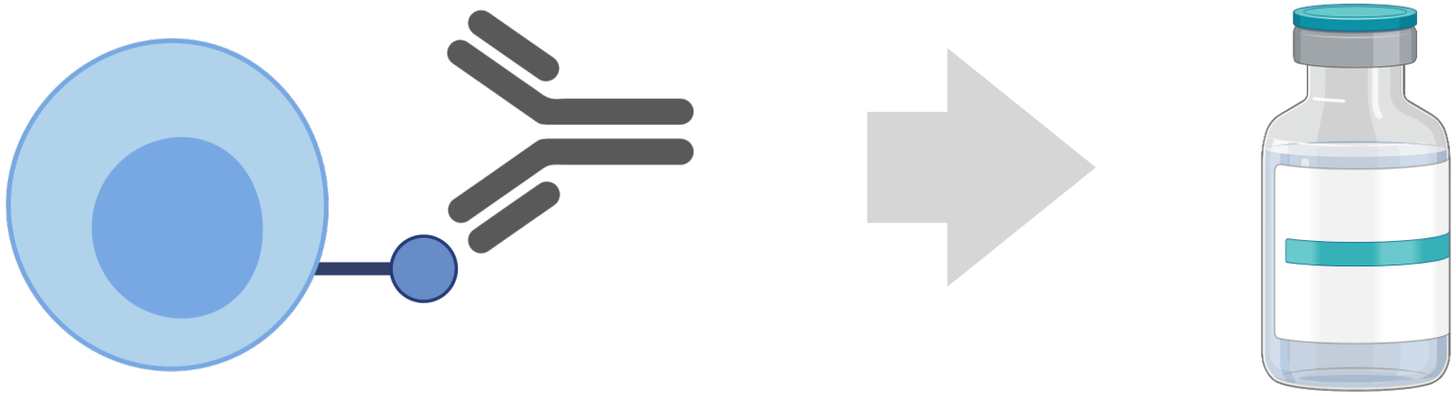
Antibodies and their Therapeutic Applications

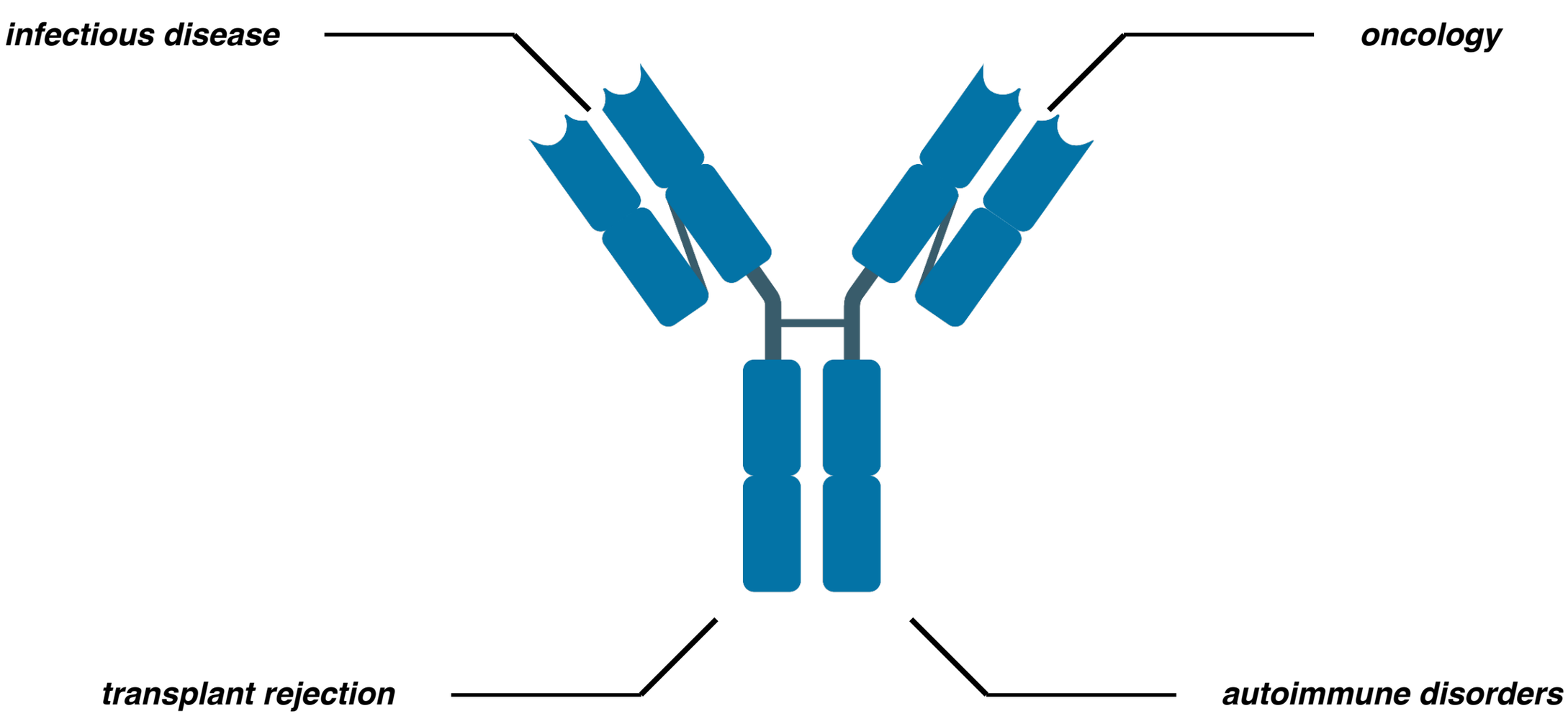


Nick Till

MacMillan Group Meeting 05/18/2020

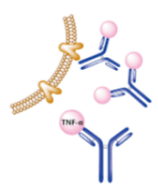
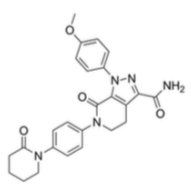
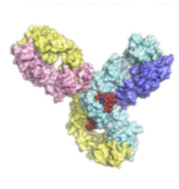
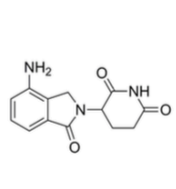
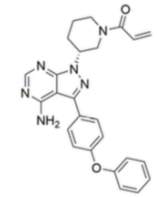
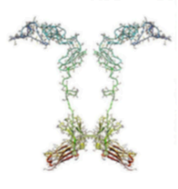
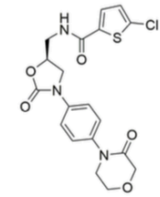
Role of Antibody Therapeutics in Medicine

– antibodies and their derivatives are powerful agents for modulating extracellular protein function



Role of Antibody Therapeutics in Medicine

- 6 of 10 top selling drugs in 2019 are antibody therapeutics (or derivatives thereof)

1 Humira (Adalimumab)	2 Eliquis (Apixaban)	3 Keytruda (Pembrolizumab)	4 Revlimid (Lenalidomide)	5 Imbruvica (Ibrutinib)	6 Opdivo (Nivolumab)	7 Eylea (Aflibercept)	8 Avastin (Bevacizumab)	9 Enbrel (Etanercept)	10 Xarelto (Rivaroxaban)
									
$19.723 Billion	$12.149 Billion	$11.084 Billion	$9.378 Billion	$8.085 Billion	$8.015 Billion	$7.363 Billion	$7.285 Billion	$7.216 Billion	$6.81 Billion
Immunology	Cardiovascular Diseases	Oncology	Oncology	Oncology	Oncology	Ophthalmology	Oncology	Immunology	Cardiovascular Diseases

muromonab-CD3

1986

first FDA approval of
an antibody therapeutic
(mouse antibody)

Humira

2003

first fully human antibody,
$137bn in sales by 2019

McGrath, N. A.; Brichacek, M.; Njardarson, J. T. *J. Chem. Educ.* **2010**, *87*, 1348–1349.

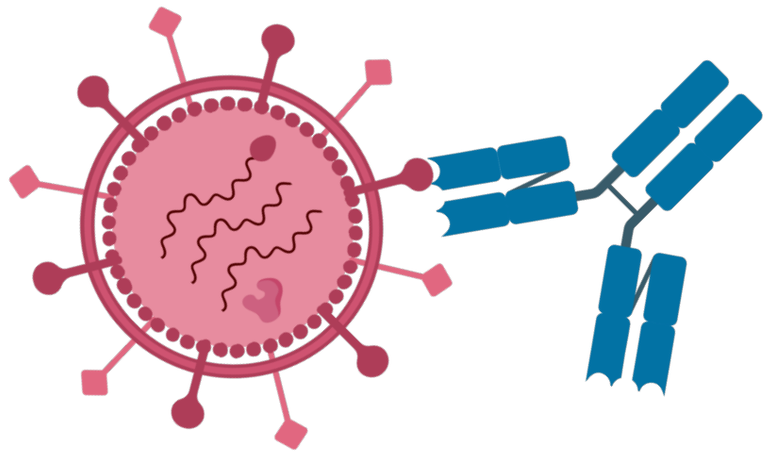
Outline of the Talk

- Part 1: the native function of antibodies in the immune response to infection
  - How antibodies are generated/origin of diversity
  - How antibodies exert their immunological effects
- Part 2: therapeutic antibodies and their development
  - technology used for antibody discovery and production
  - selected examples of mechanisms of action
- Part 3: antibody derivatives as therapeutics and future outlook

Native Immunological Function of Antibodies

foreign pathogen

(virus, bacterium, parasite)



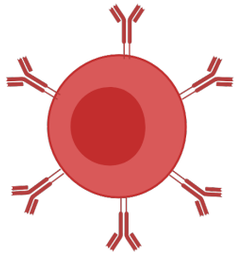
antibody

(binds foreign pathogen)

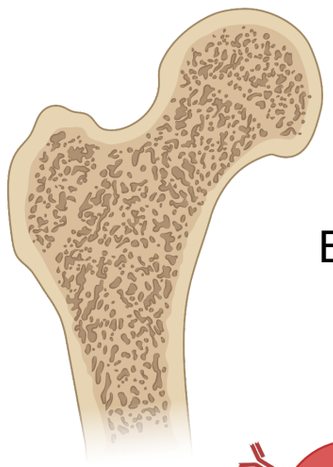
antibody response mechanisms

- opsonization (marking for phagocytosis)
- neutralization (important for toxins)
- complement activation (engages innate immunity)

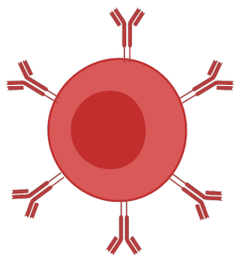
Native Immunological Function of Antibodies

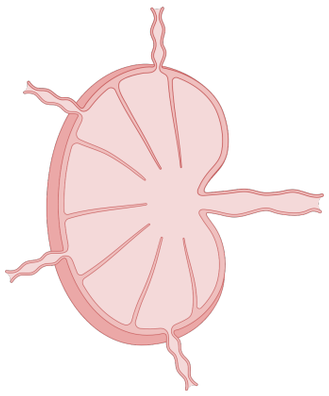


– B cells are antibody-producing lymphocytes, and crucial to adaptive immunity



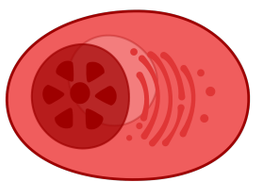
B cells begin development
in the bone marrow

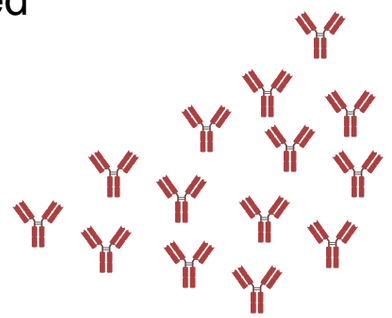




B cell maturation is completed
in the lymphatic system

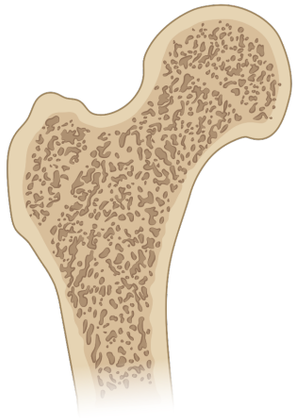
plasma cells produce
antibodies and can be long-lived

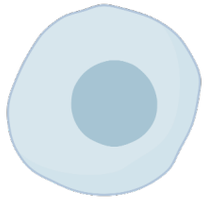




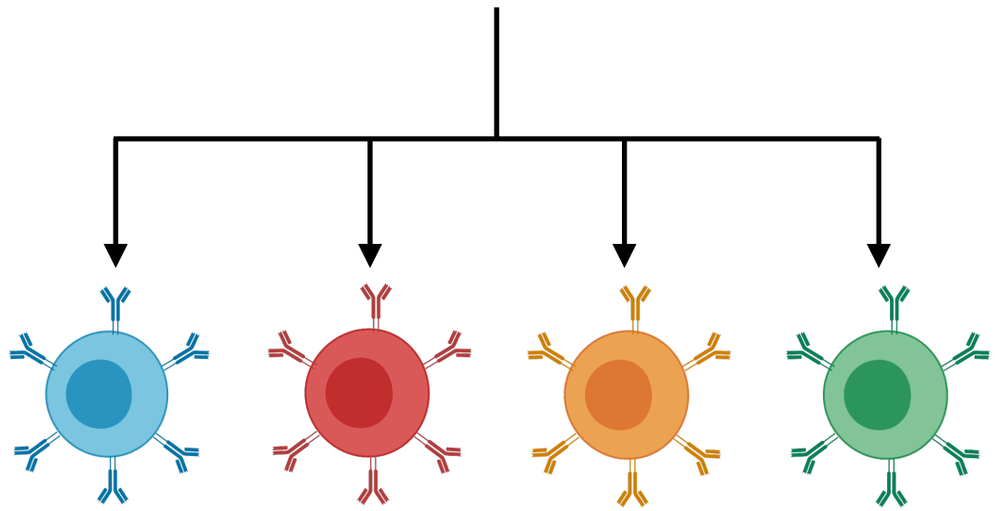
Native Immunological Function of Antibodies

First step of diversity generation takes place in the bone marrow (early B cell development)



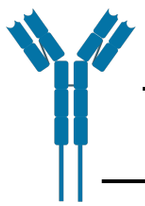


lymphoid progenitor cell



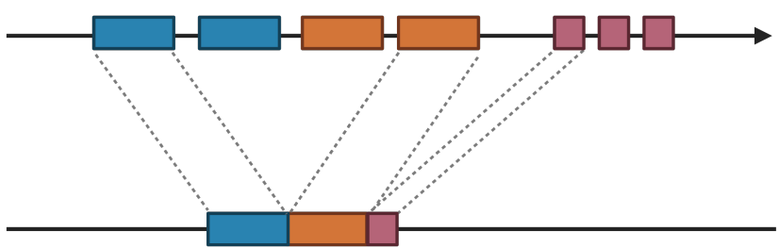
B cell repertoire: diverse set of B cells, each with a unique *BCR*

B cell receptor (BCR)



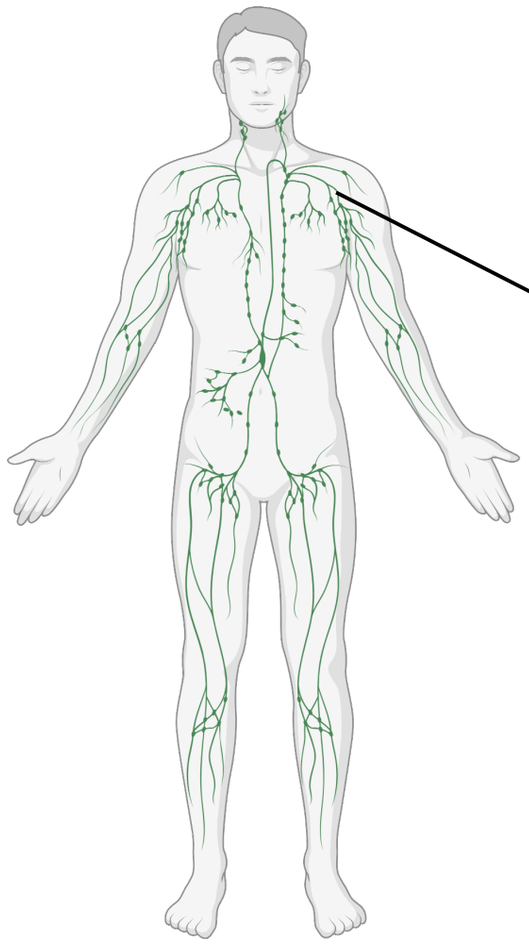
immunoglobulin domain

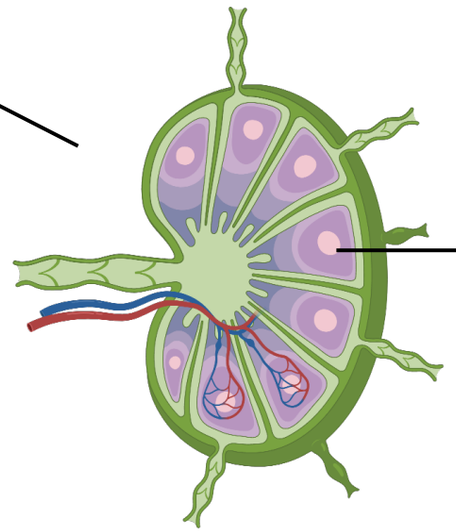
integral membrane domain

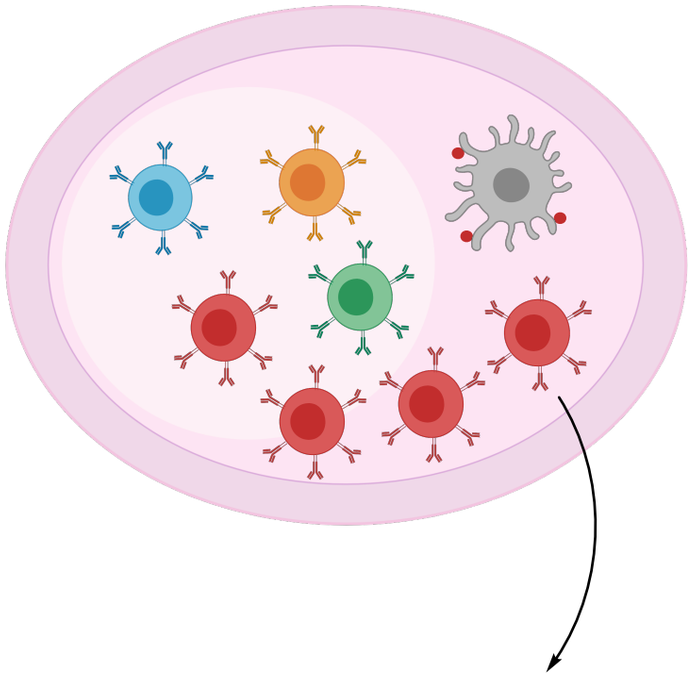


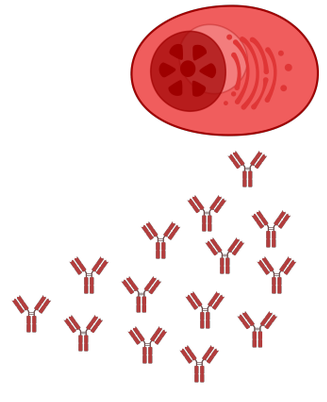
V(D)J recombination: genome rearrangement generates diverse set of BCRs (one per cell)

Native Immunological Function of Antibodies

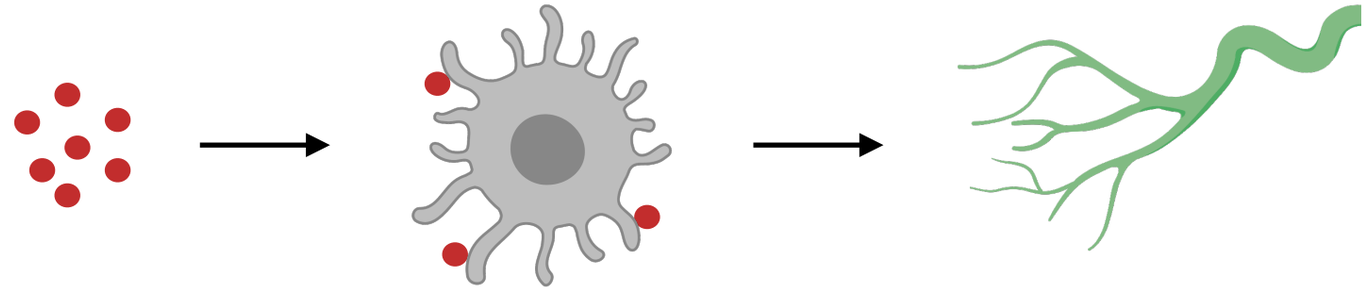








antigen trafficking during infection



foreign antigen

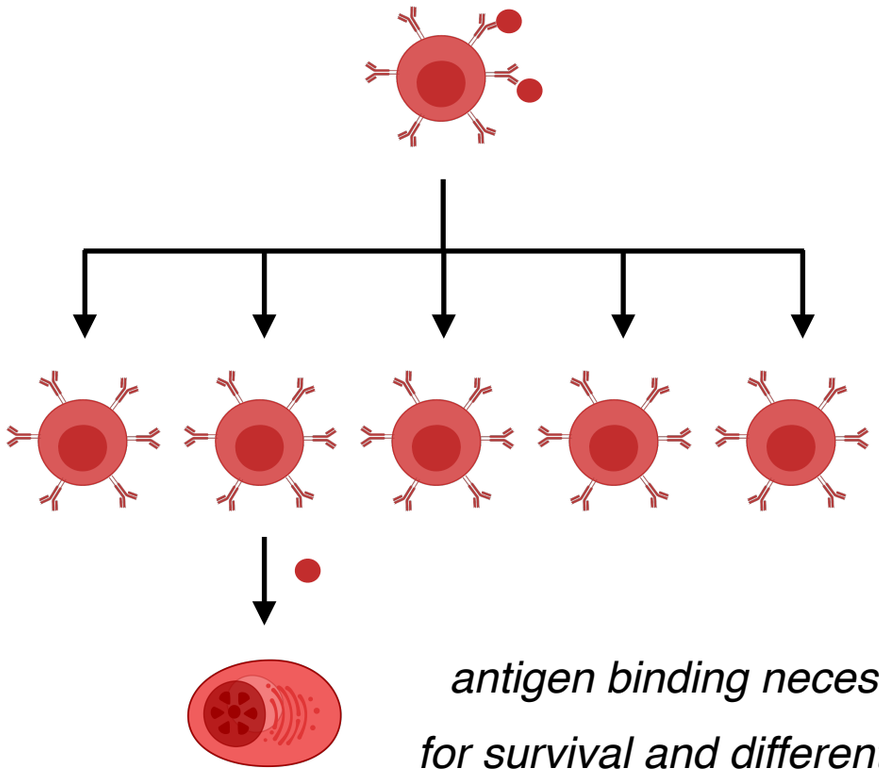
dendritic cell

DC enters lymphatic

high affinity B cell selected

Native Immunological Function of Antibodies

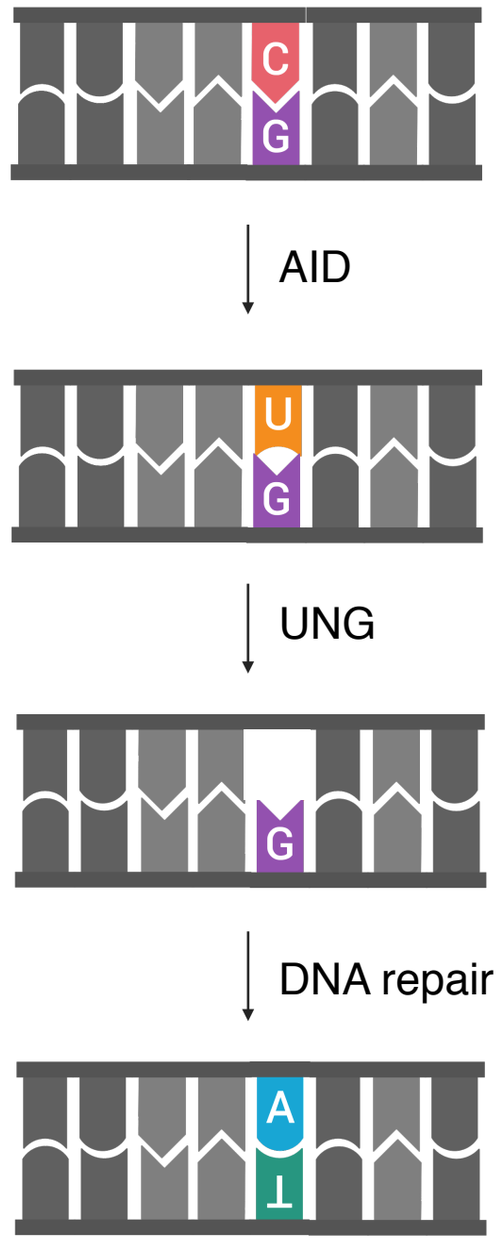
affinity maturation (positive selection process)



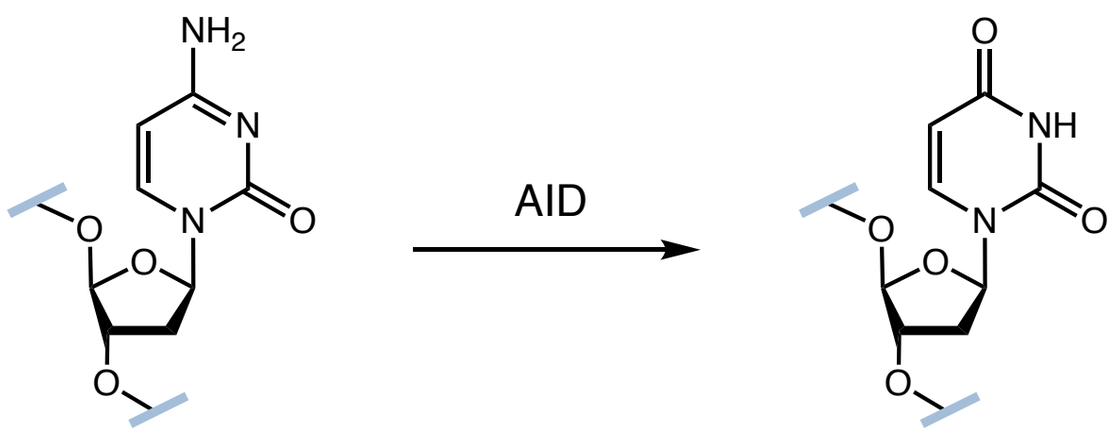
antigen binding necessary
for survival and differentiation

somatic hypermutation

(mechanism for genetic diversification)



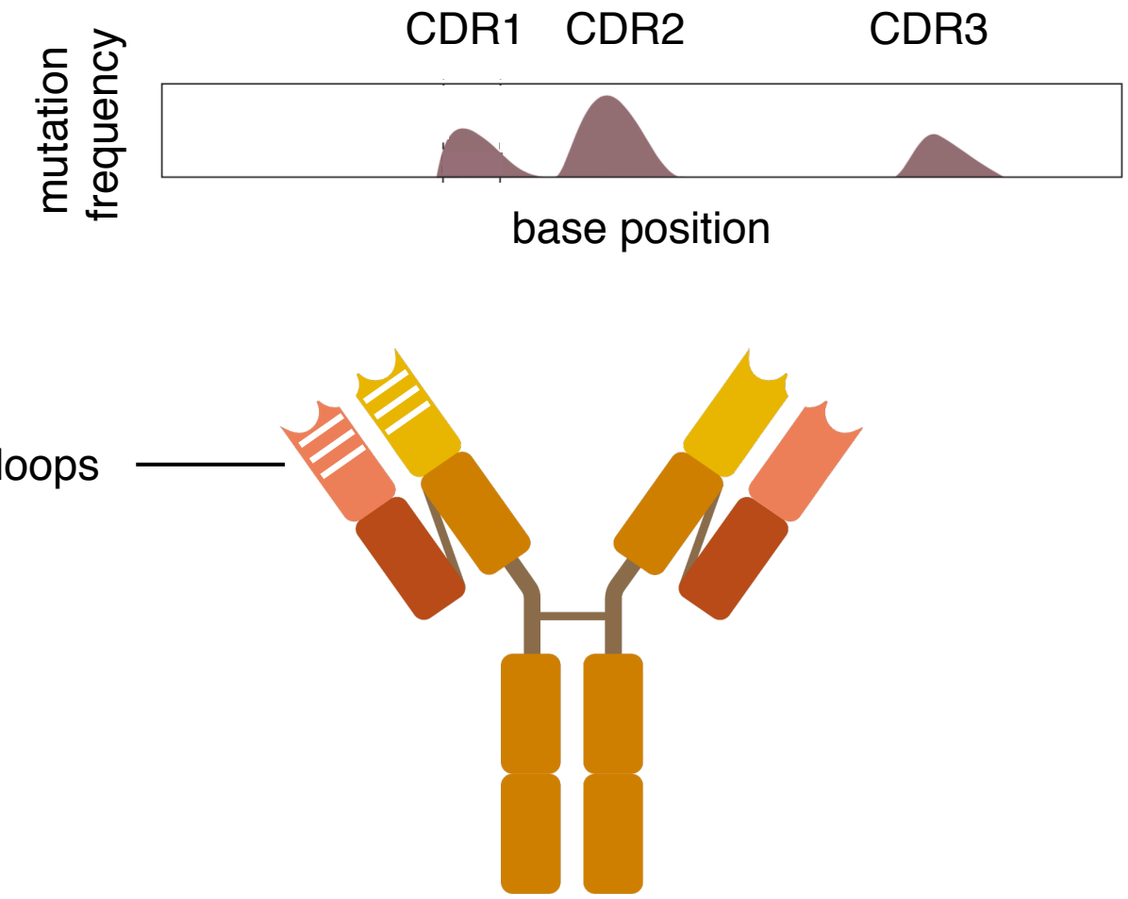
chemical mechanism of mutation generation



Di Noia, J. M.; Neuberger, M. S. *Annu. Rev. Biochem.* **2007**, *76*, 1–22.

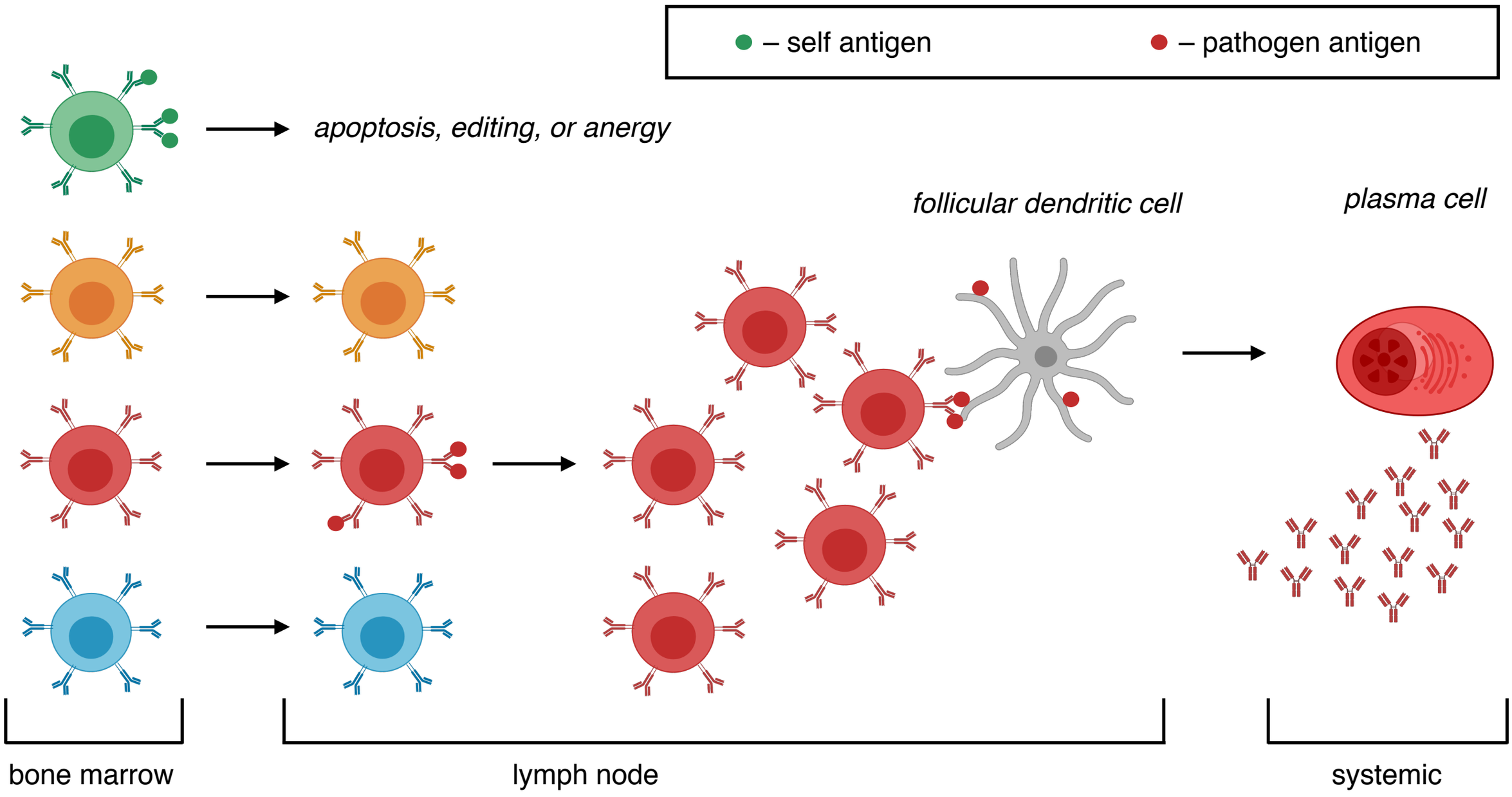
Native Immunological Function of Antibodies

sites of mutation are not random – localized to CDRs



complementarity-determining regions (CDRs): important for antigen binding, highly variable

Native Immunological Function of Antibodies



negative selection: self-reactive B cells do not progress to the proliferative phase

follicular dendritic cell: presents antigen to proliferating B cells, promotes binding-dependent growth

plasma cell: mature antibody-generating B cell (can be long-lived “memory B cell”)

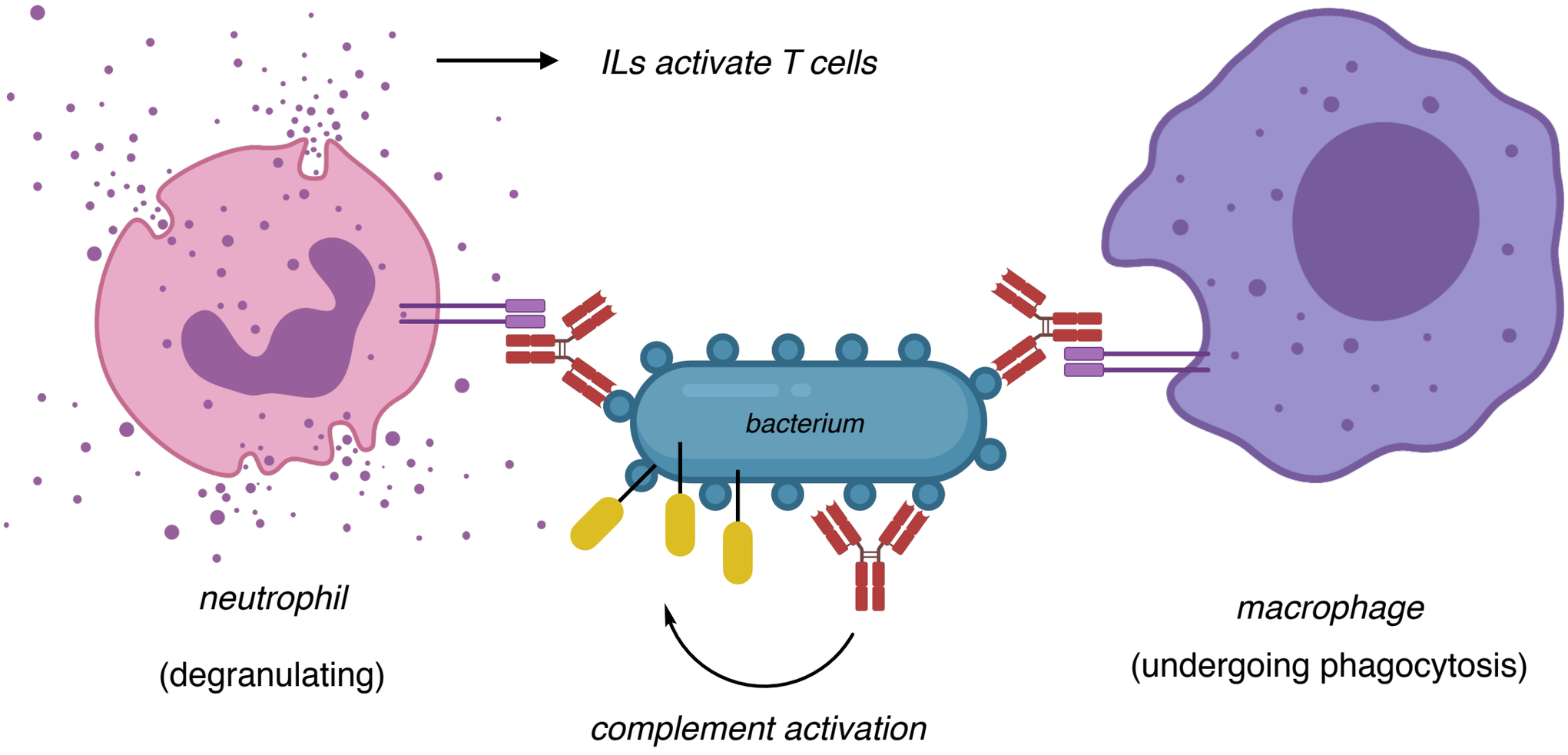
Native Immunological Function of Antibodies

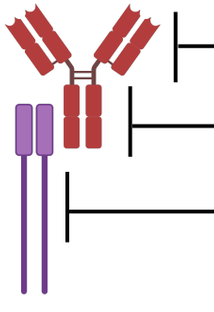
Part 1A: how does antigen specificity arise?

Part 1B: how do antibodies exert their immunological effector functions?

Native Immunological Function of Antibodies

Antibody-antigen binding causes varied and fluxional immune responses





F_{ab} (antigen binding fragment): determines antigen specificity

F_c (crystallizable fragment): determines immunological response

F_cR (F_c receptor): binding to antibody elicits immune effector function

Native Immunological Function of Antibodies

IgM – first isotype produced, low affinity,

its pentamers activate complement

IgM pentamer

IgA – common in mucosal tissues, dimers

important for antigen trafficking

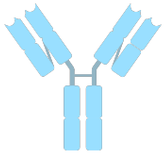
IgA dimer

IgE – Mast cell activation, important for parasite

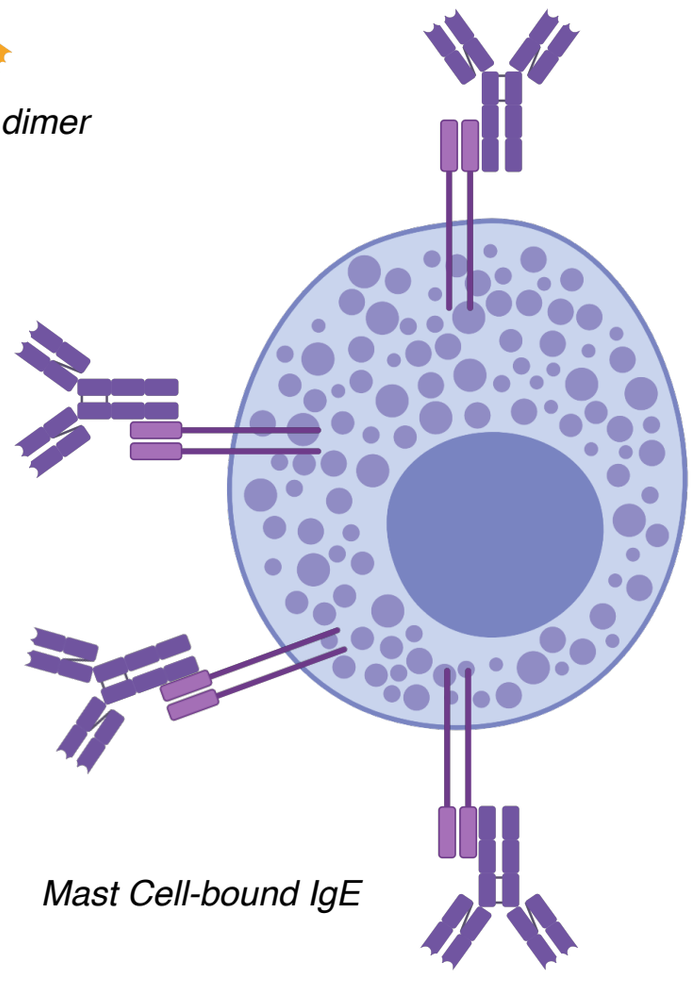
defense and allergic reactions

IgG – most abundant isotype, T cell activation,

complement activation, neutralization



IgD – basophil activation, less understood

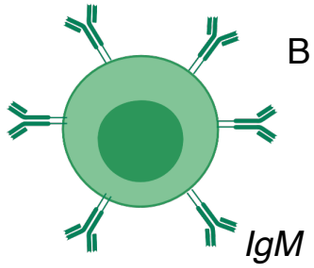


Mast Cell-bound IgE

Native Immunological Function of Antibodies

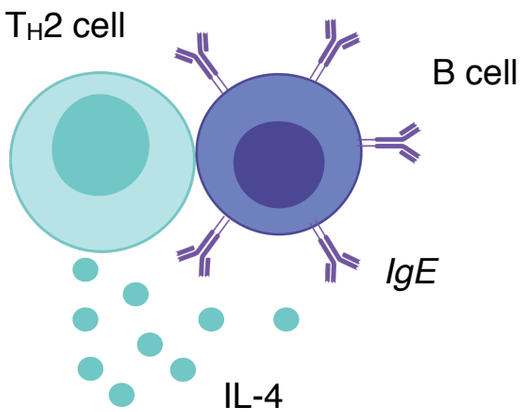
B cells produce IgM before

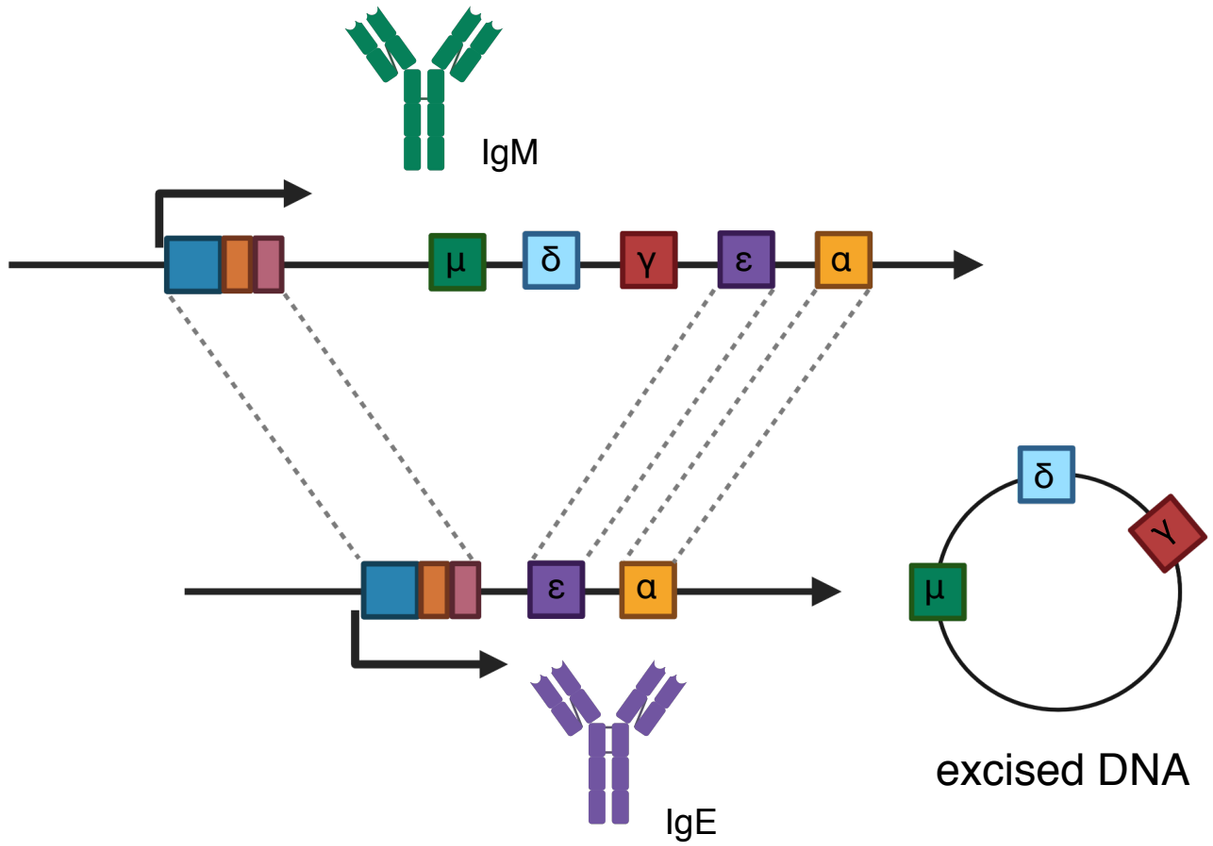
class-switching is activated



B cell activation by T cell

induces IgE production





excised DNA

no IgM, IgD, IgG

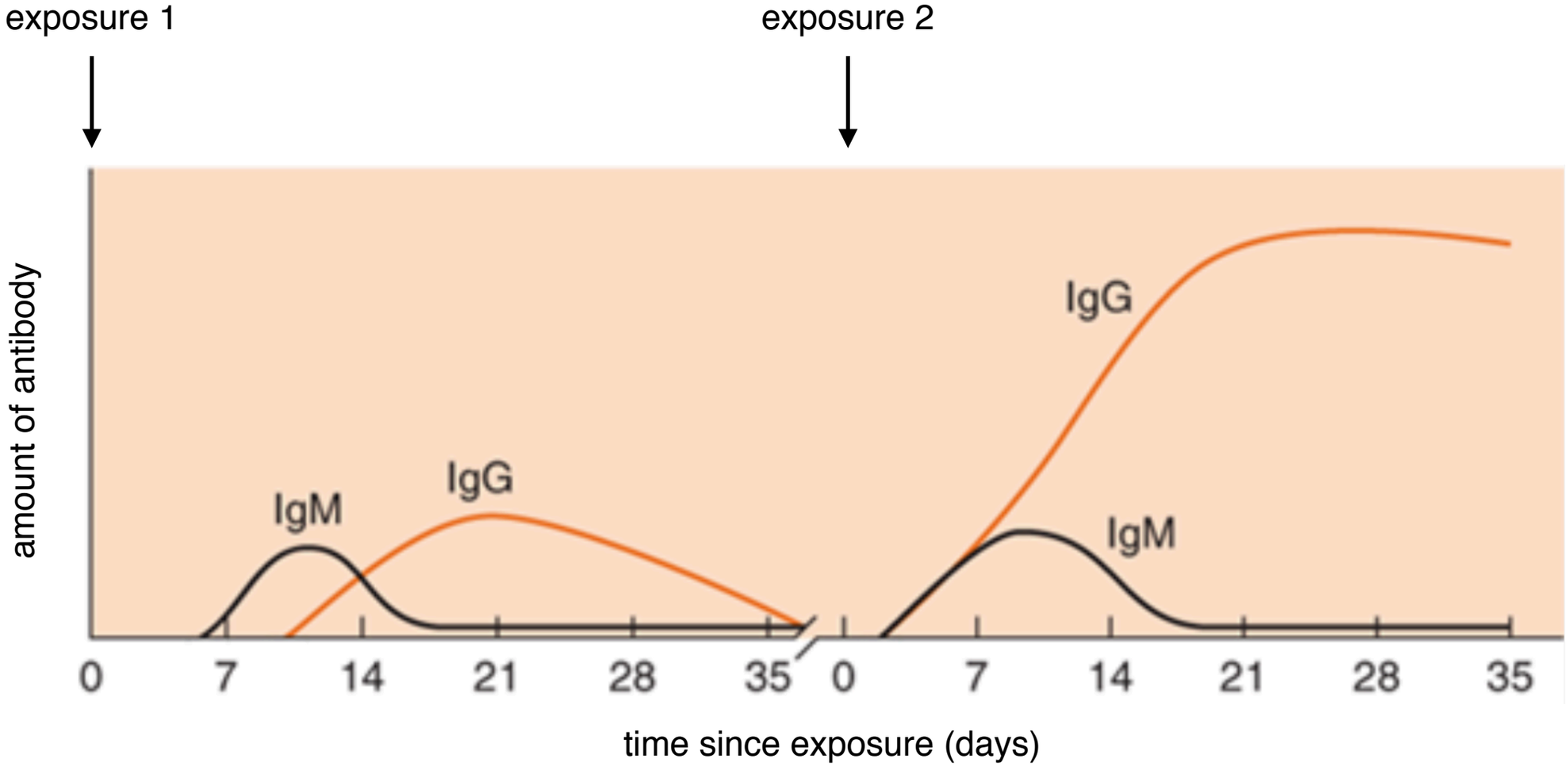
class-switch recombination: F_{ab} stays constant, but F_c isotype is changed

immune effector functions can change while keeping target constant

Native Immunological Function of Antibodies

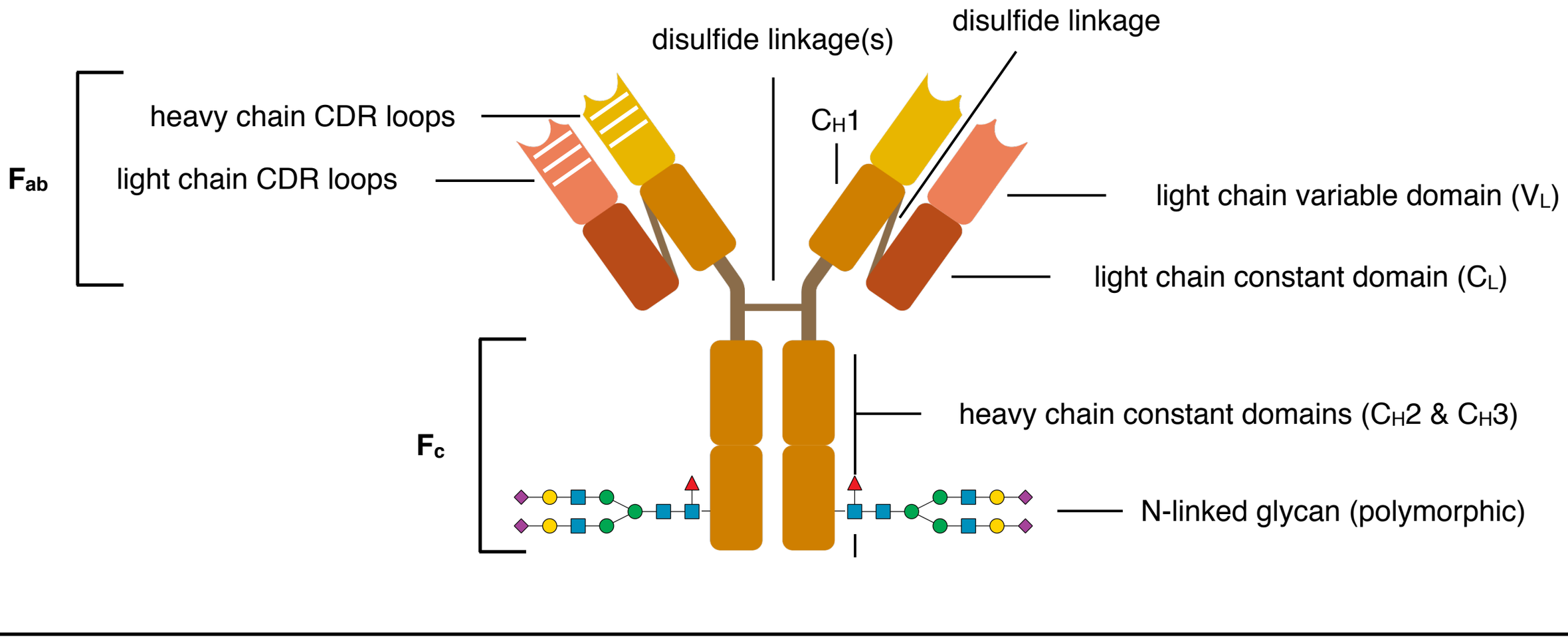
IgM: first to form in response to infection, but low affinity

IgG: slower to form in response to infection, but higher activity



Native Immunological Function of Antibodies

anatomy of an antibody

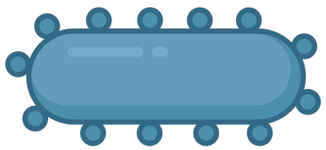


Development of Antibodies as Therapeutics

- Part 2: therapeutic antibodies and their development
  - technology used for antibody discovery and production
  - selected examples of mechanisms of action

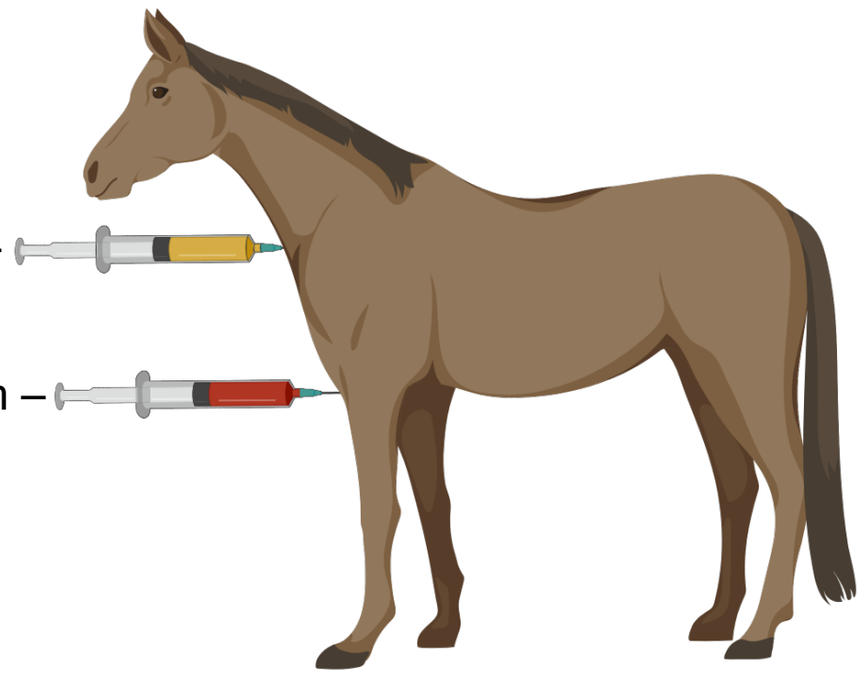
Development of Antibodies as Therapeutics

1890 – Emil Adolf von Behring develops the first antibody therapeutic, “chemical antitoxin”



inoculate with diphtheria bacterium –

collect anti-diphtheria antibodies serum –

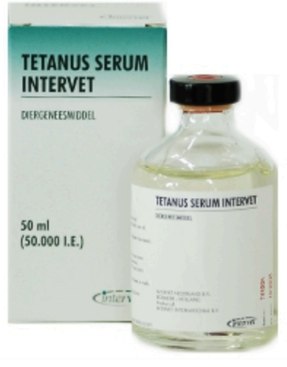


1891 – anti-diphtheria serum injected into 8 y.o. boy, curing him

1901 – von Behring awarded first Nobel prize in medicine

antibodies are still commonly used

as antivenins and antitoxins

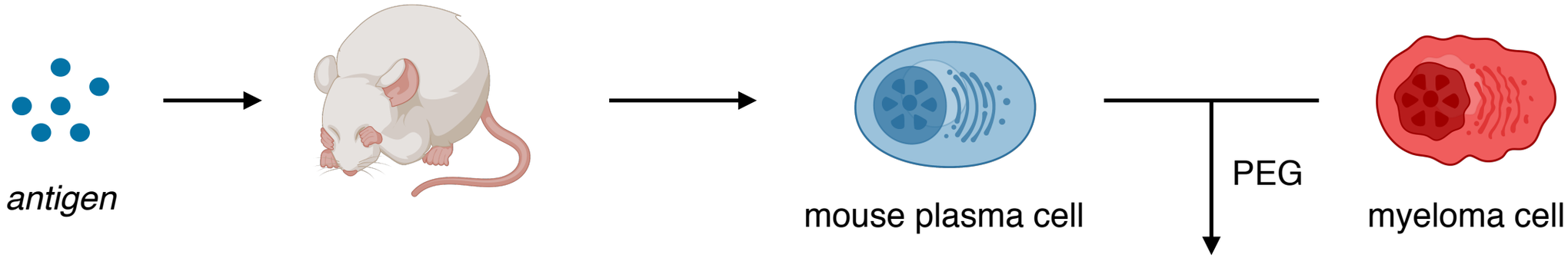


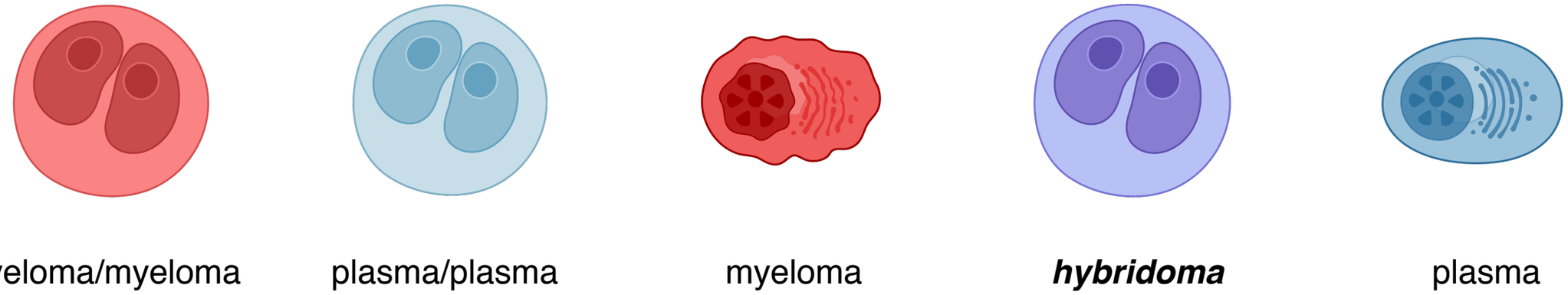
Raju, T. N. K. *Acta Paediatrica* **2006**, *95*, 258–259.

Development of Antibodies as Therapeutics

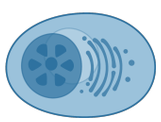
plasma cell – efficient Ab production, short-lived

myeloma cell – immortal cancer cell, no specific Ab production

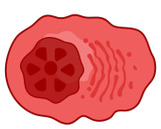




hybridoma formation is unselective – only a fraction of cells are desired plasma/myeloma hybridoma



– naturally limited number of cell cycles, dies



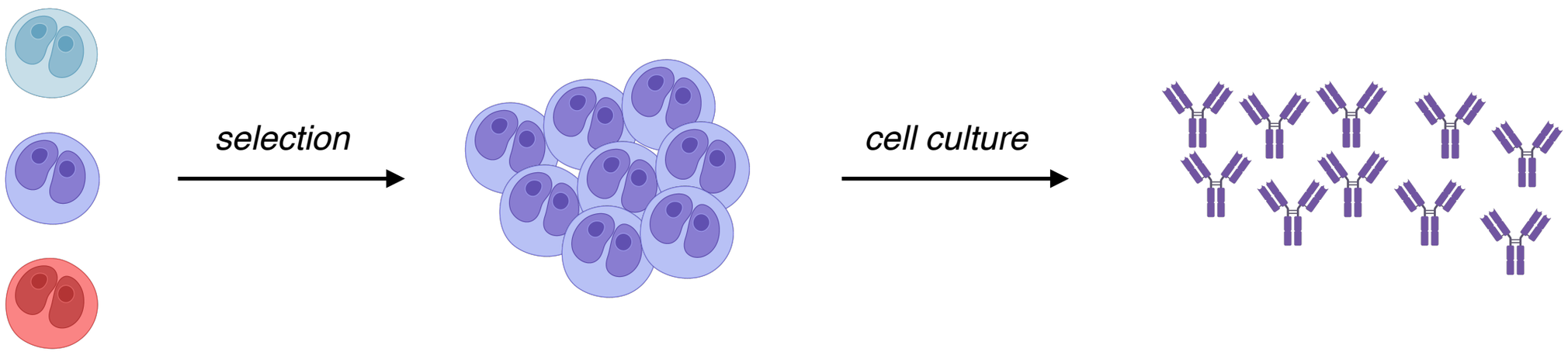
– thymidine kinase KO, *de novo* DNA synthesis inhibitor, dies

– thymidine kinase restored,

cell cycles unlimited

Köhler, G.; Milstein, C. *Nature* **1975**, *256*, 495–497.

Development of Antibodies as Therapeutics

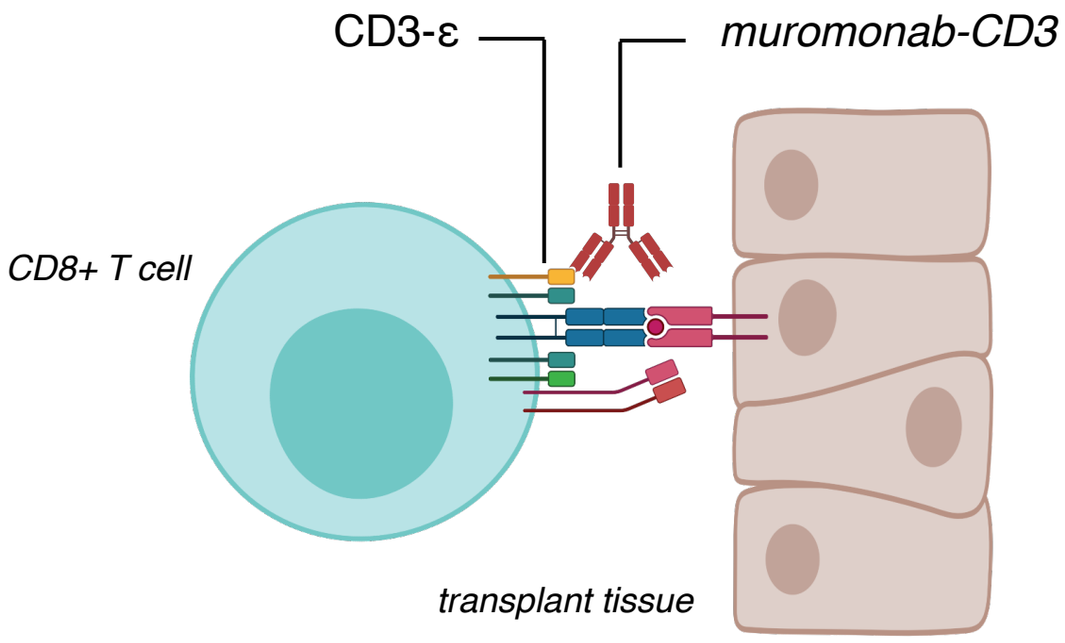


hybridoma selection – antigen affinity is identified with an ELISA assay

cell culture – with a hybridoma cell line, large scale mAb production possible

Development of Antibodies as Therapeutics

transplant rejection – T cells recognize and react to “non-self” tissue, causing often severe inflammation



- CD3 is critical T cell signaling and cytotoxic activity
- muromonab blocking of CD3-ε is immunosuppressive
- **1986:** first mAb approved by FDA, powerful treatment for glucocorticoid-resistant transplant rejection

muromonab-CD3

- muromonab-CD3 is a purely mouse antibody, elicits anti-mouse immune response
- human anti-mouse antibodies (**HAMA**) inactivate the drug, anaphylaxis possible

voluntarily withdrawn from the market in 2011

Development of Antibodies as Therapeutics

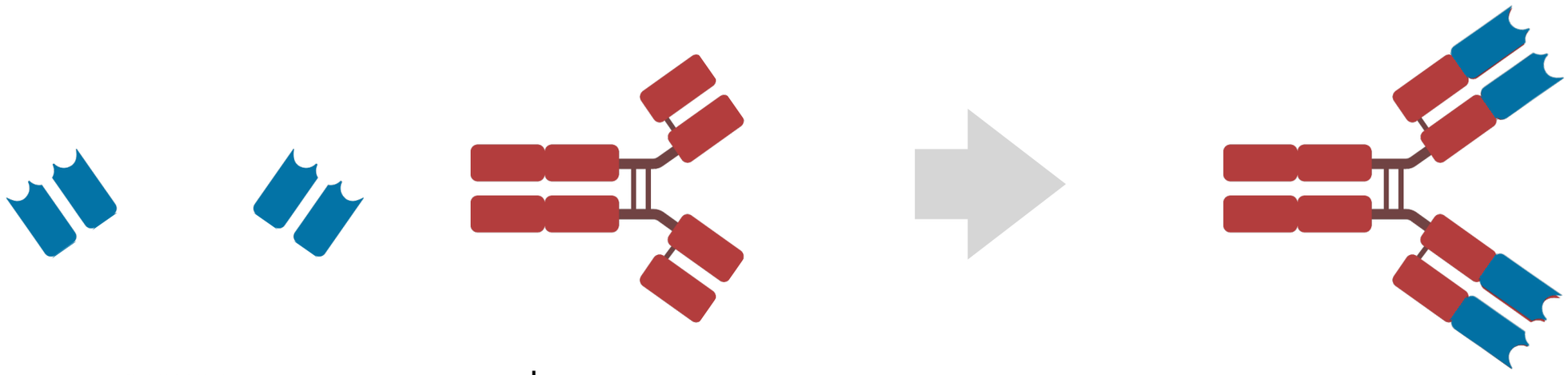
mouse antibodies challenging to develop as long-term treatments (cancer therapy, autoimmune disease)

developing antibodies with less immunogenicity, or more “humanness” became

a central focus of antibody therapeutic development from 1980s onward

Development of Antibodies as Therapeutics

mouse/human chimeric antibodies



mouse

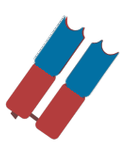
variable domains

human

constant domains

chimeric antibody

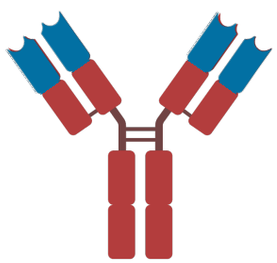
1984: chimeric antibodies can now be produced using hybridoma technology + recombinant DNA



1994: abciximab approved

first chimeric antibody

platelet aggregation inhibitor



1997: rituximab approved

anti-CD20 antibody

B cell lymphomas and more

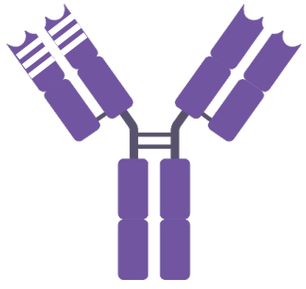
- 6 further chimeric antibodies have been approved since rituximab
- 4 more anti-CD20 antibody approvals followed the rituximab success

Morrison, S. L.; Johnson, M. J.; Herzenberg, L. A.; Oi, V. T. *PNAS* **1984**, *81*, 6851–6855.

Development of Antibodies as Therapeutics

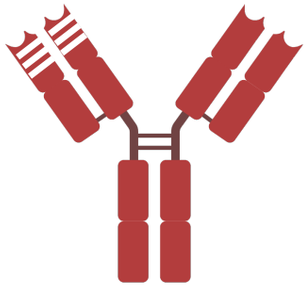
1986: Winter introduces “CDR grafting” technique – resultant antibodies are **“humanized antibodies”**

mouse hybridoma



mouse Ab

sequence mouse Ab, engineer CDRs into human Ab with rDNA



human Ab, mouse CDRs

CDR grafting often leads to large losses in potency

affinity maturation: process of fine-tuning target affinity (typically < 1 nM)

- site-directed mutagenesis in CDRs
- random mutagenesis libraries + selection

Jones, P. T.; Dear, P. H.; Foote, J.; Neuberger, M. S.; Winter, G. *Nature* **1986**, *321*, 522–525.

Development of Antibodies as Therapeutics

daclizumab

(Zinbryta)

1997

first humanized mAb

anti-CD25 for MS

trastuzumab

(Herceptin)

1998

anti-HER2

breast cancer

pembrolizumab

(Keytruda)

2014

anti-PD1

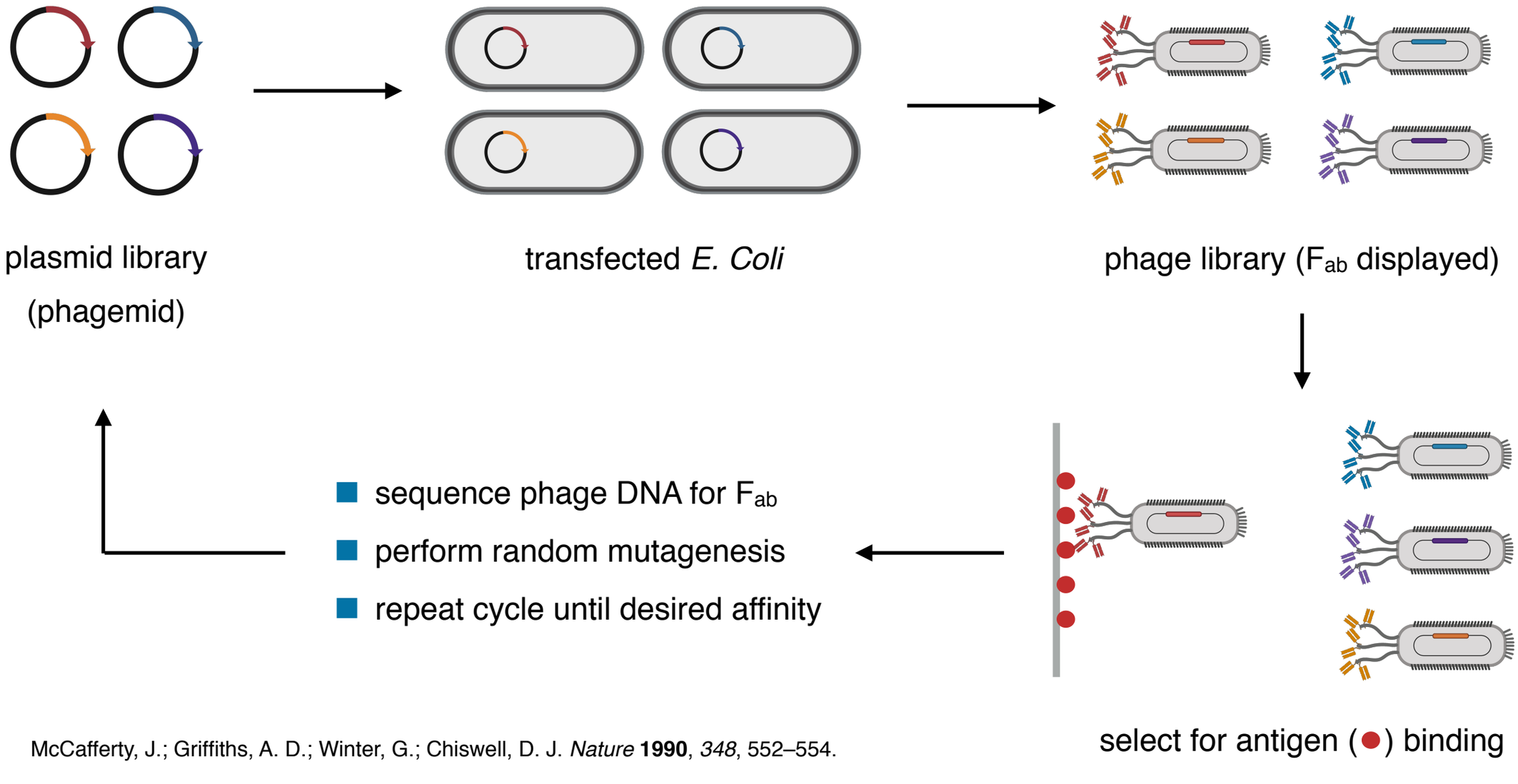
cancer (multiple)

- 38 humanized antibodies are currently on the market
- Genentech is the biggest player in the humanized mAb area

Development of Antibodies as Therapeutics

an ideal antibody therapeutic would be entirely human

phage display offers a selection system to identify high affinity antibodies without mice



McCafferty, J.; Griffiths, A. D.; Winter, G.; Chiswell, D. J. *Nature* **1990**, *348*, 552–554.

Development of Antibodies as Therapeutics

Humira

2003

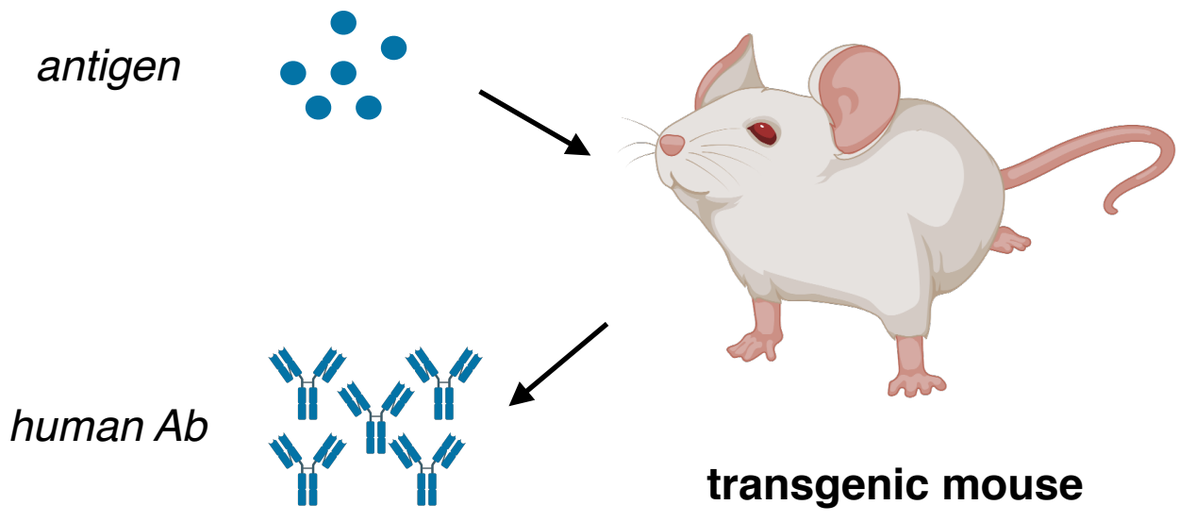
first fully human antibody,

$137bn in sales by 2019

- **Humira** (adalimumab) was developed using phage display technology

- **2018 Nobel Prize in Chemistry:** George Smith (1/4) and Gregory Winter (1/4) for developing phage display for mAb synthesis

Development of Antibodies as Therapeutics



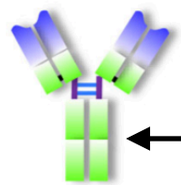
- human VDJ and C regions inserted
- mouse VDJ and C regions knocked out
- ***mouse B cells produce human Ig***

*transgenic mouse splenic B cells harvested and used in **hybridoma technology** for production*

Green, L. L.; Hardy, M. C.; Maynard-Currie, C. E.;… Jakobovits, A. *Nature Genetics* **1994**, *7*, 13–21.
Lonberg, N.; Taylor, L. D.; Harding, F. A.; Trounstine, M.; … Huszar, D. A *Nature* **1994**, *368*, 856–859.

Development of Antibodies as Therapeutics

major transgenic organism platforms for antibody generation

Company	Product	Reference	hVH[a]	hVK[b]	Constant	Country	Structure
Medarex	HuMAbMouse	1994 Lonberg *et al.*	4	4	Human (Cμ)	US	
Abgenix	XenoMouse	1994 Green *et al.* 1997 Mendez *et al.*	17	17	Human (Cμ-Cδ-Cγ2)	US	
Ligand	OmniRat	2013 Osborn *et al.*	22	12	Rat	US	
Kymab	KyMouse	2014 Lee *et al.*	43	37	Mouse	UK	
Regeneron	VelocImmune	2014 Murphy *et al.*	47	23	Mouse	US	
Harbour Antibodies BV	H2L2 Mouse	https://harbourantibodies.com	18	11	Mouse	US	
Trianni	Trianni Mouse	https://trianni.com/	44	39	Mouse	US	

Medarex: acquired by Bristol-Myers Squibb in 2009, later leads to approvals of **Yervoy** and **Opdivo**

Abgenix: acquired by Amgen in 2005, later leads to multiple antibody therapeutic approvals

Lu, R.-M.; Hwang, Y.-C.; Liu, I.-J.; Lee, C.-C.; Tsai, H.-Z.; Li, H.-J.; Wu, H.-C. *Journal of Biomedical Science* **2020**, *27* , 1.

Development of Antibodies as Therapeutics

phage display led to the first and most successful human mAb therapeutic (**Humira**)

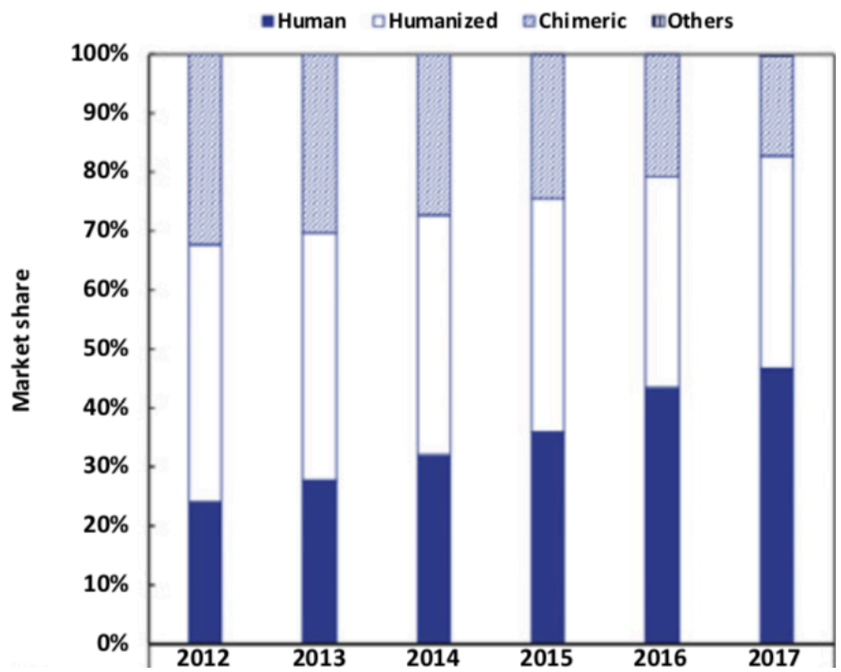
but

many more approvals have been gained using transgenic mice

phage display: 9 approvals

transgenic mouse: 19 approvals

the monoclonal antibody therapeutic landscape is increasingly human



Grilo, A. L.; Mantalaris, A. *Trends in Biotechnology* **2019**, *37*, 9–16

Development of Antibodies as Therapeutics

antibody generic name suffix indicates origin

- –**mo**mab: murine (mouse)
- –**xi**mab: chimeric
- –**zu**mab: humanized
- –**um**ab: fully human

Keytruda

pembrolizumab

–zumab: humanized monoclonal antibody

–li: refers to immunological target

Development of Antibodies as Therapeutics

Part 2A: importance of F_{ab} engineering for therapeutics

Part 2B: the F_c domain in therapeutic antibody development

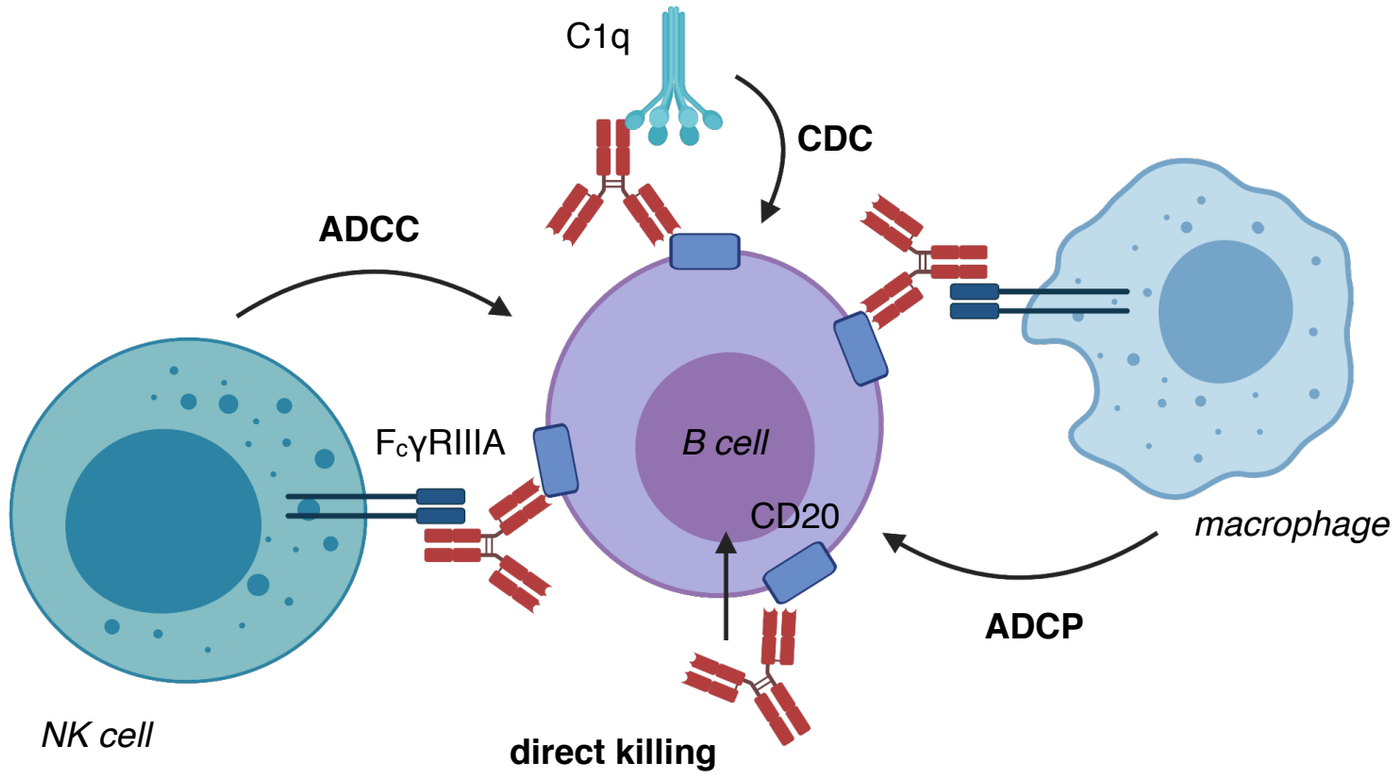
Development of Antibodies as Therapeutics

obinutuzumab (Gazyva)

anti-CD20 mAb

chronic lymphocytic leukemia

Genentech, **2013**



direct killing – apoptosis signaling induced by obinutuzumab binding CD20

ADCC (antibody-dependent cellular cytotoxicity) – cytotoxic immune effector cell kills cell

ADCP (antibody-dependent cellular phagocytosis) – phagocytic immune effector cell kills cell

CDC (complement-dependent cytotoxicity) – activating the complement system leads to cell death

the specific IgG isotype (Fc structure) determines immune response

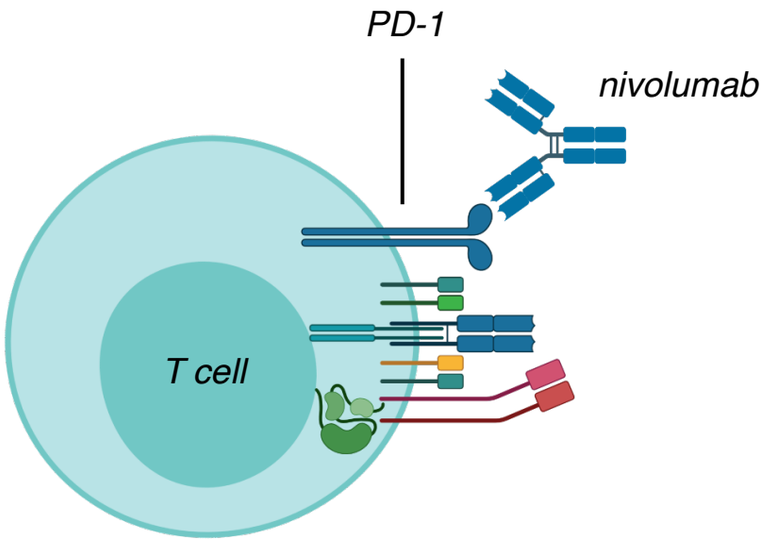
Mössner, E.; Brünker, P.; Moser, S.;…Umaña, P. *Blood* **2010**, *115*, 4393–4402.

Development of Antibodies as Therapeutics

Properties	**IgG1**	**IgG2**	**IgG3**	**IgG4**
Approximate molecular weight (kDa)	146	146	165	146
Hinge length (number of amino acids)	15	12	62	12
Antibody-dependent cell-mediated cytotoxicity	+++	+/−−	++	+/−−
Antibody-dependent cell-mediated phagocytosis	+	+	+	+/−−
C1q binding	+	+/−	+++	−
Complement-mediated cytotoxicity	++	+/−	++	−
FcRn binding	+	+	+/−	+
Plasma half-life (days)	21	21	5–7.5	21
Approximate average plasma concentration (mg ml^{-1})	9	3	1	0.5

obinutuzumab (Gazyva)

nivolumab (Opdivo)



- PD-1 blockade stops PD-L1 binding
- IgG4 does not activate cell-killing
- S228P mutation prevents F_{ab} exchange

Wang, C.; Thudium, K. B.; Han, M.;…Korman, A. J.. *Cancer Immunol Res* **2014**, *2*, 846–856.
Almagro, J. C.; Daniels-Wells, T. R.; Perez-Tapia, S. M.; Penichet, M. L. *Front. Immunol.* **2018**, *8*.

Development of Antibodies as Therapeutics

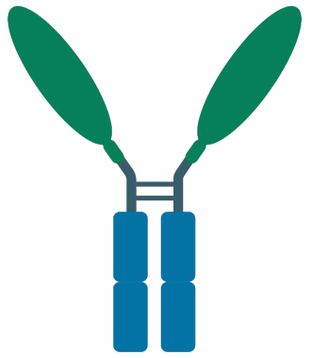
- Part 3: antibody derivatives as therapeutics and future outlook

Development of Antibodies as Therapeutics

nanobody

(caplacizumab, **2019**)

Ablynx



F_c-fusion protein

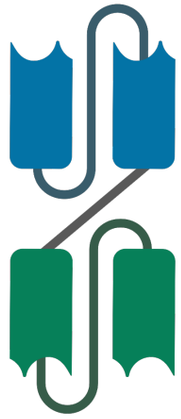
(etanercept, **1998**)

Amgen

DuoBody

(epcoritamab, **phase I/II**)

GenMab



BiTE

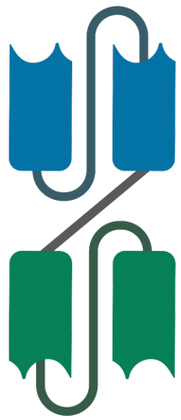
(blinatumomab, **2014**)

Amgen

Development of Antibodies as Therapeutics

blinatumomab: bispecific T cell engager (BiTE)

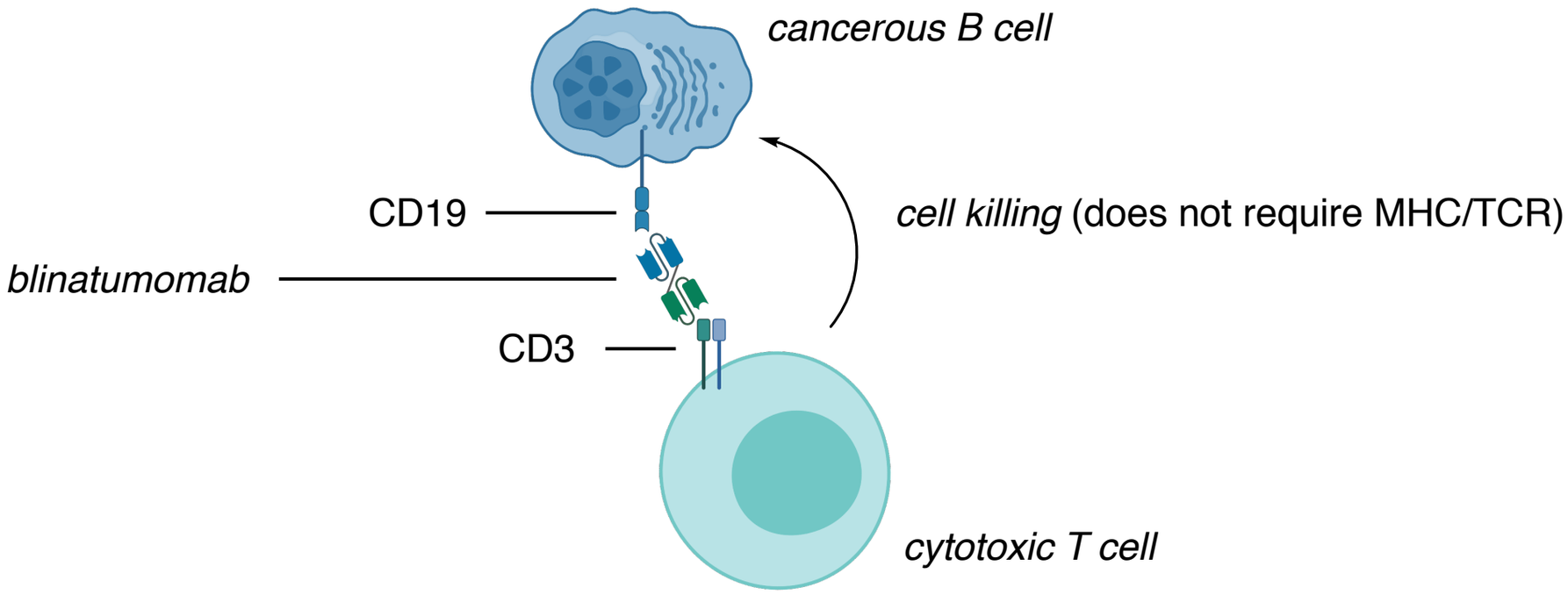
Amgen, FDA approval in **2014** for ALL



– anti-CD19 murine scF_V (B cell targeting)

– anti-CD3 murine scF_V (T cell targeting)

mechanism of action

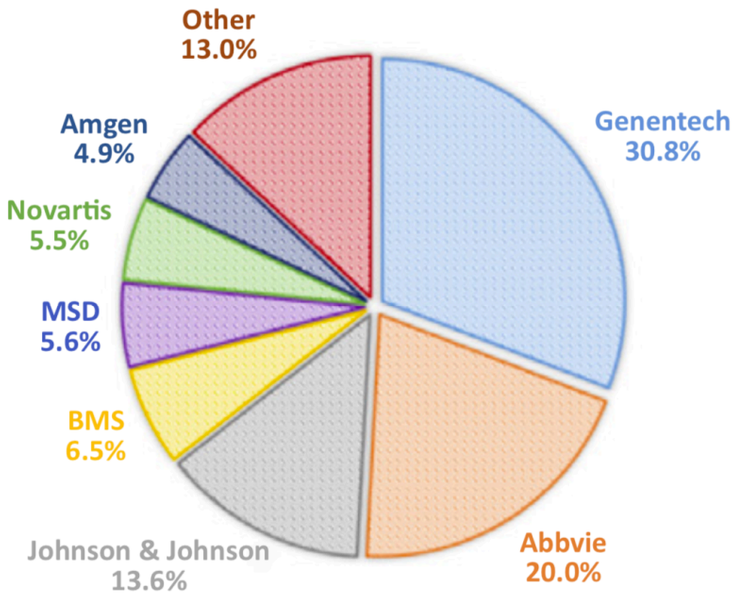


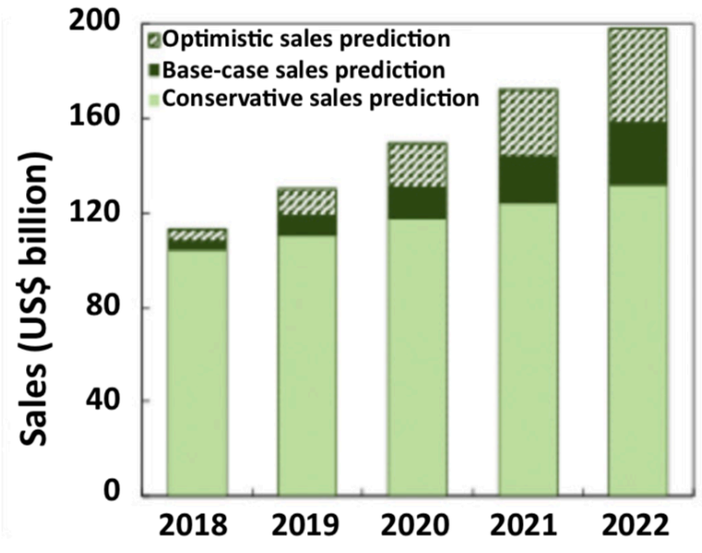
Development of Antibodies as Therapeutics

drawbacks/limitations of antibody therapeutics

- injection necessary (1-2% oral bioavailability)
- no intracellular targets accessible (low membrane permeance)
- biosimilars are not generics, require more lengthy approval process
- manufacturing costs are higher than small molecules

current and future market outlook for mAbs





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