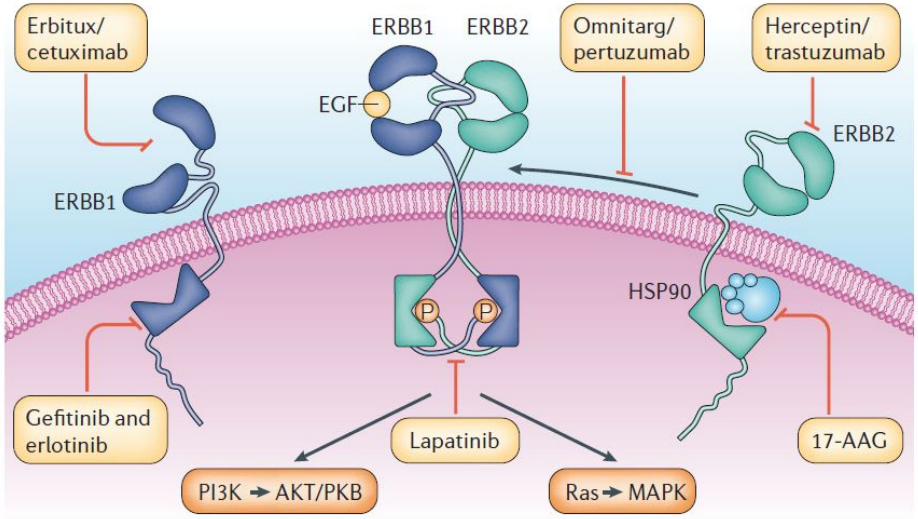
Cytoplasm

Nucleus

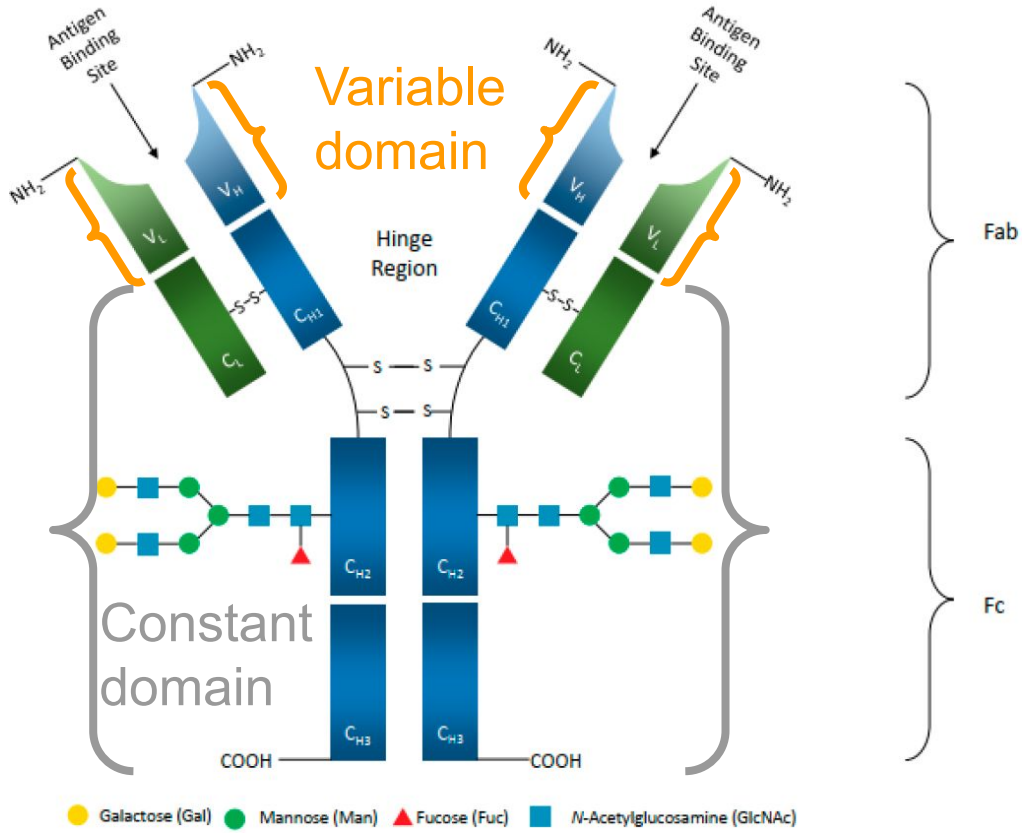
Everywhere

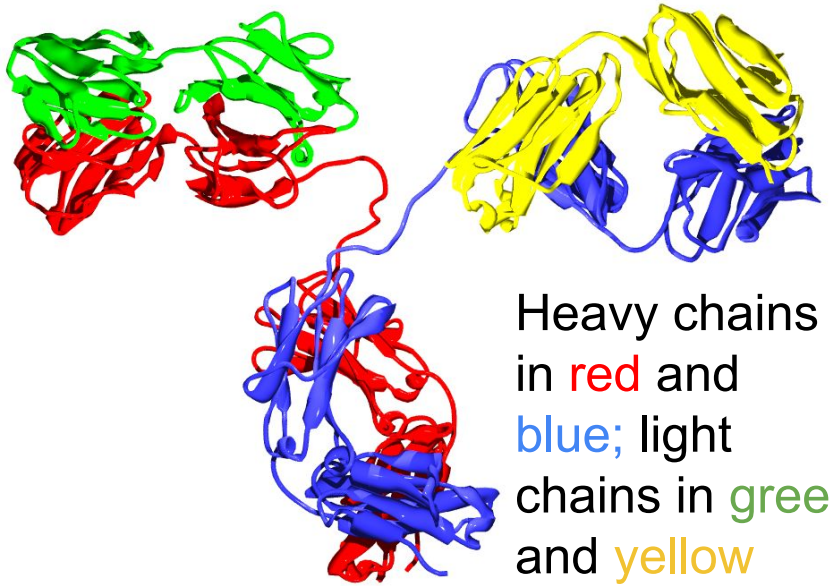
ERBB signaling system and antibody drugs





Structure of antibodies





Heavy chains in red and blue; light chains in green and yellow

Fc=fragment crystallizable region

Fab=fragment antigen binding

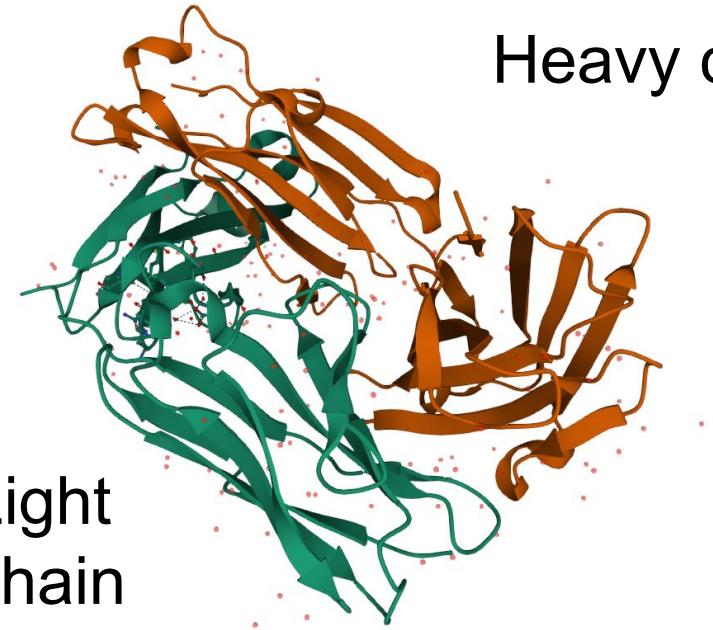
Cetuximab as an example

Variable heavy chain

QVQLKQSGPGLVQPSQSLSITCTVSGF
SLTNYGVHWVRQSPGKGLEWLGVIWSG
GNTDYNTPFTSRLSINKDNSKSQVFFK
MNSLQSNDTAIYYCARALTYYDYEFAY
WGQGTLVTVSA

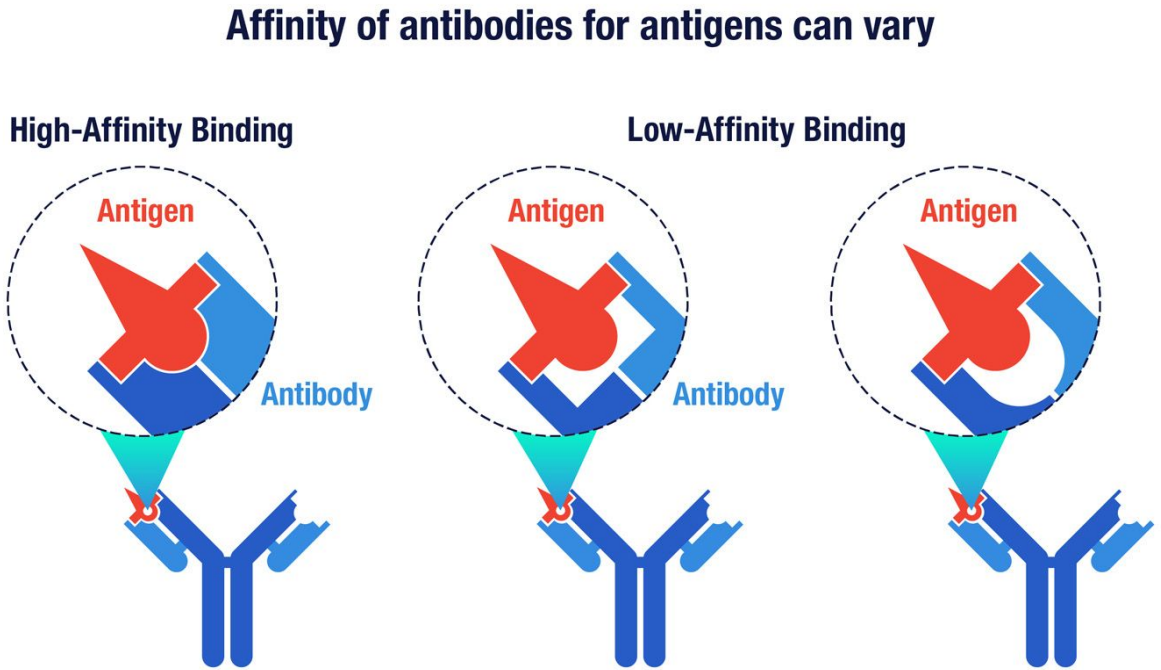
Variable light chain

DILLTQSPVILSVSPGERVSFSCRASQ
SIGTNIHWYQQRTNGSPRLLIKYASES
ISGIPSRFSGSGSGTDFTLSINSVESE
DIADYYCQQNNNWPTTFGAGTKLELK



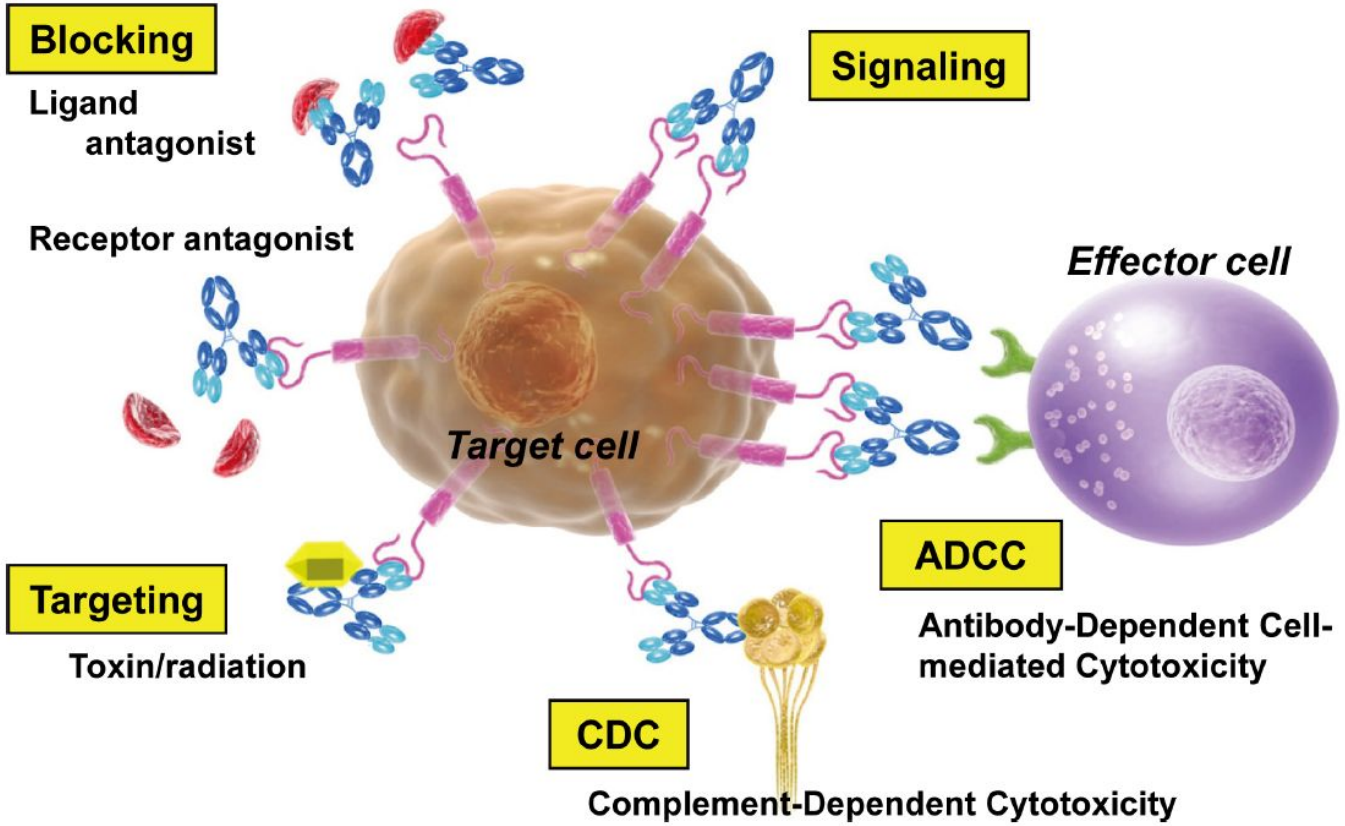
PDB 1YY8

Antibodies work by shape complementarity

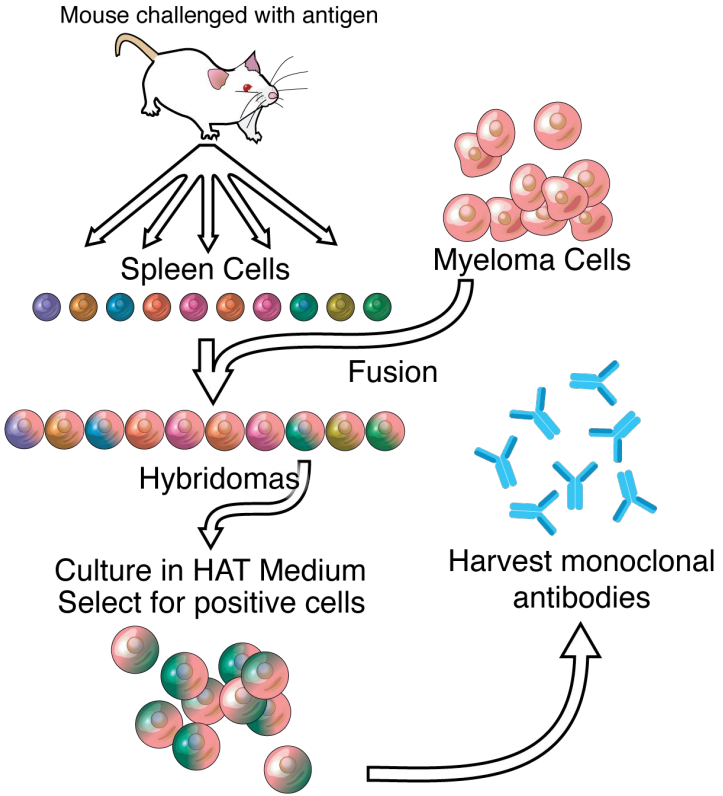


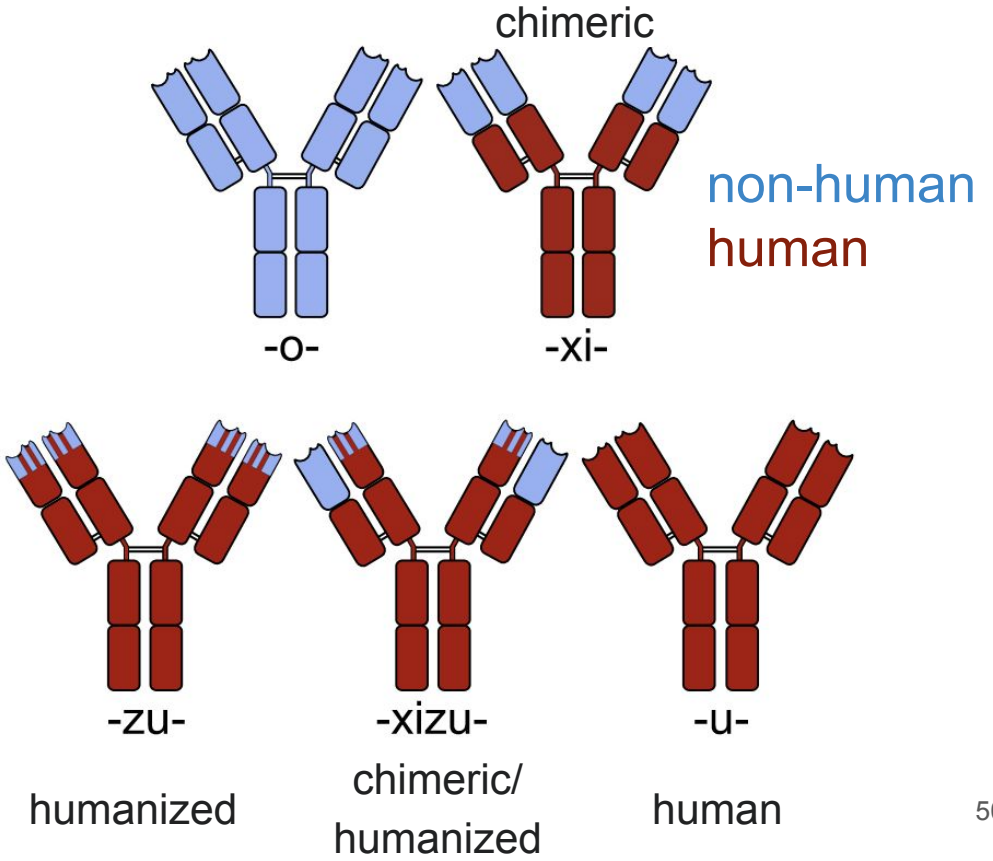
Recommended reading: 10 things to know about antibodies by Amgen

Mechanisms of action of therapeutic antibodies

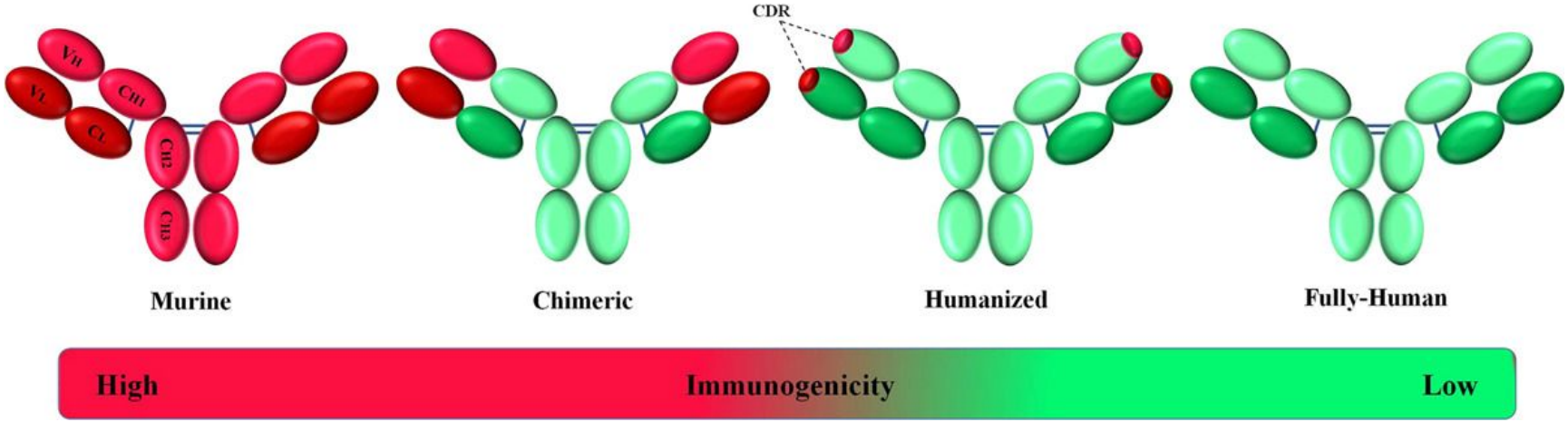


Therapeutic antibody discovery with hybridoma and humanization

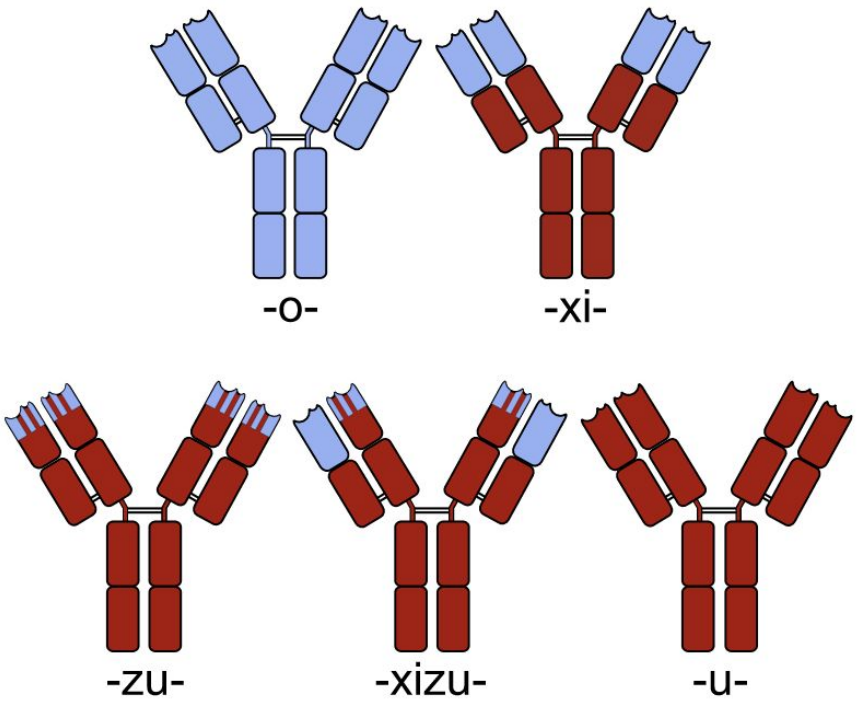




Evolution of therapeutic antibodies



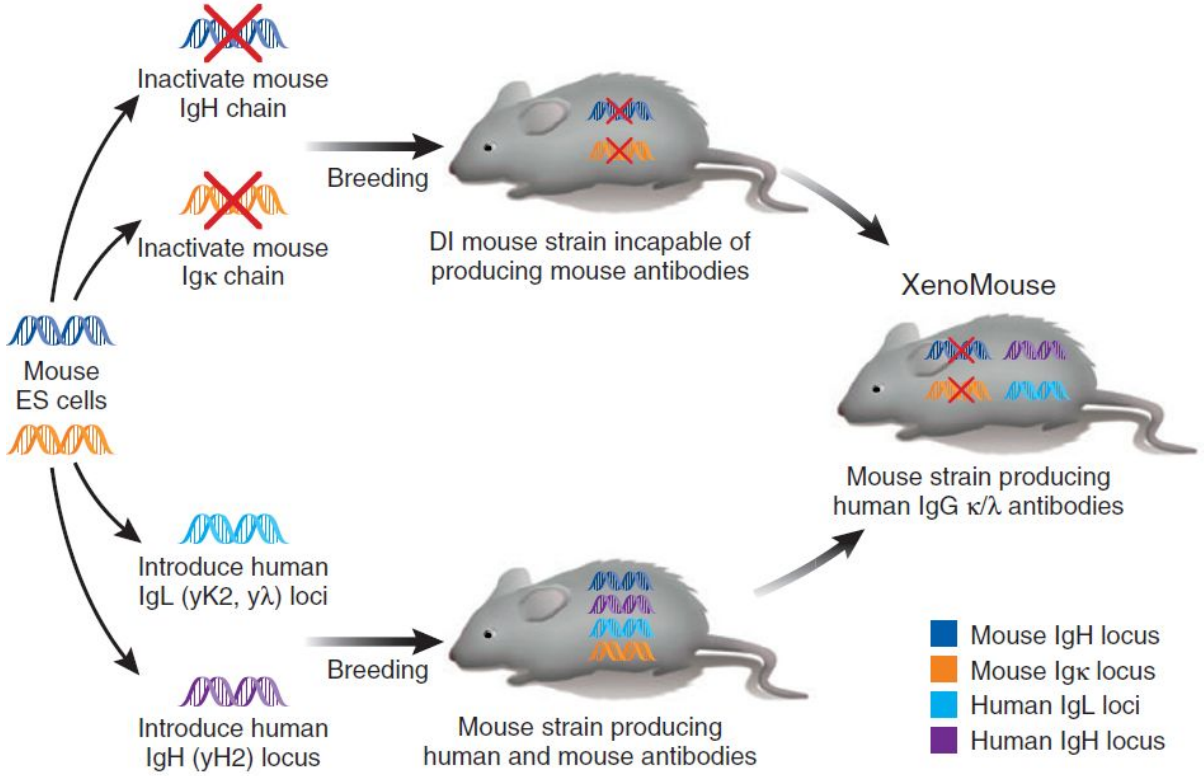
Antibody names suggest their types



- **Chimeric**: Abicximab (Ab against platelet aggregation inhibitor)
- **Humanized:** Trastuzumab (HER2)
- **Chimeric/Humanized:** Otelixizumab (CD3, a T lymphocyte receptor)
- **Human**: Adalimumab (TNF-alpha)

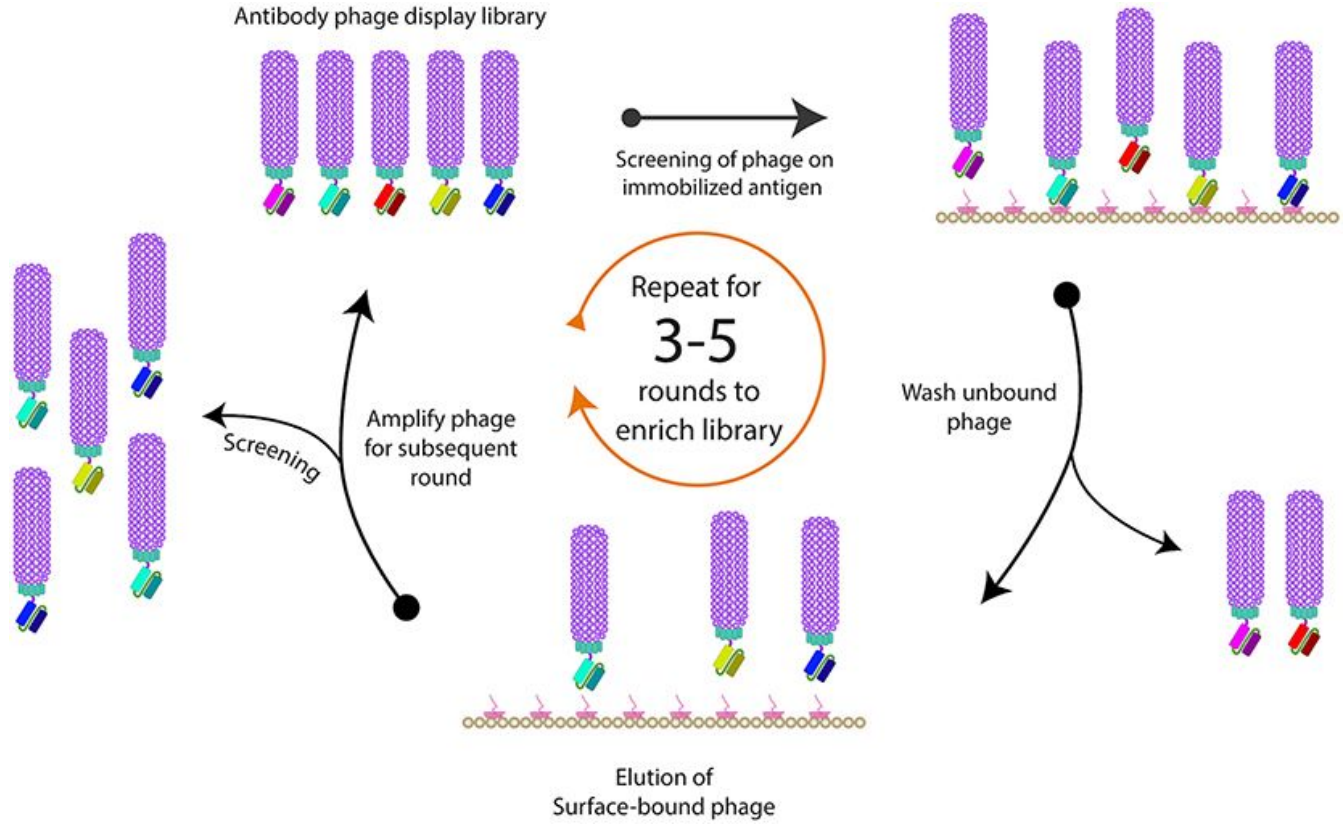
Therapeutic antibody discovery with transgenic animals

The XenoMouse model, which led to the discovery of panitumumab (Vectibix). Panitumumab targets EGFR for advanced colorectal cancer.



The principle of phage display

A protein-encoding gene is inserted into the phage coat protein gene, causing the phage to **display** the protein, which can be screened *in vitro* iteratively.



Antibody discovery with phage display

Convalescent or patient blood plasma

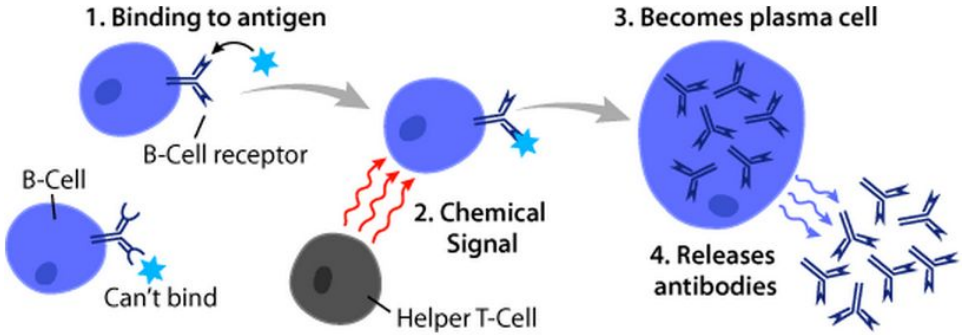
RNA → cDNA

Copy antibody-coding sequences

Paste sequences into phage coats

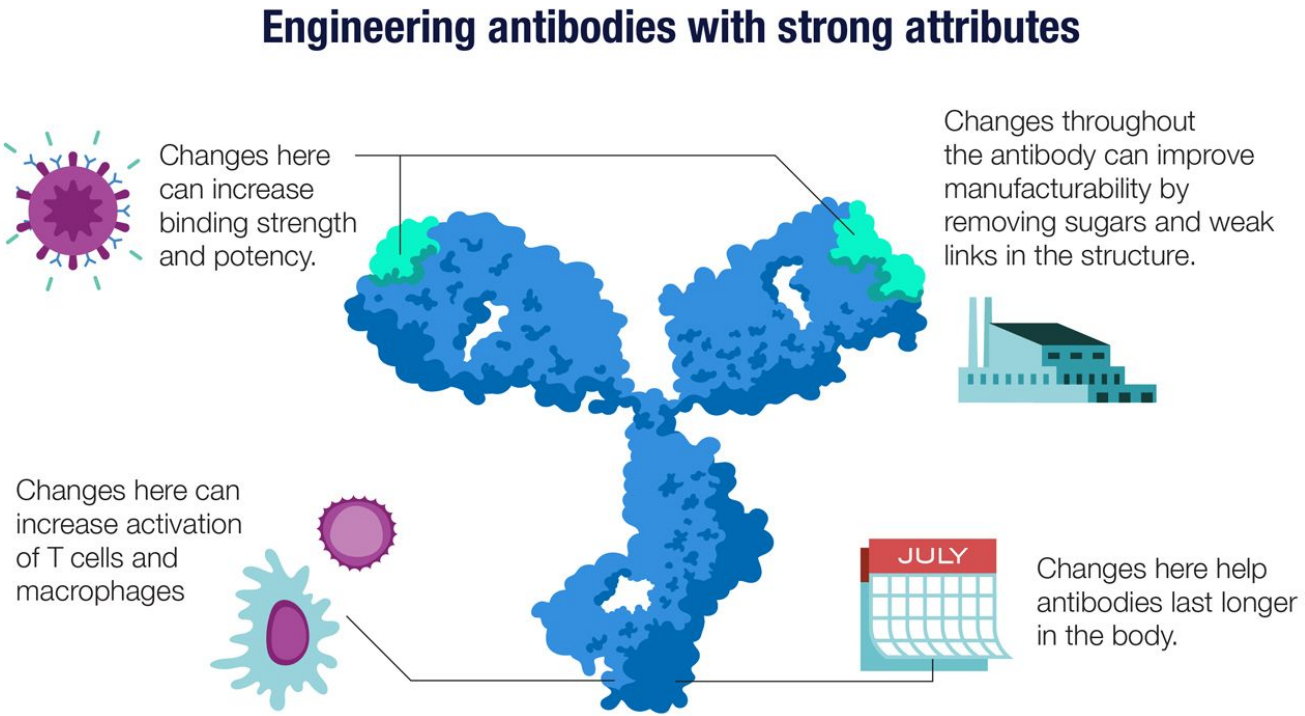
Antibody selection

Copy/Paste: PCR and cloning



Aim: Reverse-engineering existing antibodies

Discovered antibodies need further development



Major challenges of antibody discovery and development

- *Lack of quantitative rules of developability*
- Immunogenicity of therapeutic proteins (see backup)

Biophysical properties of clinical-stage antibodies (N=137 by ~2017)

Name	Light chain class	Type	Original mAb Isotype or Format	Clinical Status	Phage[c]	Year Name Proposed
abituzumab	kappa	ZU	IgG2	Phase 2	No	2013
abrilumab	kappa	H				
adalimumab	kappa	H				
alemtuzumab	kappa	Z				
alirocumab	kappa	H				
anifrolumab	kappa	H				

Name	VH	VL	LC Class	Source	Source Detailed[a]
abituzumab	QVQLQQSGGELAKPGASVKVSCKASG	DIQMTQS	kappa	WHO-INN	PL109
abrilumab	QVQLVQSGAEVKKPGASVKVSCKVSG	DIQMTQS	kappa	WHO-INN	PL111
adalimumab	EVQLVESGGGLVQPGRSLRLSCAASGF	DIQMTQS	kappa	PDB	4NYL
alemtuzumab	QVQLQESGPGLVRPSQTLSLTCTVSGF	DIQMTQS	kappa	PDB	1BEY
alirocumab	EVQLVESGGGLVQPGGSLRLSCAASGF	DIVMTQS	kappa	WHO-INN	PL107

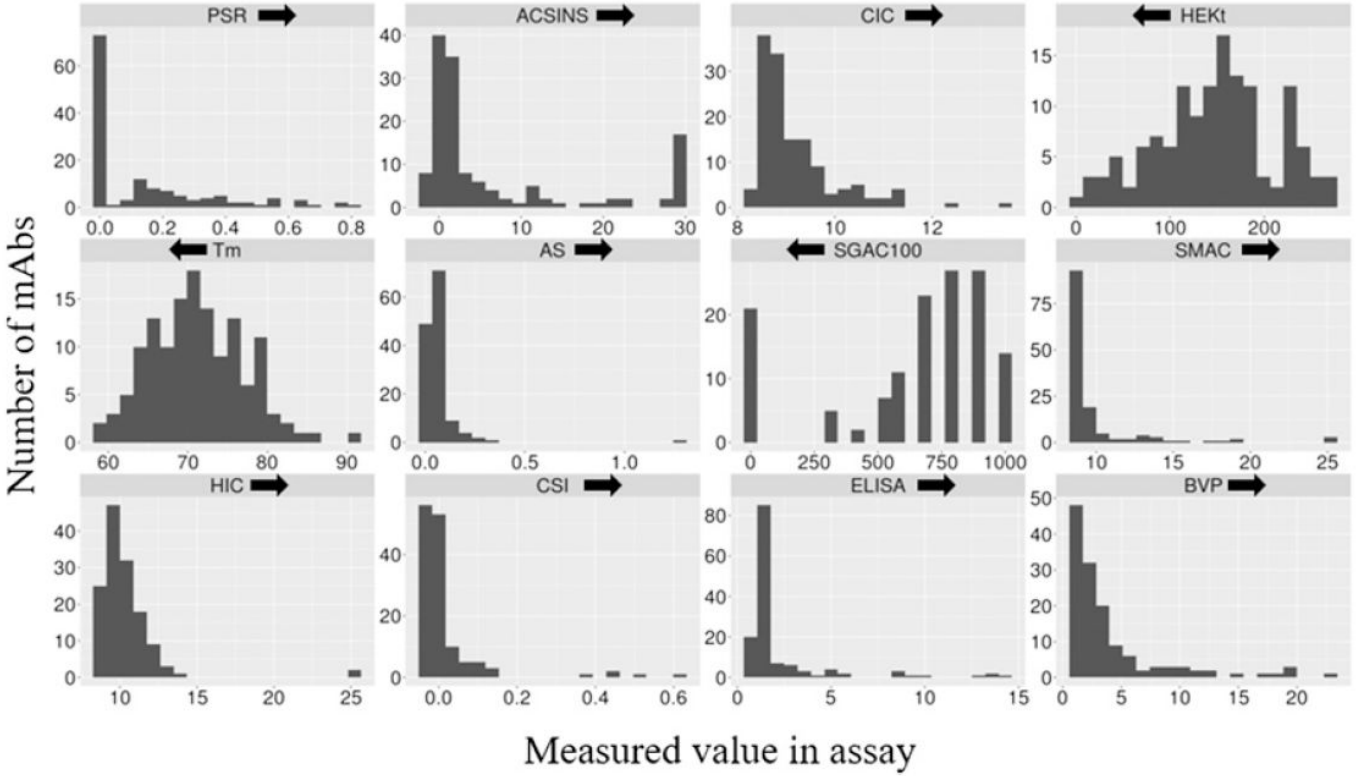
Name	HEK Titer (mg/L)	Fab Tm by DSF (°C)	SGAC-SINS AS100 ((NH4)2SO4 mM)	HIC Retention Time (Min)[a]	SMAC Retention Time (Min)[a]	Slope for Accelerated Stability	Poly-Specificity Reagent (PSR) SMP Score (0-1)	Affinity-Capture Self-Interaction Nanoparticle Spectroscopy (AC-SINS) Δλmax (nm) Average	CIC Retention Time (Min)	CSI-BLI Delta Response (nm)	ELISA	BVP ELISA
abituzumab	89.6	75.5	900.0	9.2	8.7	0.06	0.17	1.5	8.6	0.00	1.14	2.72
abrilumab	100.2	71.0	900.0	9.4	8.7	0.03	0.00	-0.9	8.4	-0.02	1.12	1.82
adalimumab	134.9	71.0	900.0	8.8	8.7	0.05	0.00	1.1	8.9	-0.01	1.08	1.49
alemtuzumab	144.7	74.5	1000.0	8.8	8.7	0.06	0.00	-0.8	8.5	-0.02	1.16	1.46
alirocumab	69.2	71.5	900.0	9.0	8.7	0.03	0.00	1.2	8.8	-0.01	1.20	2.18
anifrolumab	82.0	62.5	700.0	8.8	8.6	0.07	0.00	-0.6	8.5	-0.02	1.16	1.62
atezolizumab	164.1	73.5	300.0	13.4	19.3	0.06	0.07	15.0	10.8	0.06	1.29	6.20
bapineuzumab	151.1	73.0	1000.0	8.9	8.7	0.07	0.00	-0.7	8.6	0.06	1.21	3.55
basiliximab	107.5	60.5	0.0	9.6	8.6	0.05	0.40	28.8	9.4	0.00	1.20	2.14
bavituximab	45.1	59.5	0.0	11.5	12.7	0.04	0.56	29.9	11.4	-0.01	1.32	1.69
belimumab	10.5	60.0	800.0	10.5	9.3	0.13	0.00	0.8	8.6	-0.03	3.61	12.23

Twelve different biophysical assays

Code	Name	Purpose
AC-SINS	Affinity-capture self-interaction nanoparticle spectroscopy	Self-interaction
CSI	Clone self-interaction by biolayer interferometry	Self-interaction
PSR	Poly-specificity reagent	Cross-interaction
BVP	Baculovirus particle	Cross-interaction
CIC	Cross-interaction chromatography	Cross-interaction
ELISA	Enzyme-linked immunosorbent assay with commonly used antigens	Cross-interaction

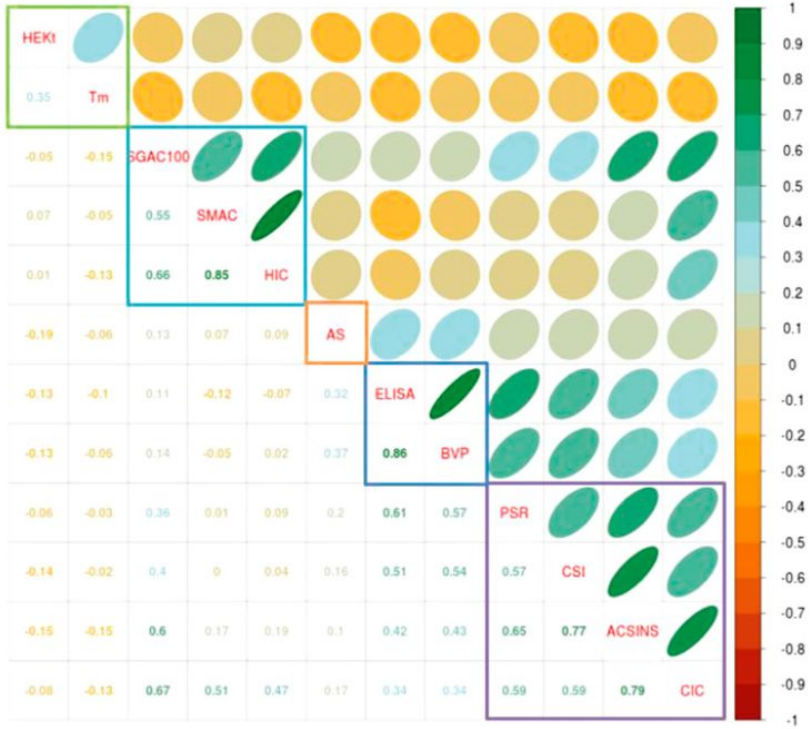
Code	Name	Purpose
HEK	Expression titer in HEK cells	Expression
Tm	Melting temperature	Thermostability
HIC	Hydrophobic interaction chromatography	Species separation and analysis
SAGC-SINS	salt-gradient affinity-capture self-interaction nanoparticle spectroscopy	Species separation and analysis
SMAC	standup monolayer adsorption chromatography	Developability
AS	Size-exclusion chromatography in accelerated stability	Stability

Distribution of results from biophysical assays for 137 monoclonal antibodies

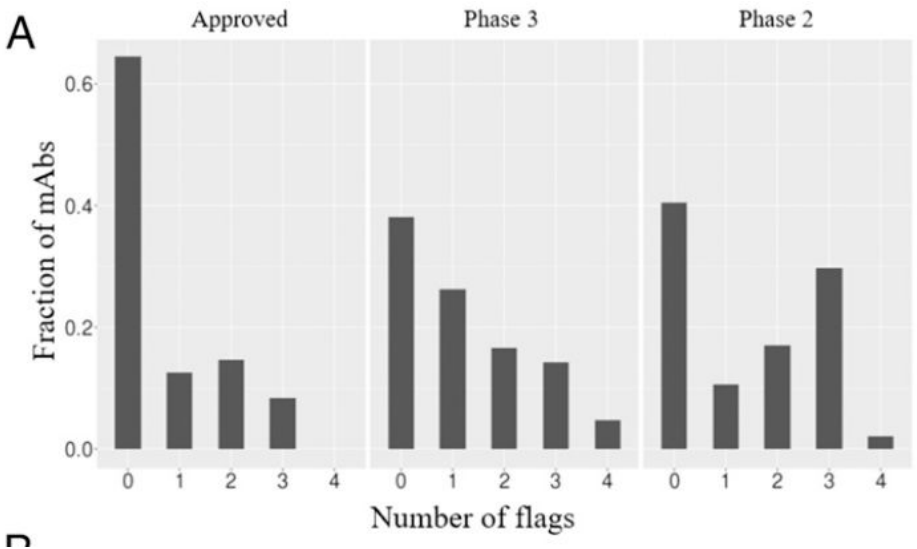


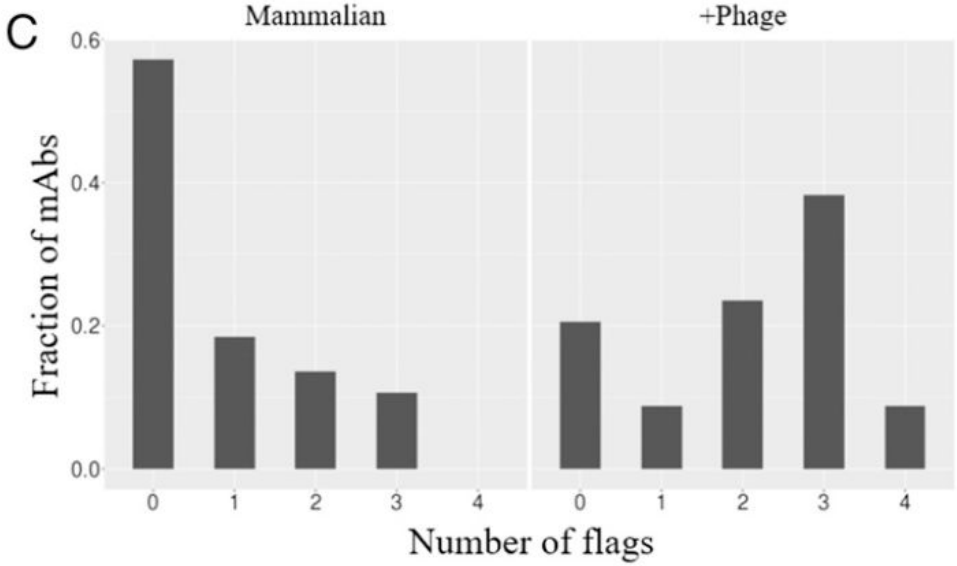
Unsupervised clustering analysis reveals related assays

Group	Assay	Worst 10% threshold
Group 1	PSR	0.27 ± 0.06
	ACSINS	11.8 ± 6.2
	CSI	0.01 ± 0.02
	CIC	10.1 ± 0.5
Group 2	HIC	11.7 ± 0.6
	SMAC	12.8 ± 1.2
	SGAC-SINS	370 ± 133
Group 3	BVP	4.3 ± 2.2
	ELISA	1.9 ± 1.0
Group 4	AS	0.08 ± 0.03



Approved antibodies and antibodies discovery not via phage display tend to have fewer flags





Conclusions

- Given mechanistic understanding of biological processes underlying diseases, we can develop different modalities as therapeutics.
- Mathematical and computational biology
  1. reveals how drug candidate work and ranks them
  2. helps with molecule design
  3. contributes to modality selection

Offline activities

Reading the review draft on leveraging protein turnover for drug discovery, and sharing questions, criticism, and feeback.

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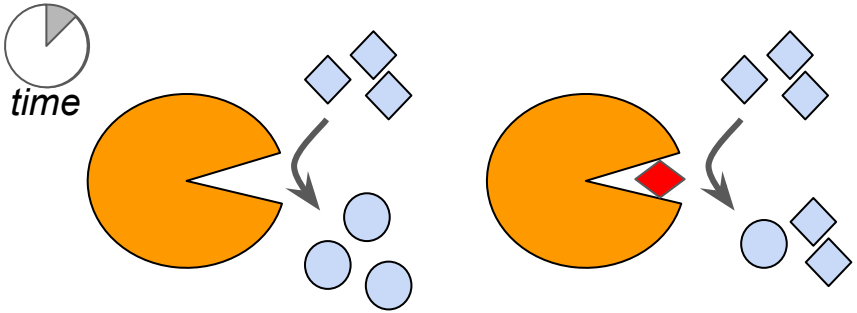
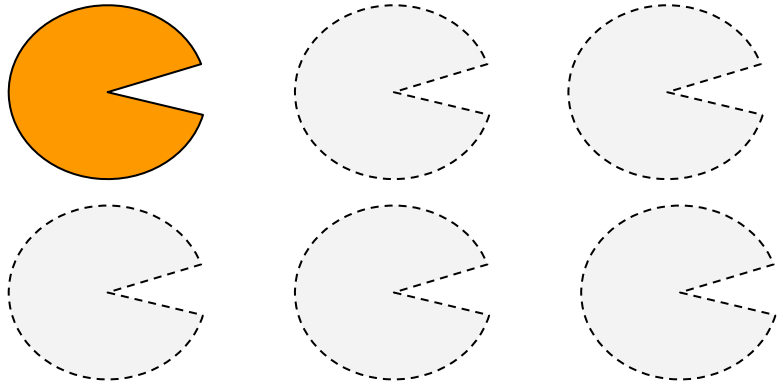
Supplementary Information

Competitive inhibitors reduce reaction rate; antisense oligonucleotides modulate protein abundance



A competitive inhibitor (red diamond) reduces the rate of product generation in an enzymatic reaction.



Antisense oligonucleotides reduce the abundance of the enzyme protein.

Enzymic and genetic inhibition have distinct impact on reaction dynamics

A

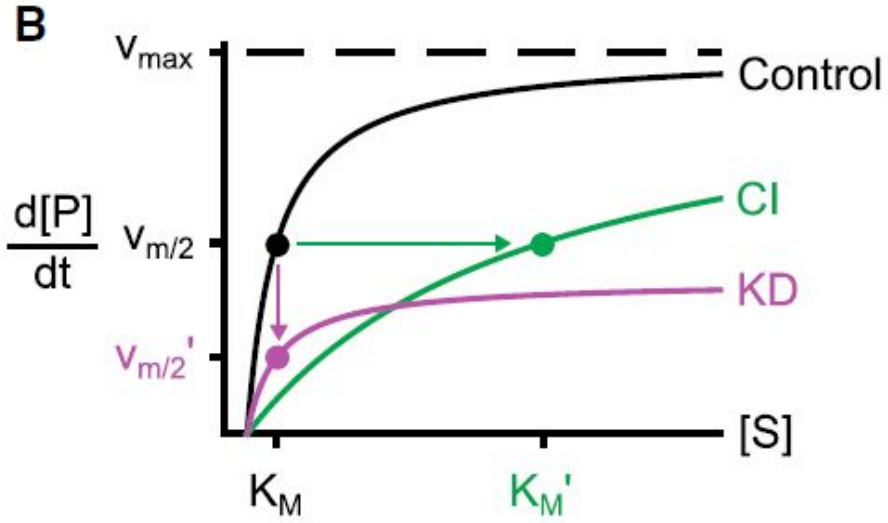
$$[E] + [S] \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} [E\text{-}S] \xrightarrow{k_{cat}} [E] + [P]$$

$$\frac{d[P]}{dt} = v_{max}\frac{[S]}{[S] + K_M}$$

$$K_M = \frac{k_{-1} + k_{cat}}{k_1} \qquad v_{max} = k_{cat}[E]_o$$

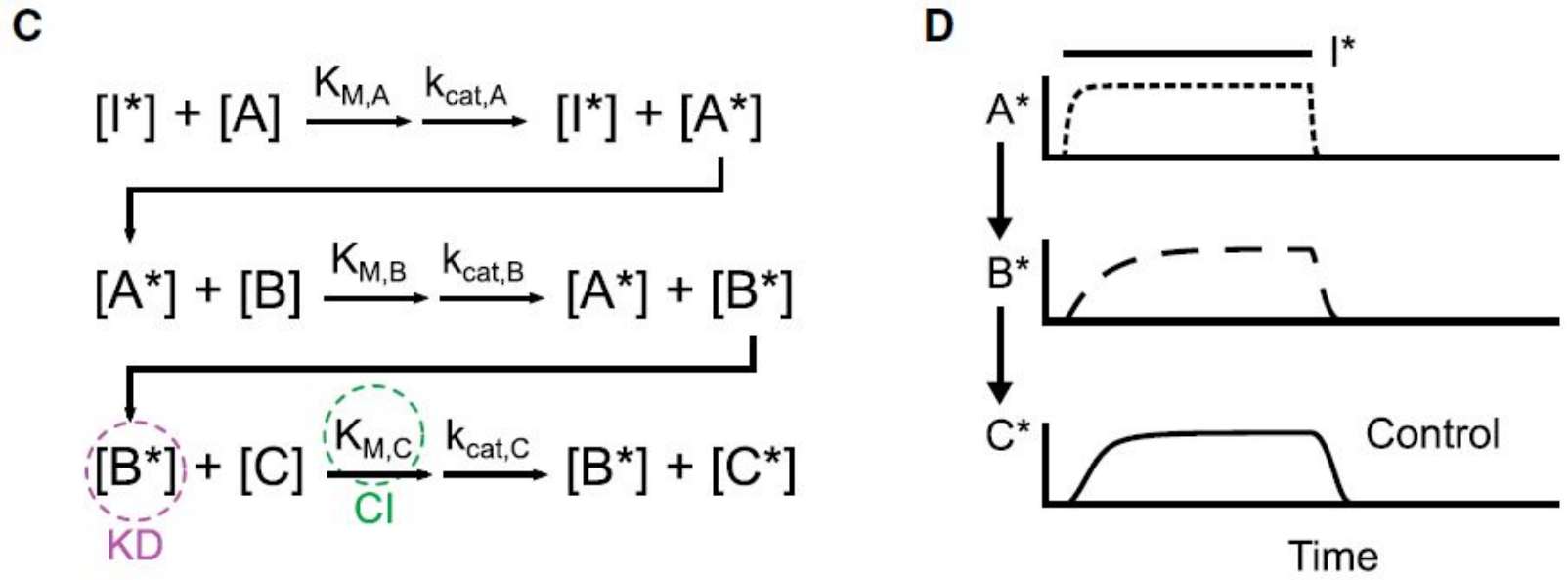
The Michaelis-Menten Equation

B



Competitive inhibition (CI) versus knockdown (KD)

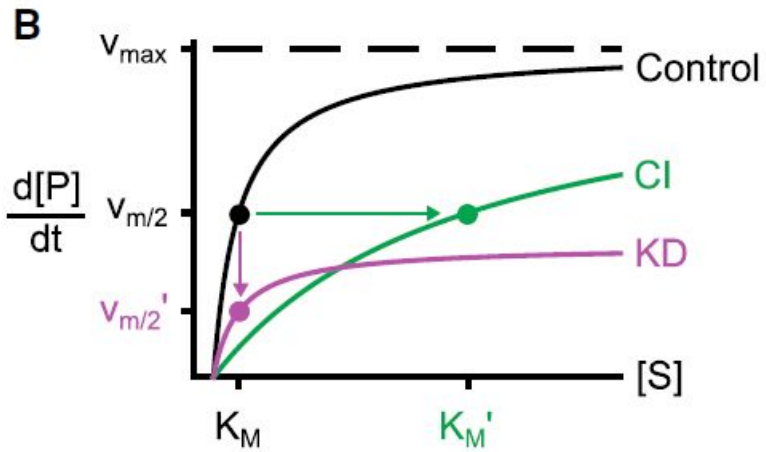
A linear system simulating enzymatic reactions

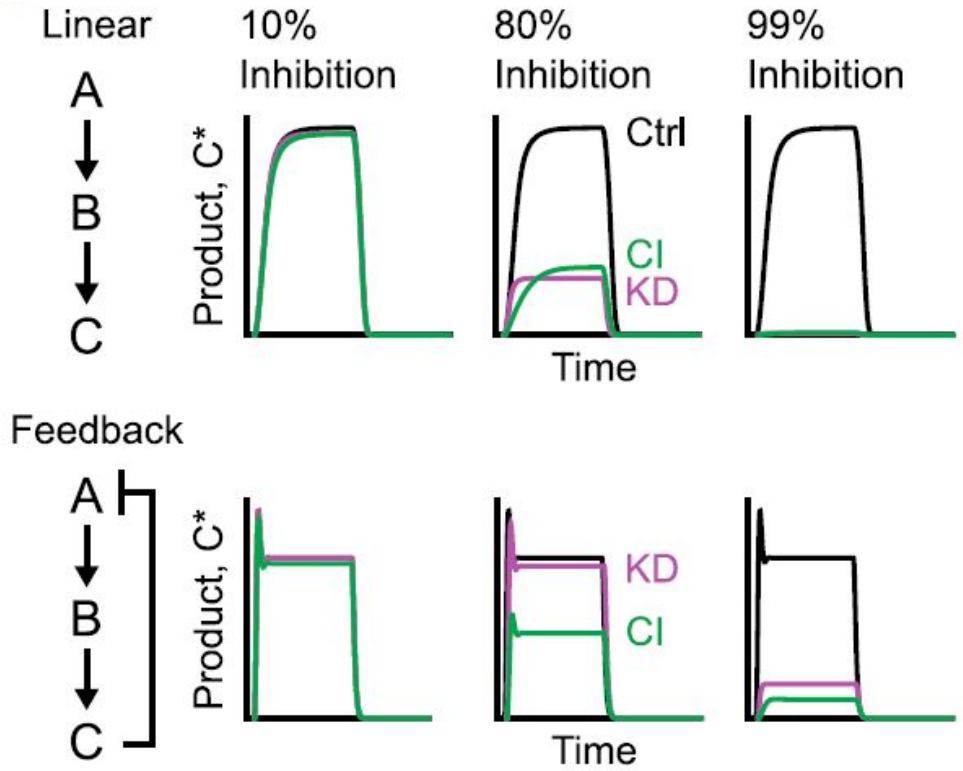


I*: upstream input; A/A* and B/B*: inactivated and activated enzyme; C*: product

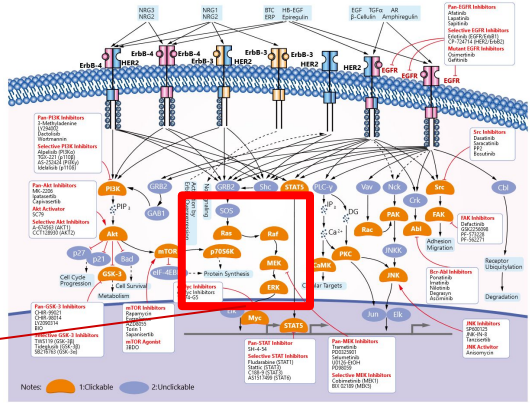
Adding a negative feedback may differentiate effects of enzymatic and genetic inhibition

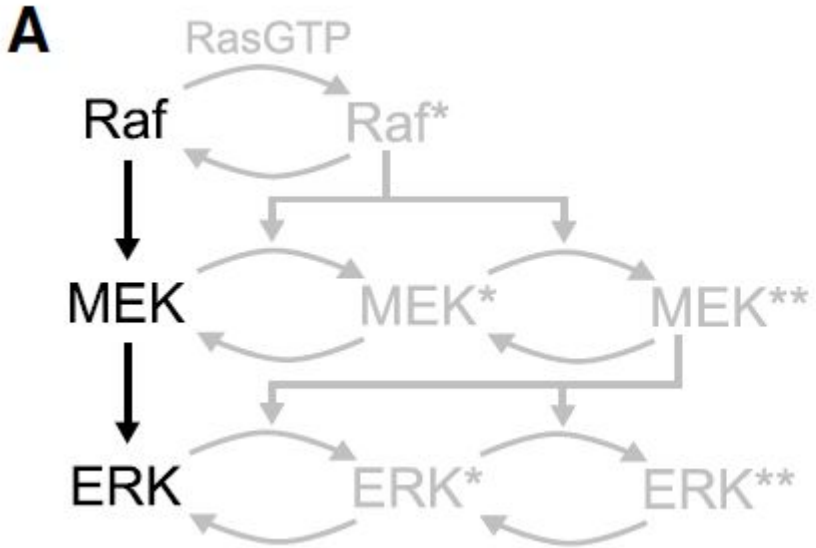
Intuition: when [B*] stays low, CI leads to **slower** accumulation of C* than KD.

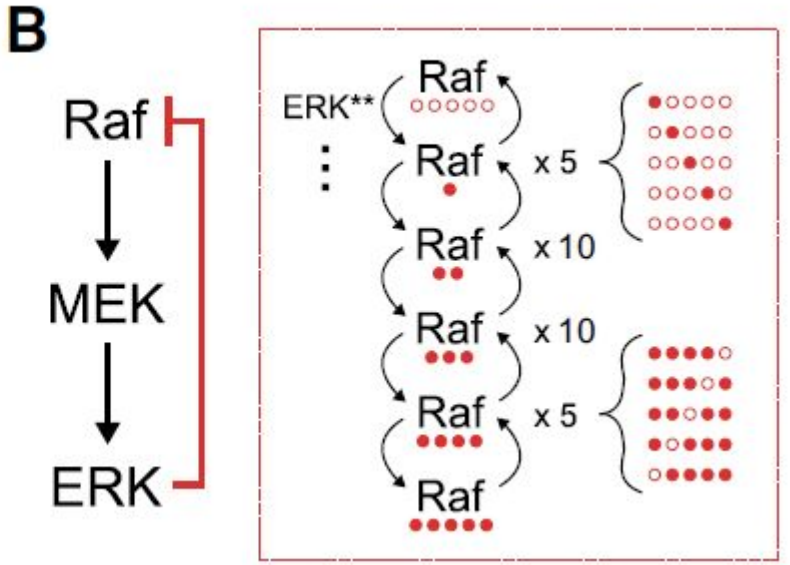




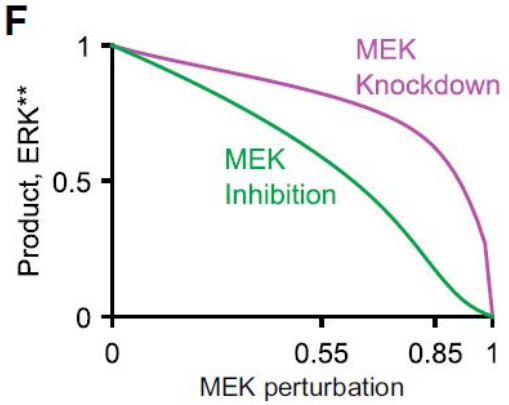
The MAPK/ERK pathway downstream of EGFR signalling

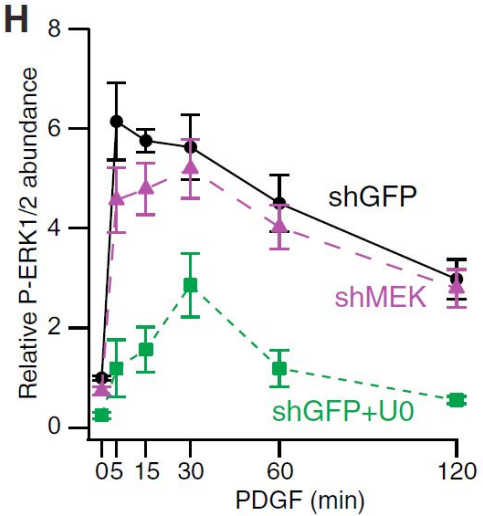


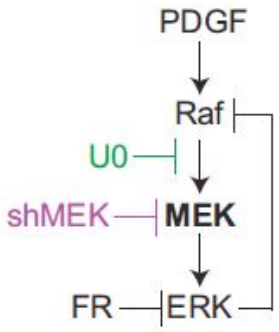


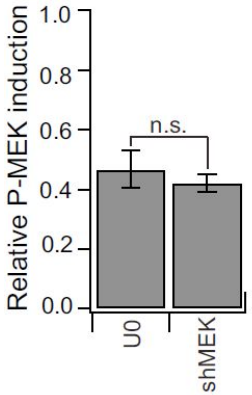


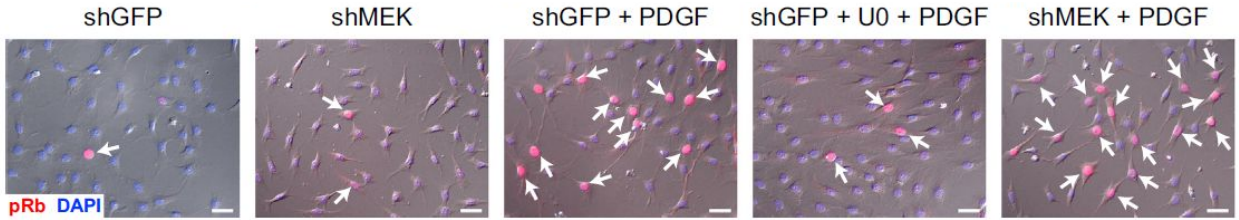
Confirmation of predicted difference of KD and CI



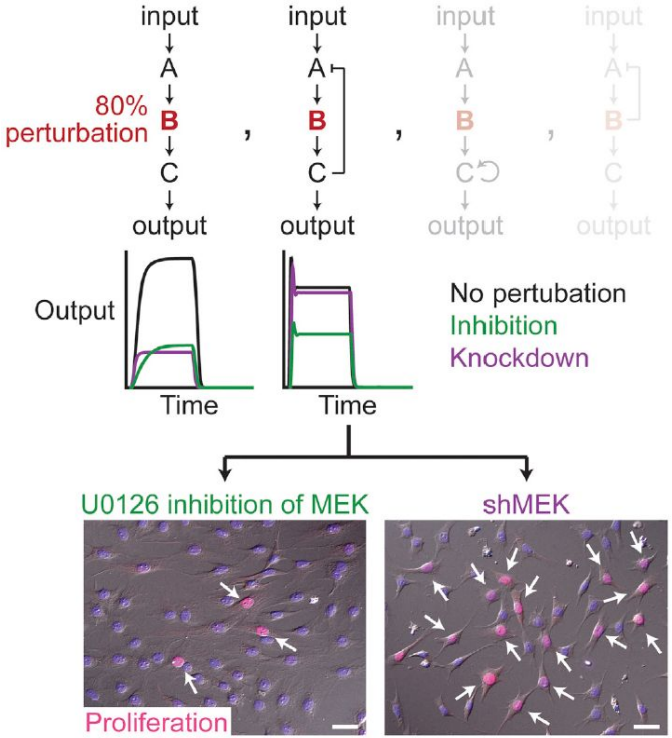


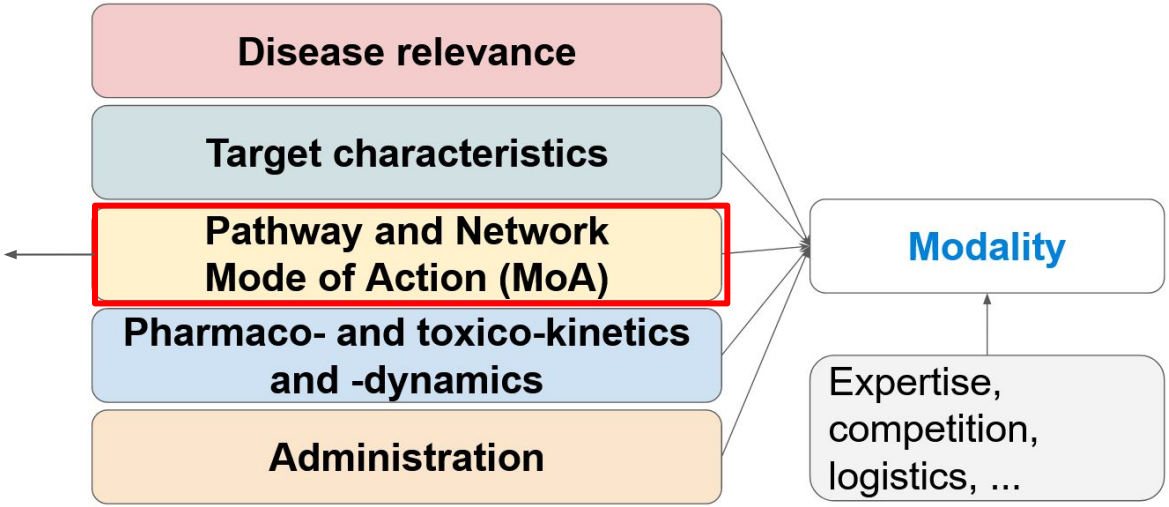






Computational biology may empower our choice of modality

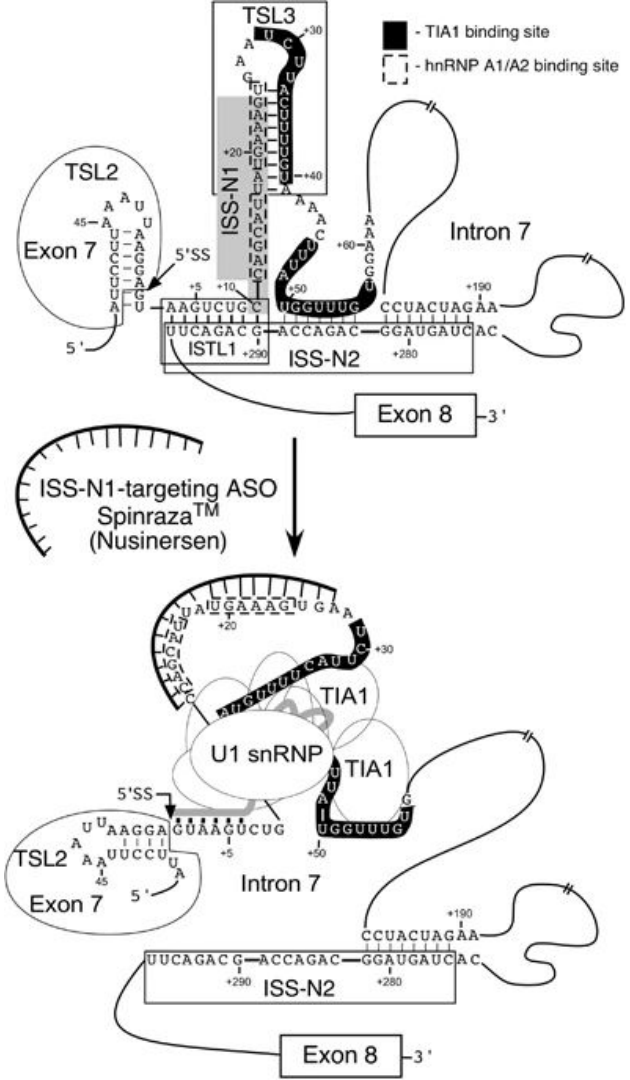




How Spinaraza (nusinersen) works, base by base

Nusinersen binds to ISS-N1, causing structural rearrangement and recruitment of U1 snRNP by TIA1.

- ISS-N1: Intronic splicing silencer N1;
- TIA1: TIA1 cytotoxic granule associated RNA binding protein;
- TSLs: (inhibitory) terminal stem-loop structures;
- ISTL1: internal stem formed by a long-distance interaction



Clinical-stage siRNAs

The growth of siRNA-based therapeutics

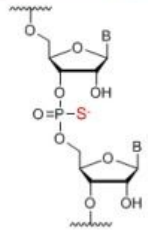
FDA-approved siRNA drugs: Patisiran | Givosiran | Lumasiran

siRNA drugs in clinical trials: Vutrisiran | Nedosiran | Inclisiran | Fitusiran | Teprasiran | Cosdosiran | Tivanisiran

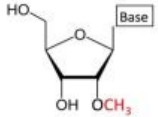
Drug	Alternative name	Company	Disease	Updated status
Patisiran	ONPATTRO	Alnylam	Hereditary transthyretin mediated amyloidosis	FDA approval in 10/08/2018 210922Orig1s000*
Givosiran	GIVLAARI	Alnylam	Acute hepatic porphyria	FDA approval in 11/20/2019 212194Orig1s000
Lumasiran	ALN-GO1	Alnylam	Primary hyperoxaluria type 1 (PH1)	FDA approval on 11/23/2020 214103Orig1s000
Vutrisiran	ALN-TTRsc02	Alnylam	Hereditary transthyretin mediated amyloidosis	Phase 3 trials ELIOS-A (NCT03759379)** HELIOS-B (NCT04153149)
Nedosiran	DCR-PHXC	Dicerna Alnylam	Primary hyperoxaluria	Phase 3 trial PHYOX 3 (NCT04042402)
Inclisiran	ALN-PCSSC	Alnylam Novartis	Hypercholesterolemia	Phase 3 trials ORION-9 (NCT03397121) ORION-10 (NCT03399370) ORION-11 (NCT03400800)
Fitusiran	ALN-AT3sc ALN-APC SAR439774	Alnylam Sanofi Genzyme	Hemophilia A and B	Phase 3 trials ATLAS-A/B (NCT03417245) ATLAS-INH (NCT03417102) ATLAS-PPX (NCT03549871) ATLAS-PEDS (NCT03974113) ATLAS-OLE (NCT03754790)
Teprasiran	AKIi-5, DGFi, I-5NP, QPI-1002	Quark Novartis	Acute kidney injury Delayed graft function	Phase 3 trial ReGIFT (NCT02610296)
Cosdosiran	QPI-1007	Quark	Non-arteritic anterior ischemic optic neuropathy (NAION)	Phase 2/3 trial NCT02341560
Tivanisiran	SYL-1001	Sylentis	Dry eyes Ocular pain	Phase 3 trial HELIX (NCT03108664)

* FDA application number.
** ClinicalTrials.gov identifier number at https://clinicaltrials.gov/ct2/

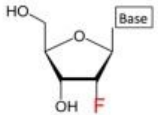
Drug	Chemical modifications				Delivery platform
	Backbone	Sugar			
	PS	2'-OMe	2'-F	2'-MOE	
Patisiran	-	+ (11)	-	-	LNP
Givosiran	+ (6)	+ (28)	+ (16)	-	GalNAc
Lumasiran	+ (6)	+ (34)	+ (10)	-	GalNAc
Vutrisiran	+ (6)	+ (35)	+ (9)	-	GalNAc
Nedosiran	+ (6)	+ (35)	+ (19)	-	GalNAc
Inclisiran	+ (6)	+ (32)	+ (11)	+ (1)	GalNAc
Fitusiran	+ (6)	+ (23)	+ (21)	-	GalNAc
Teprasiran	-	+ (19)	-	-	None
Cosdosiran	-	+ (9)	-	-	None
Tivanisiran	-	-	-	-	None



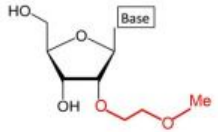
Phosphorothioate (PS)



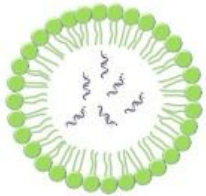
2-O-methyl (2'-OMe)

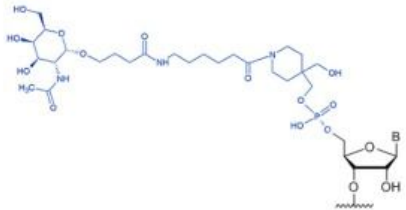


2'-fluoro (2'-F)



2'-O-methoxyethyl (2'-MOE)

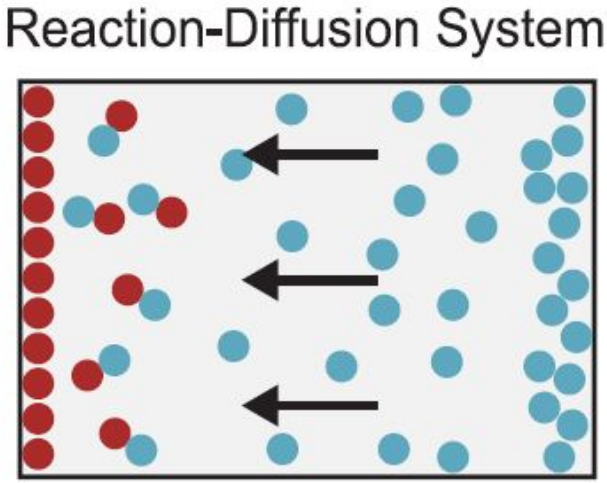

Lipid nanoparticle (LNP)



N-acetylgalactosamine (GalNAc)

Chemically induced proximity

A reaction-diffusion model



$$\frac{\partial u}{\partial t} = \underbrace{D\frac{\partial^2 u}{\partial x^2}}_{\text{Diffusion}} + \underbrace{ku}_{\text{Binding}}$$

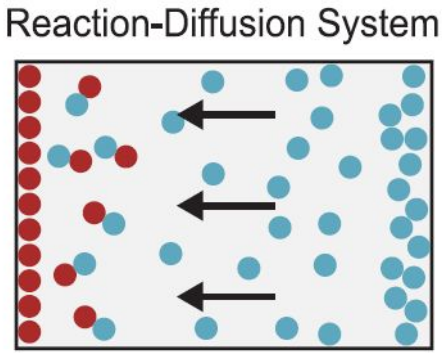
x: position

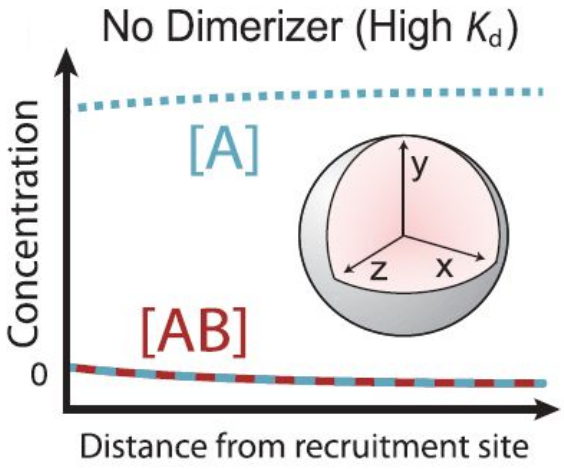
u: product concentration

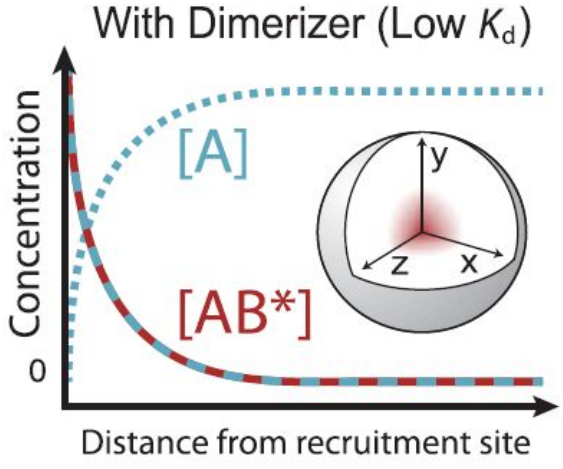
t: time

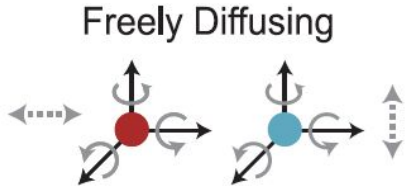
The diffusion term follows *Fick's second law of diffusion*; the binding term describes the reaction.

Kinetic and thermodynamic contributions of chemically induced proximity

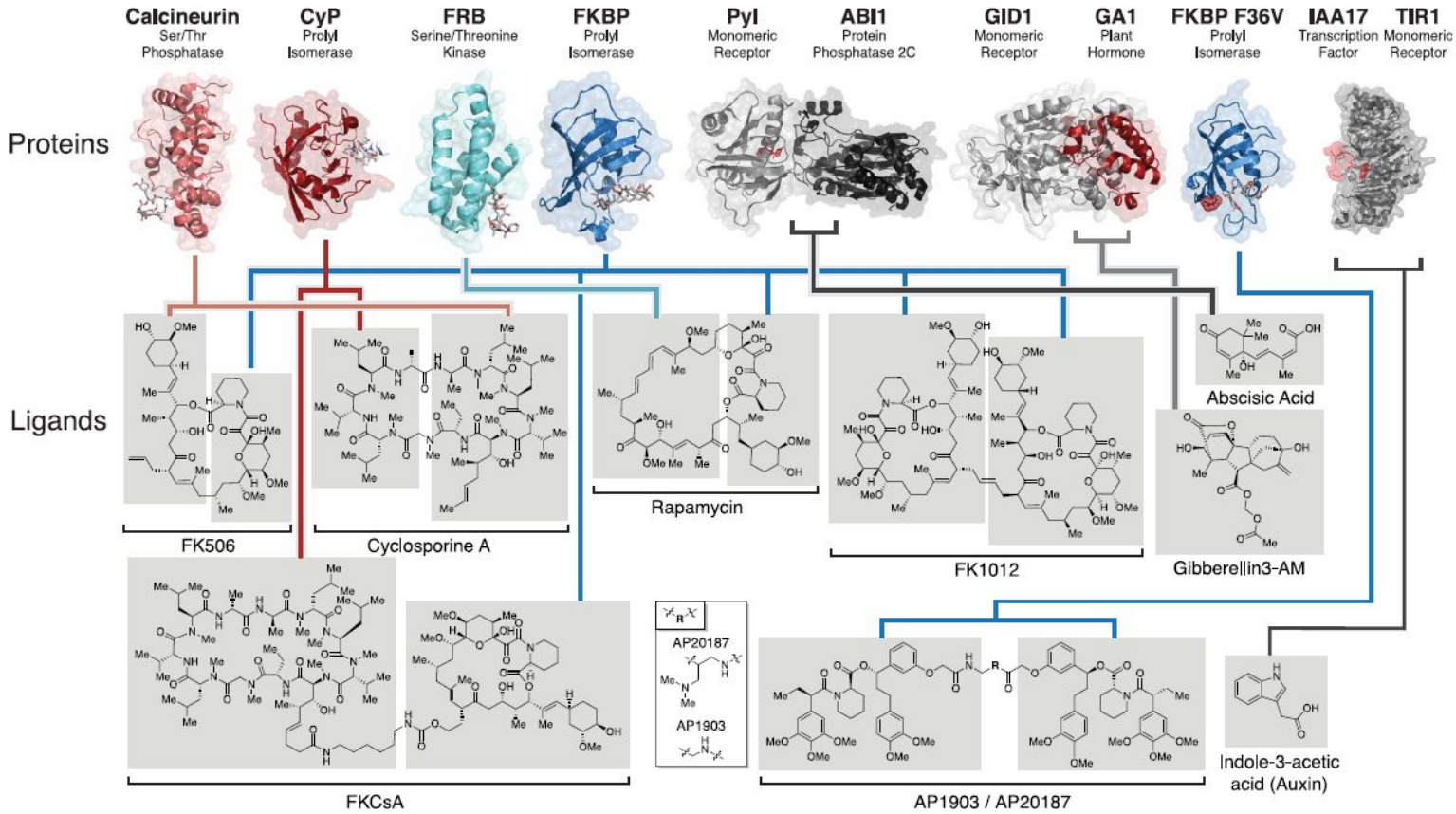






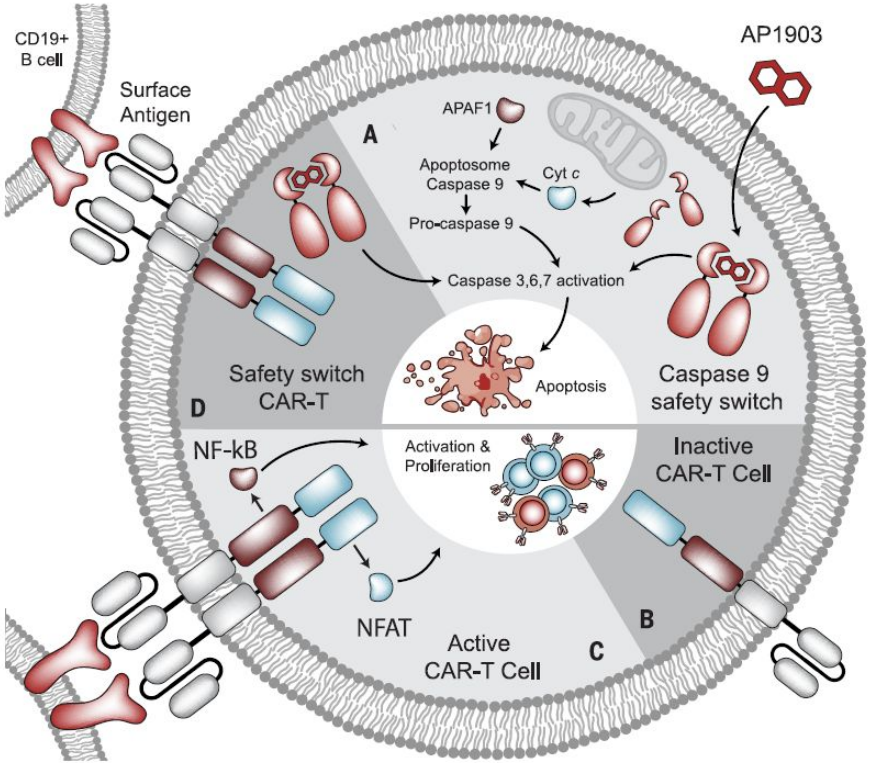


Chemically induced proximity



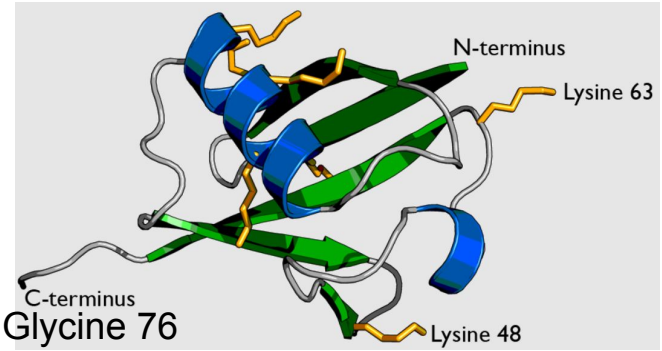
Chemically induced proximity as ‘safety switch’ for cell therapy

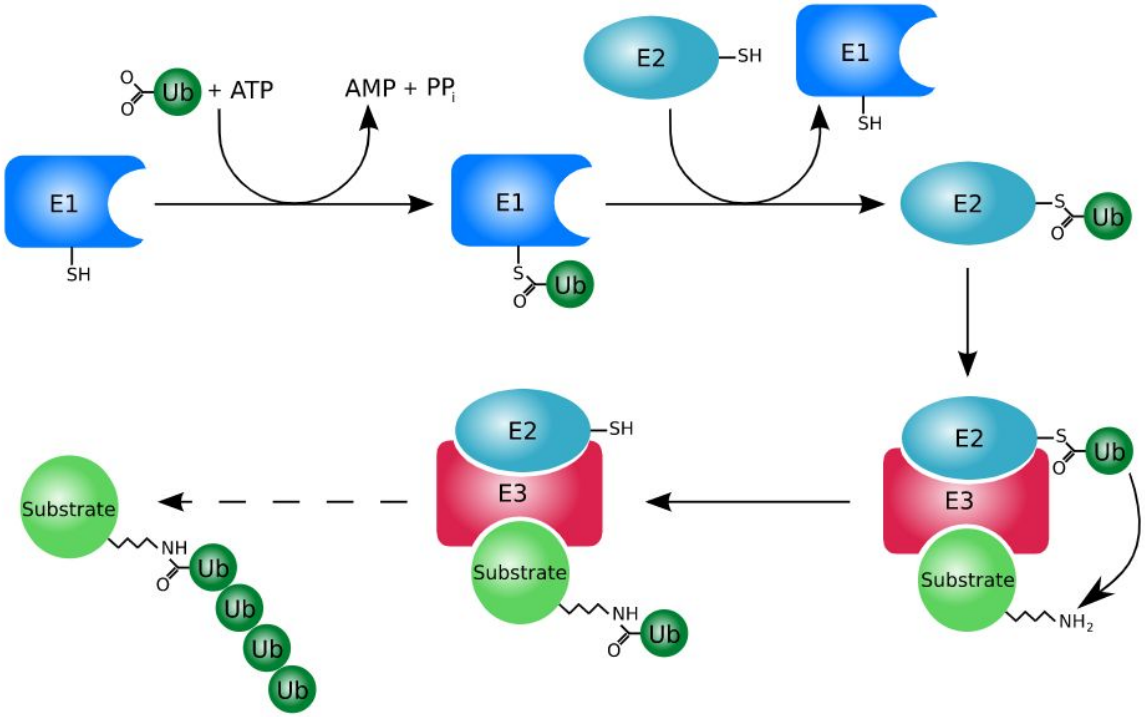
- Too many or too active CAR-T cells may induce serious side effects (cytokine release syndrome, B cell aplasia, *etc.*)
- *Bioinert* small molecules (AP1903 in this case) can be used as ‘safety switch’ to kill transplanted CAR-T cells.



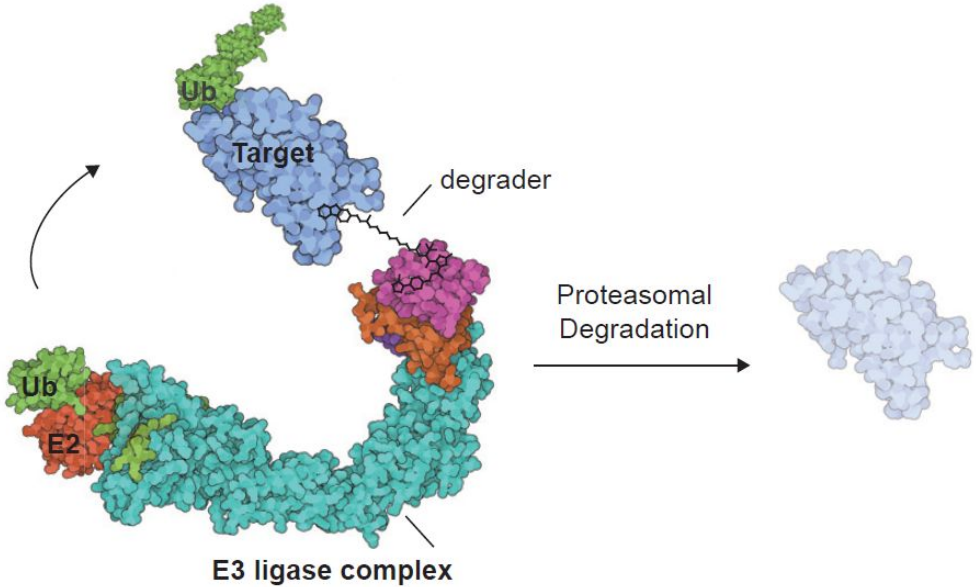
Therapeutic use of protein degradation

Ubiquitination marks proteins to be degraded

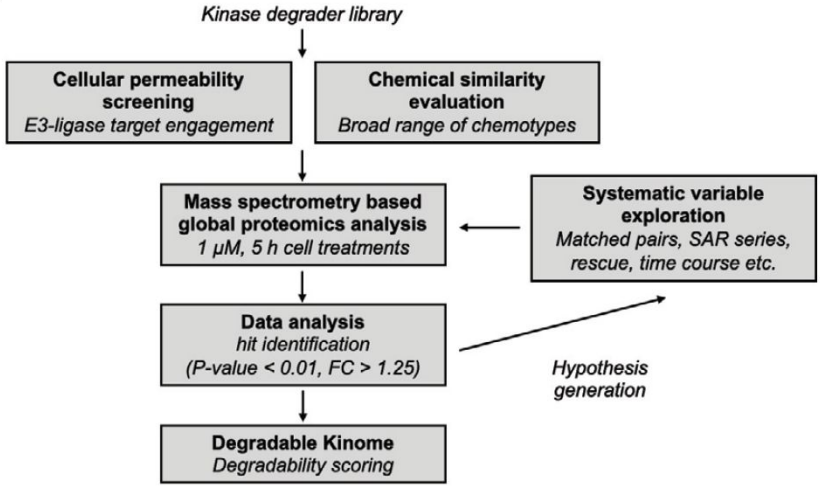


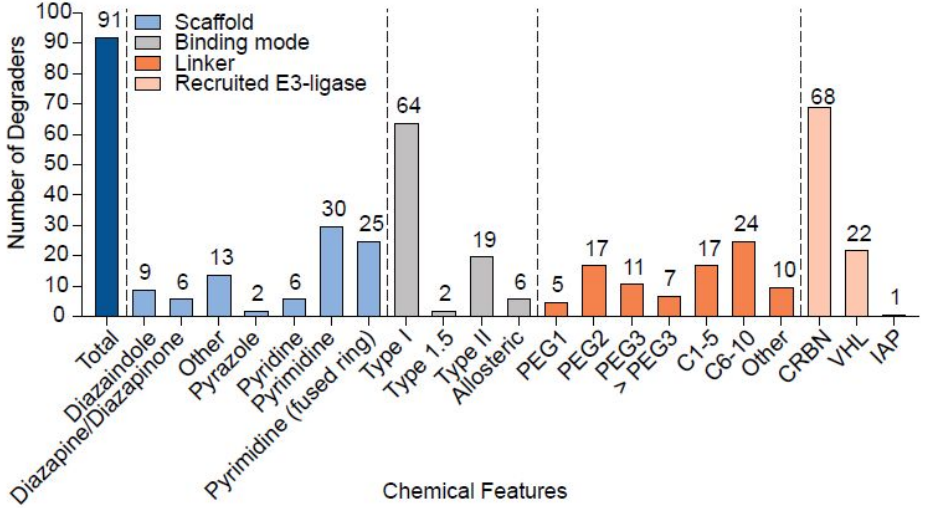


Donovan *et al.* (2020) reports screening results with 91 kinase degraders



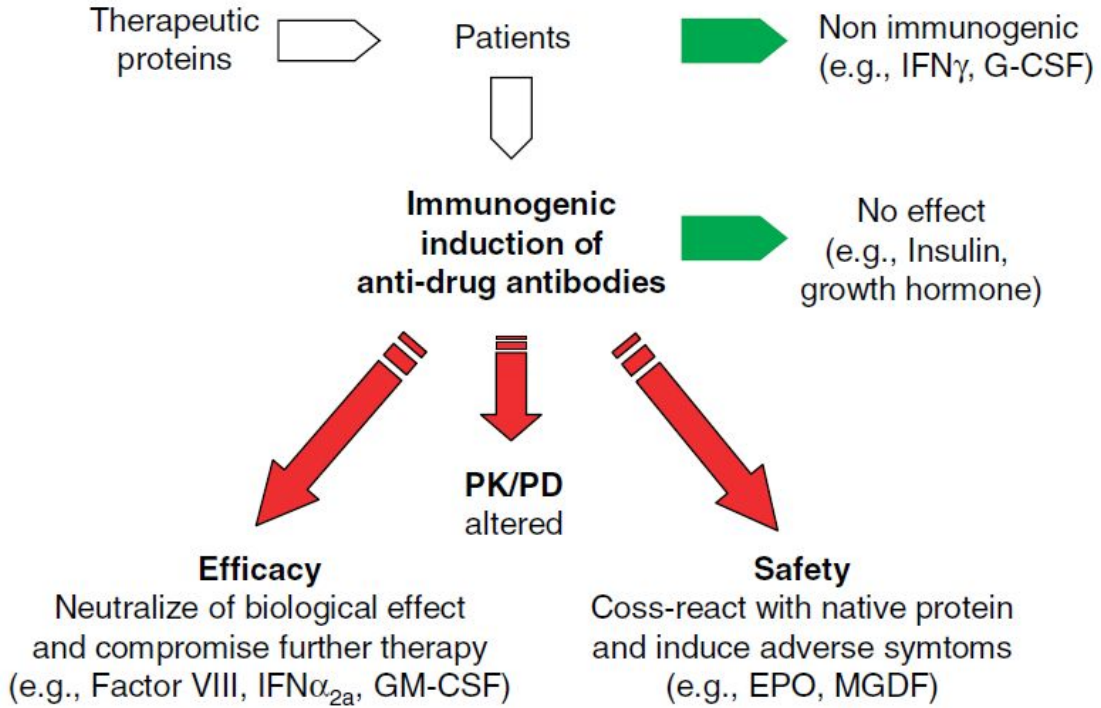
Donovan *et al.* (2020) reports screening results with 91 kinase degraders





Immunogenicity of therapeutic proteins

Immunogenicity affects both efficacy and safety



Immune response underlies immunogenicity

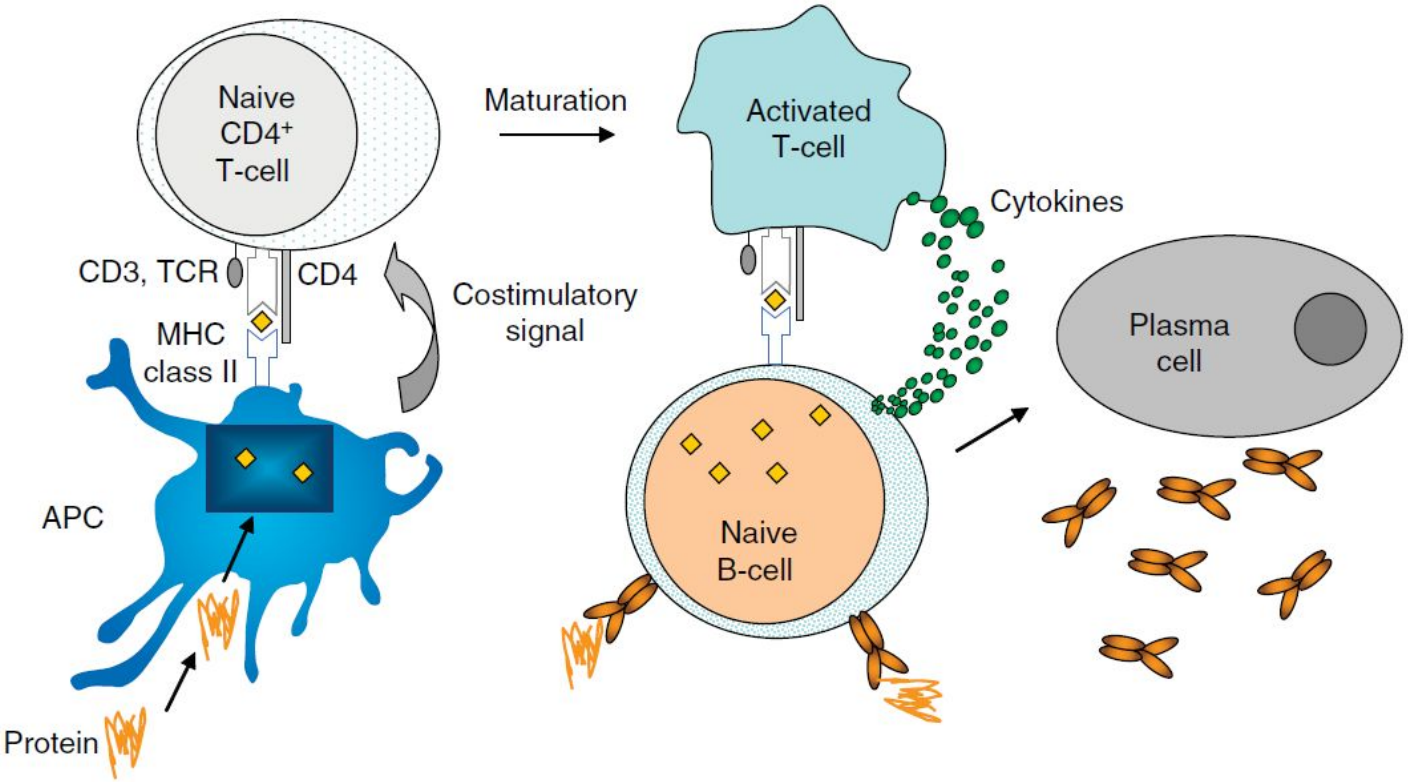


Table 2. The factors contributing to immunogenicity are divided into three groups.

	Immunogenicity potential
Drug product	
Host	Non-human: ▲
Immunomodulatory properties	▲?
Glycosylation	▲
Aggregation	▲
Size	Molecular mass < 10 kDa: ▼
Formulation	Polymers ▼
Excipients/stabilizers	To be characterized: silicone oil ▲
Impurities	Product/process related: ▲
Post-translational mod.	Oxidation, deamidation, etc.:▲
aa Composition	Charged aa: ▲; Aromatic aa: ▼
Conjugates	▲
Patient	
Age	▼?
Disease state	Different indication/different response
Immune status	Immune compromised: ▼
	Infective disease: ▲
Patient to patient variability	Not predictable
Concomitant therapy	Earlier exposure to similar protein - crossreacting antibodies to similar proteins
Genetic factors	Defective gene
	Polymorphisms for cytokines
Administration	
Dose	Higher dose: ▲?
Route	Intravenous administration less immunogenic than subcutaneous or intramuscular
	Short-term administration less immunogenic than long-term treatment
	Continuous administration less immunogenic than intermittent
Frequency	More frequent: ▲
Duration of therapy	Short term: ▼

▲: Potential to increase immunogenicity; ▼: Potential to decrease immunogenicity; ?: Most likely; aa: Amino acid.

A mechanistic, multiscale model of immunogenicity: subcellular model

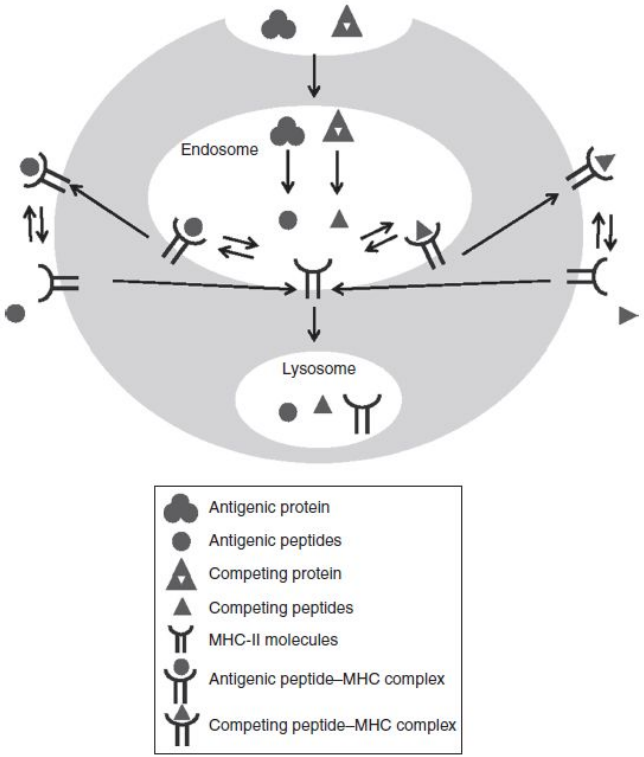


Figure 1. Model structure for the subcellular level, including processes for antigen presentation in mature dendritic cells. The symbols in the figure legends are described below, with corresponding equation number in **Supplementary Materials** shown between parentheses. : Antigenic protein, including antigenic protein in plasma (*Ag*, **Eq. 27 in Supplementary Material**) and antigenic protein in the endosome: (Ag^E, **Eq. 4 in Supplementary Material**); : antigenic peptide in endosome (p_j^E, **Eq. 5 in Supplementary Material**); : competing protein in the endosome (cp^E, **Eq. 9 in Supplementary Material**); : competing peptide in the endosome (cpt^E, **Eq. 10 in Supplementary Material**); : MHC-II molecules, including those in the endosome (M^E_k, **Eq. 6 in Supplementary Material**) and those on dendritic cell membrane (M_k, **Eq. 13 in Supplementary Material**); : antigenic peptide-MHC complex, including those in the endosome ($p_jM^E_k$, **Eq. 7 in Supplementary Material**) and those on cell membrane (p_jM_k, **Eq. 8 in Supplementary Material**); : competing peptide-MHC complex, including those in the endosome ($cptM^E_k$, **Eq. 11 in Supplementary Material**) and those on cell membrane ($cptM_k$, **Eq. 12 in Supplementary Material**).

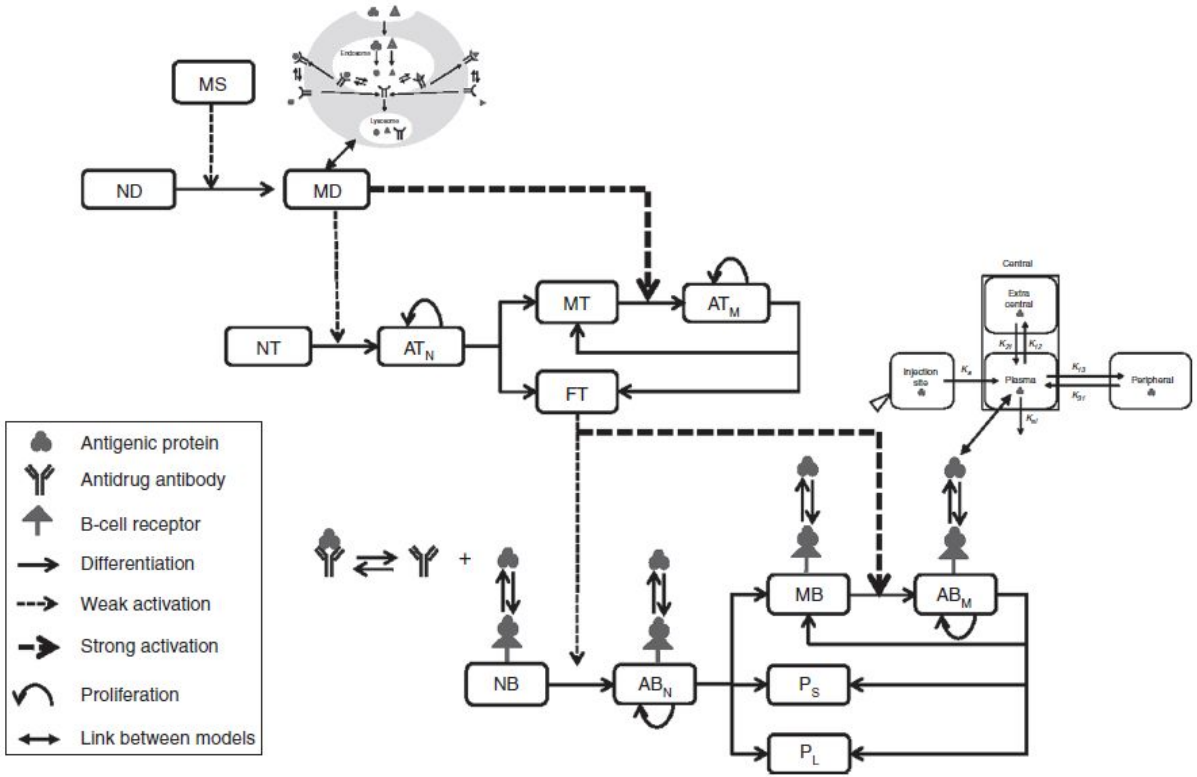


Figure 2. Model structure for the cellular level, including cells, antigen, antidrug antibody, and B-cell receptor. The links between the three levels of the multiscale model are also illustrated to help interpretation. The acronyms are explained below, along with the corresponding equation number in the **Supplementary Material** shown between parentheses. MS: maturation signal (**Eq. 1 in Supplementary Material**); ID: immature dendritic (**Eq. 2 in Supplementary Material**); MD: mature dendritic (**Eq. 3 in Supplementary Material**); NT: naïve T (**Eq. 14 in Supplementary Material**); AT_N: activated T from naïve T (**Eq. 15 in Supplementary Material**); AT_M: activated T from memory T (**Eq. 16 in Supplementary Material**); MT: memory T (**Eq. 17 in Supplementary Material**); **FT**: functional T (**Eq. 18 in Supplementary Material**); NB: naïve B (**Eq. 19 in Supplementary Material**); AB_N: activated B from naïve B (**Eq. 20 in Supplementary Material**); AB_M: activated B from memory B (**Eq. 21 in Supplementary Material**); MB: memory B (**Eq. 22 in Supplementary Material**); P_S: short-lived plasma (**Eq. 23 in Supplementary Material**); P_L: long-lived plasma cell (**Eq. 24 in Supplementary Material**).

The whole-body model

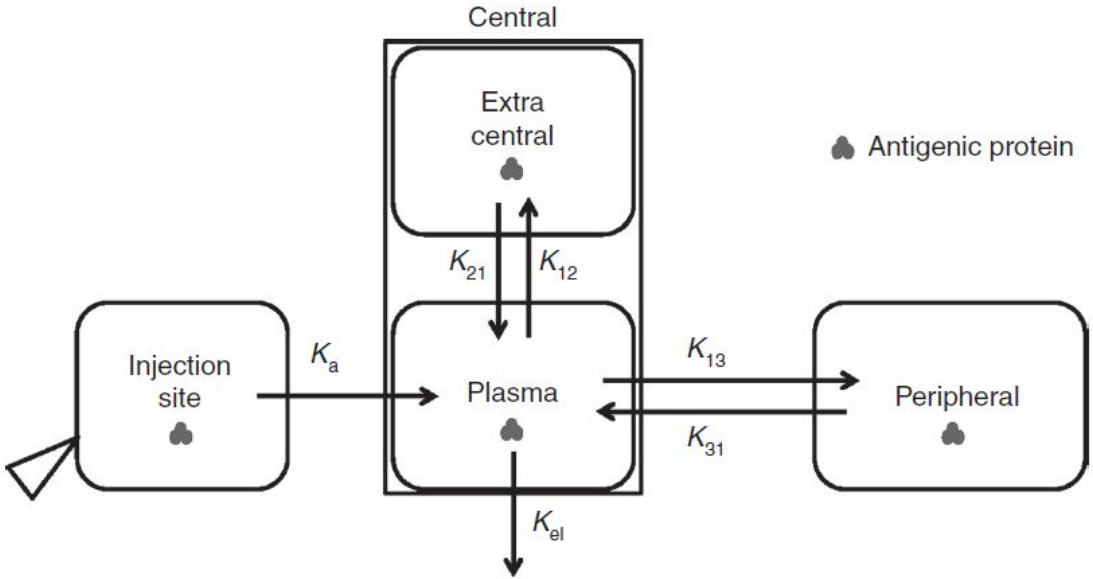


Figure 3. Model structure for the whole-body level, accounting for the *in vivo* disposition of antigenic protein. Details are described in the Results section and also by **Eqs. 26–29 in the Supplementary Materials**.

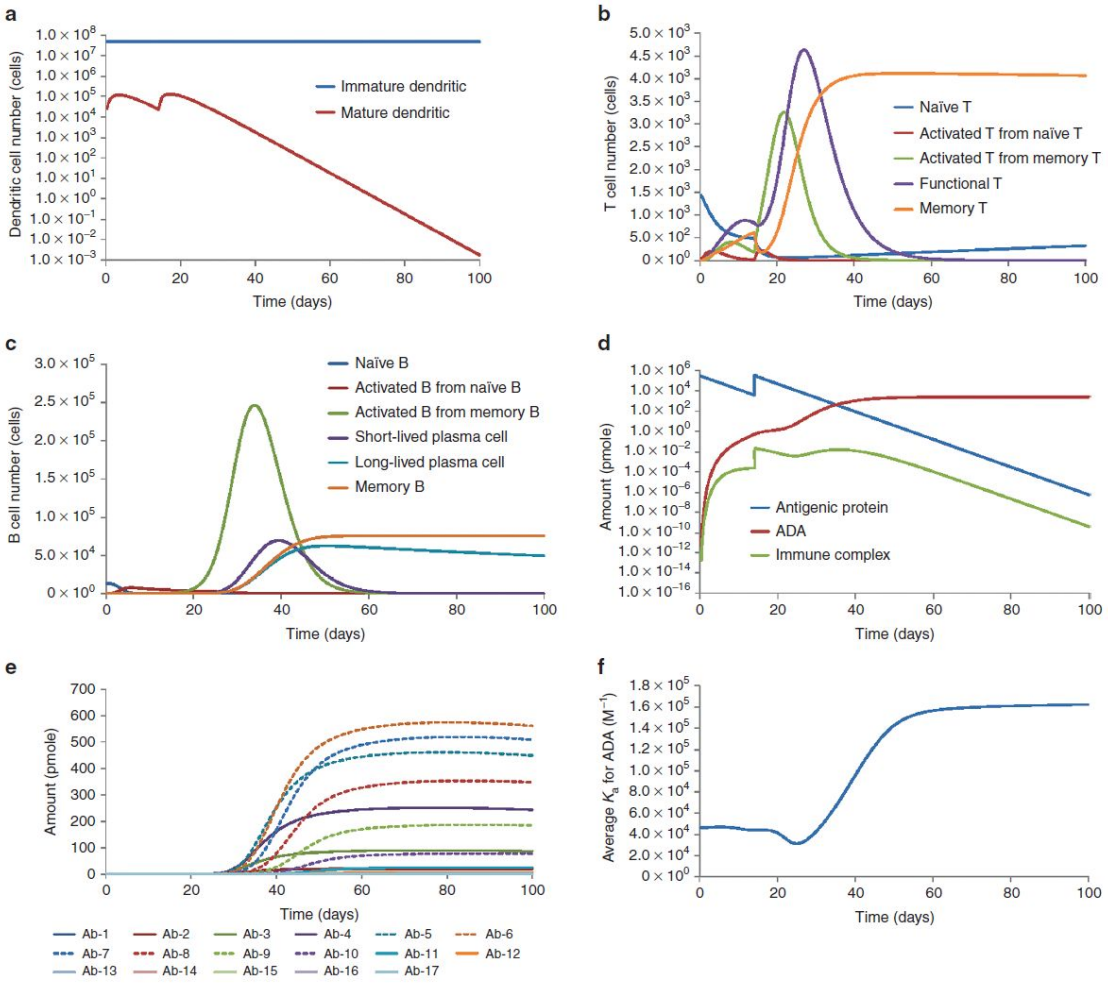
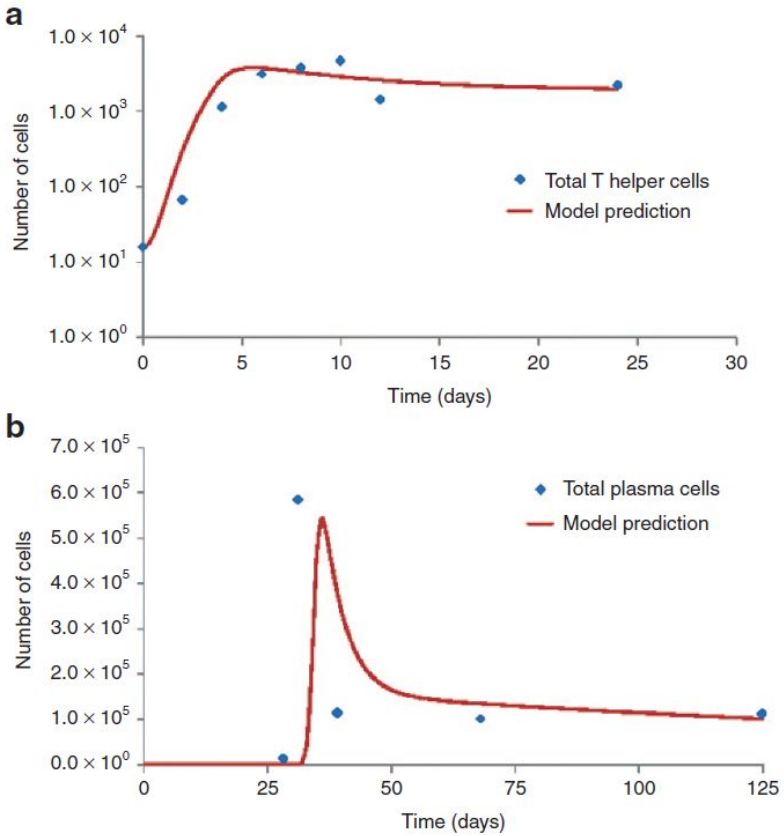


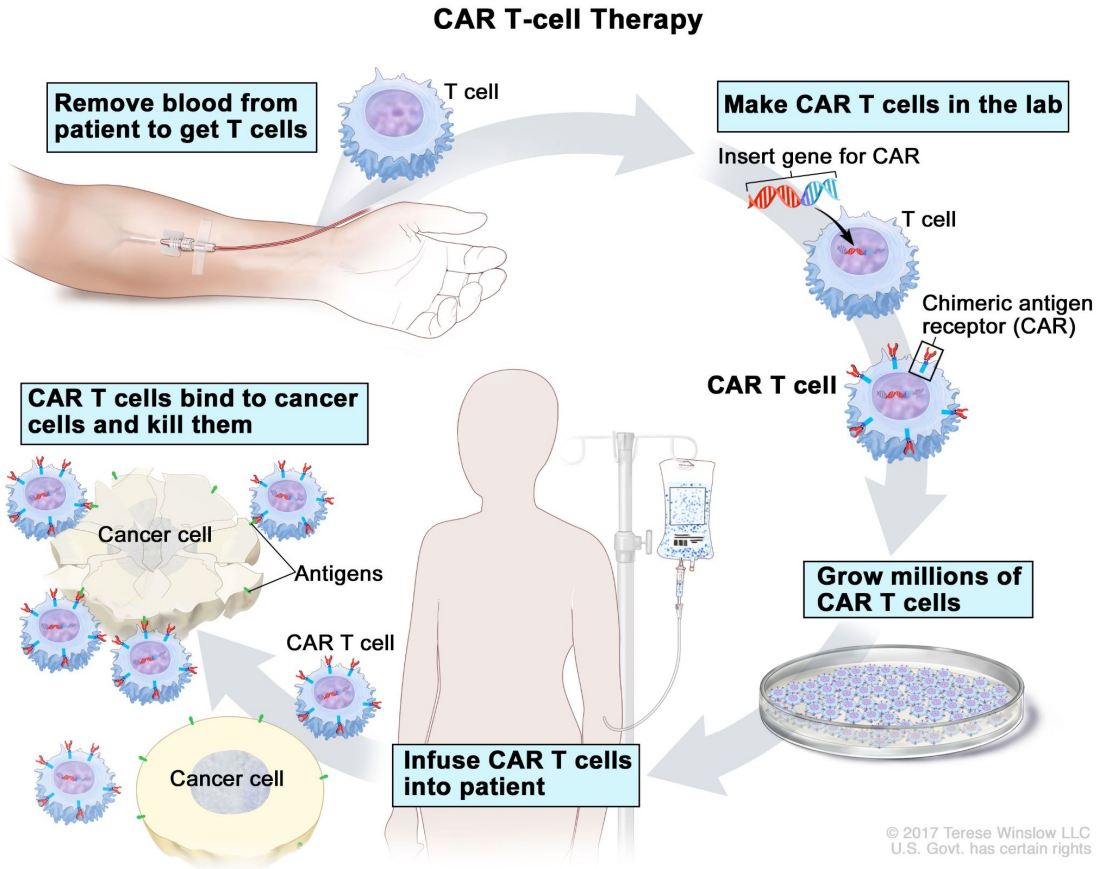
Figure 4. Simulation results of immune responses in human against a theoretical antigenic protein. The results include kinetic profiles for (**a**) dendritic cells; (**b**) helper T cells; (**c**) B cells; (**d**) antigenic protein, ADA, and immune complex; (**e**) polyclonal ADA (total 17 clones, whose antigen-binding affinity increases by twofold between clones, from clone 1 to clone 17); (**f**) average antigen-binding affinity of ADA. ADA, antidrug antibody.

Observation and model prediction

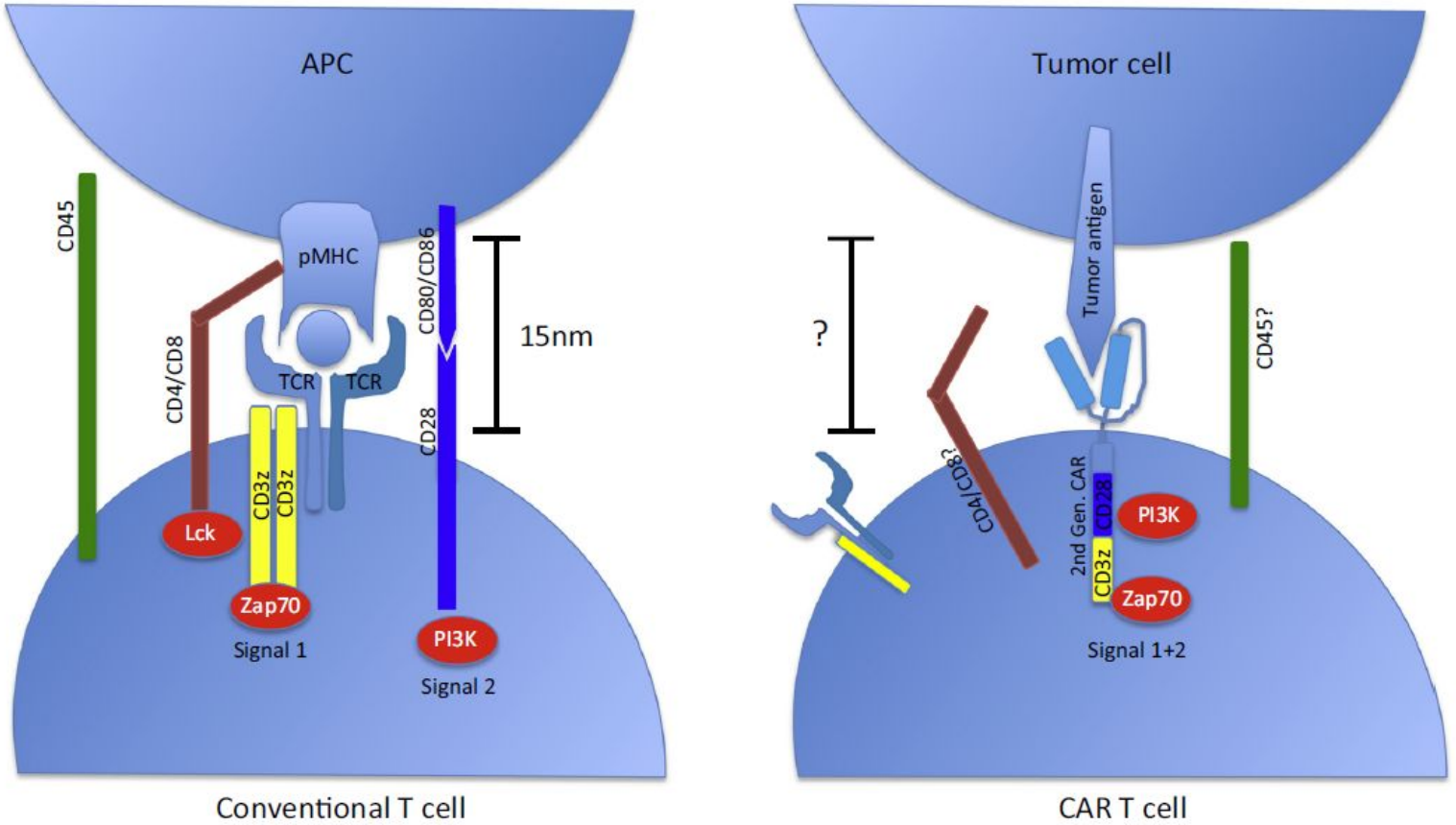


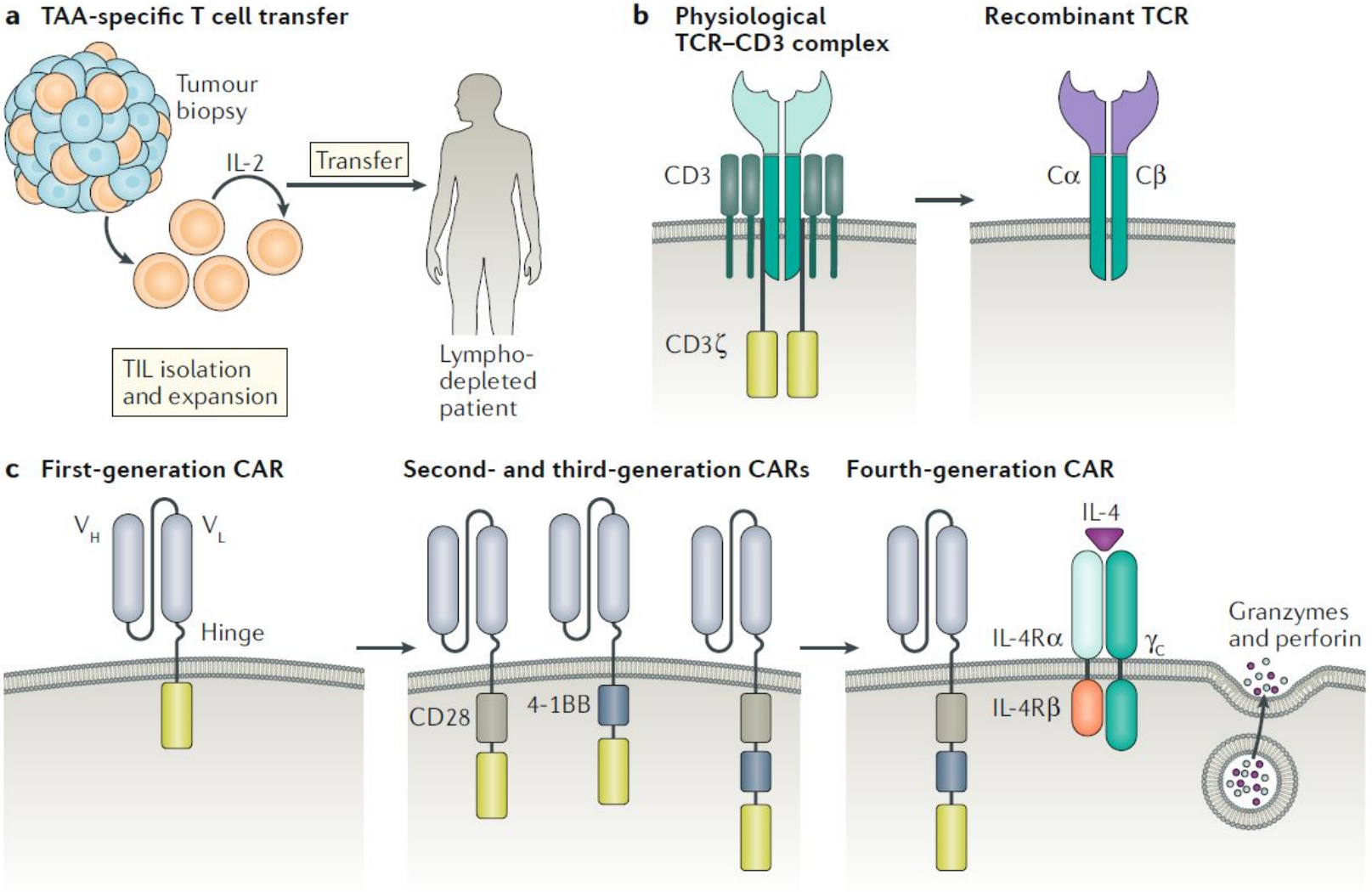
CAR-T and individualized vaccines

CAR (Chimeric Antigen Receptor) T-Cell Therapy



Signaling of conventional and CAR T cells




a TAA-specific T cell transfer
Tumour biopsy
IL-2
Transfer
TIL isolation and expansion
Lympho-depleted patient
b Physiological TCR–CD3 complex
Recombinant TCR
CD3
CD3ζ
Cα
Cβ
c First-generation CAR
Second- and third-generation CARs
Fourth-generation CAR
V_H
V_L
Hinge
CD28
4-1BB
IL-4
IL-4Rα
γ_c
IL-4Rβ
Granzymes and perforin

Towards personalized vaccine development