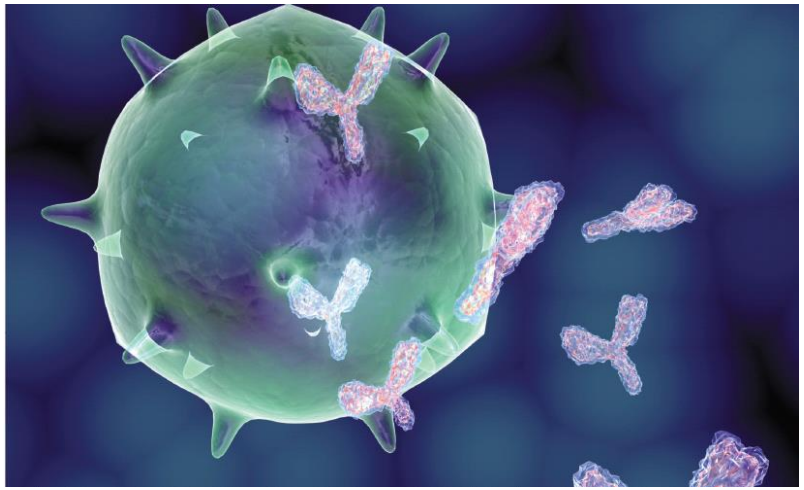
UCL CANCER INSTITUTE
Paul O'Gorman Building

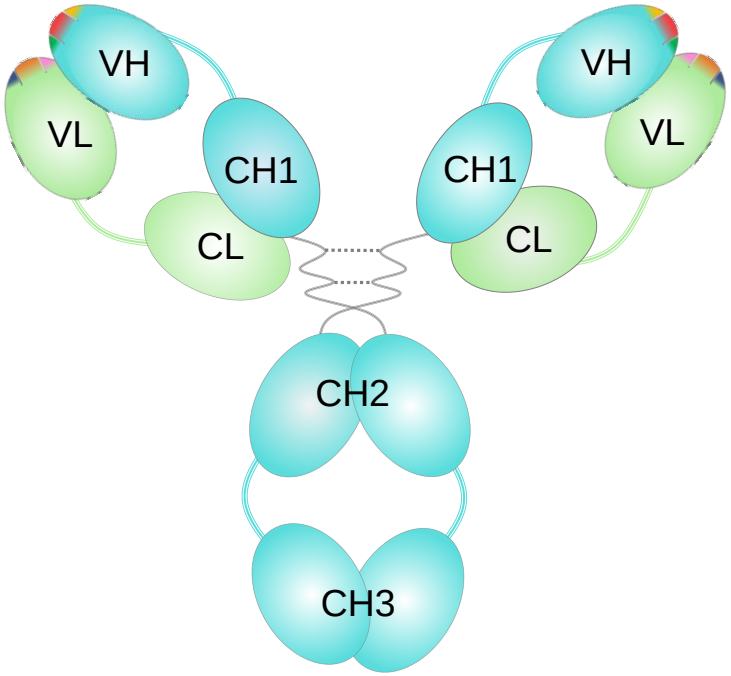
Recombinant Antibodies for Cancer Therapy: 1



Kerry Chester

Antibodies: Sequence, Structure and Designing Therapeutics 4th - 5th July 2019

The Antibody



These iconic, characteristically Y shaped proteins with virtually limitless binding diversity are expected to comprise almost half of the USD $22.7 billion biologics discovery market by 2025.

Talk Outline

PART I

Why engineered antibodies are such powerful tools

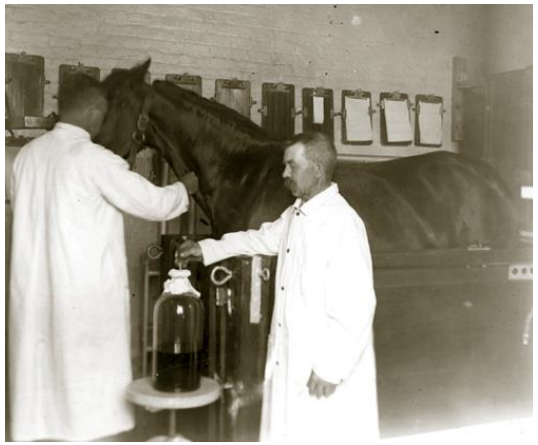
Main ways that (usually whole) engineered antibodies are used as anti-cancer drugs

PART II

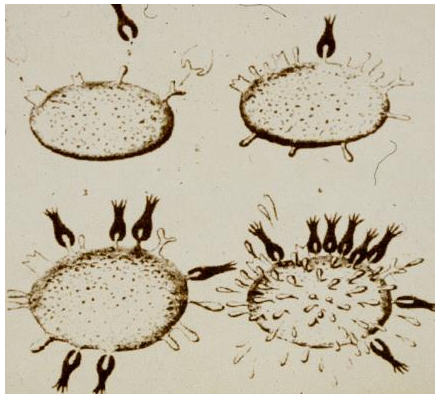
Wider scope

- Engineered Single chain Fv fragments (ScFv)
- Phage technology
- ScFv for T cell therapy (CAR-T)
- Bi-specifics and Bi-specific T-cell engagers (BiTEs)

Power of antibodies - the beginning of discovery



Practical 1890s, discovery that serum from infected animals can be used to treat infection in other animals - horse antiserum was used to treat diphtheria and tetanus. Behring & Kitasato 1901 Nobel Prize

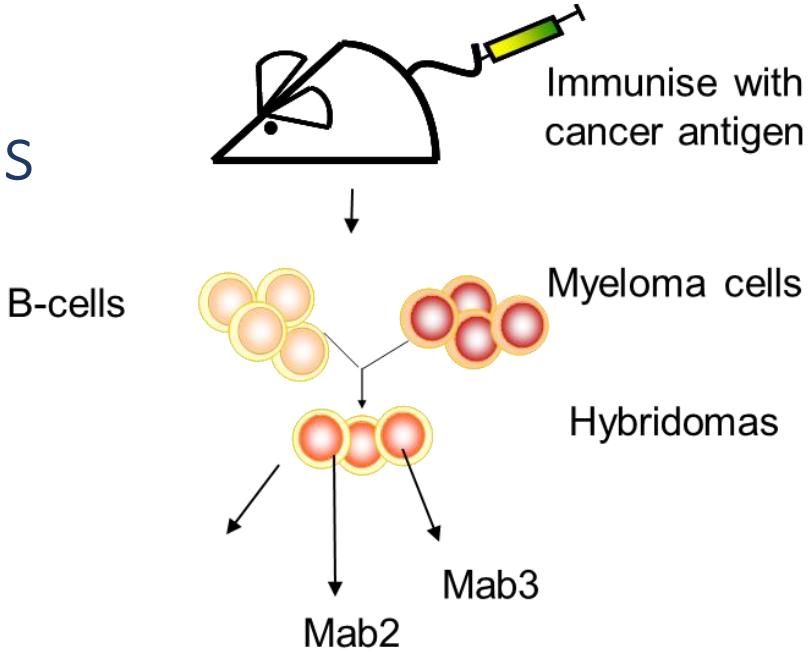


Visionary concept of creating 'magic bullets' to fight against human diseases. Paul Ehrlich proposed that cells can express a wide variety of side-chains that are shed into the blood and act as antitoxins or 'antibodies' 1908 Nobel Prize

NYS Department of Health Antitoxin Laboratory, established 1901 to standardize and manufacture antitoxin

Monoclonal antibodies

Cesar Milstein



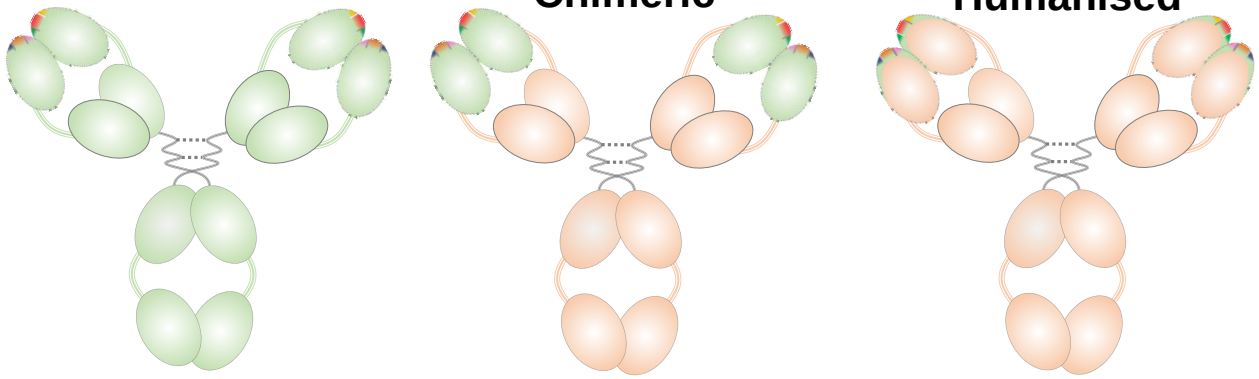
Nature 1975

By fusing B-cells with immortal (cancer) cells give monoclonal antibody-producing cell line

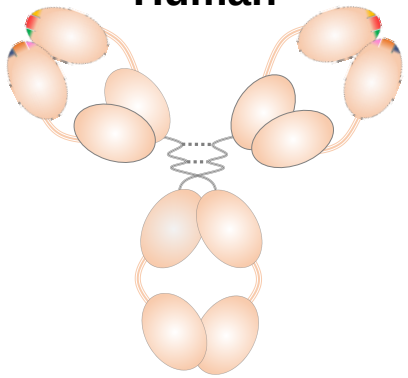
Cesar Milstein, Georges J. F. Köhler & Niels Kaj Jerne. Nobel Prize 1984

Next 30 years of Vision and Technology

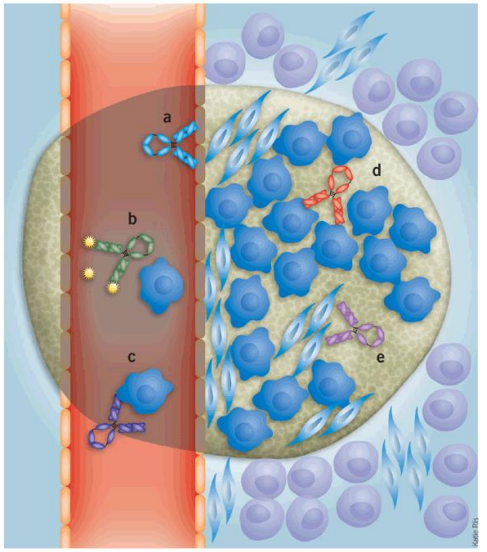
http://www.kymab.com/



By antibody engineering



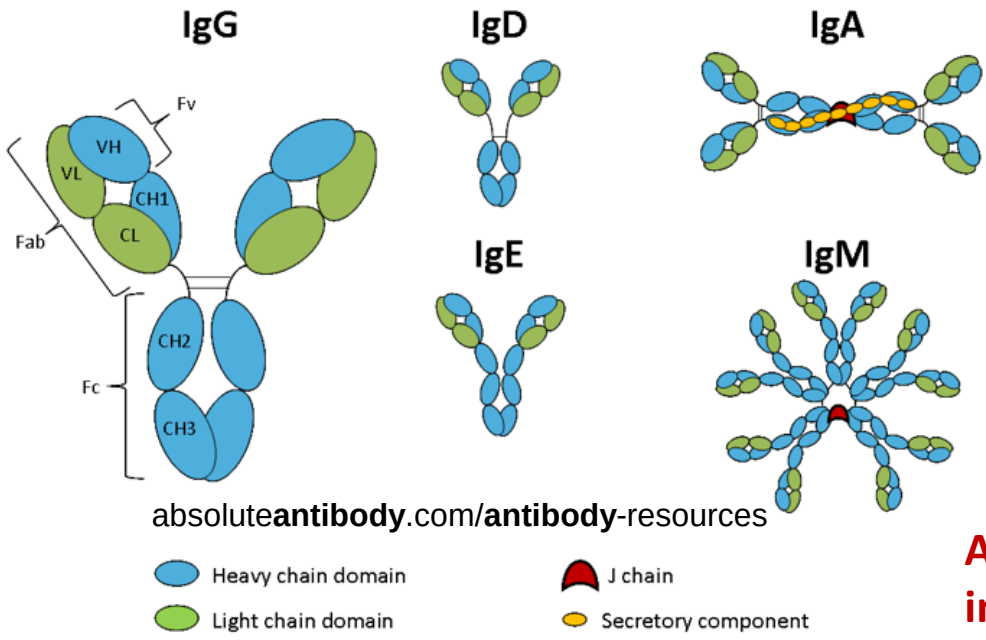
Transgenic mouse human immunoglobulin genes



Clinically applicable recombinant Mabs to many cancer targets

Antibodies – some species and unusual forms

Human/mouse

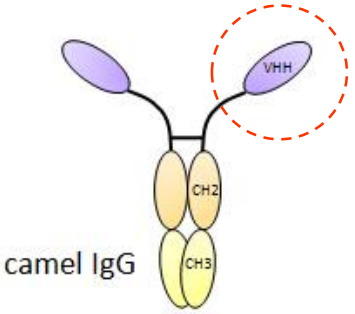


absoluteantibody.com/antibody-resources

Heavy chain domain
Light chain domain
J chain
Secretory component

Many other species

Camel/Llama



https://www.ablynx.com/

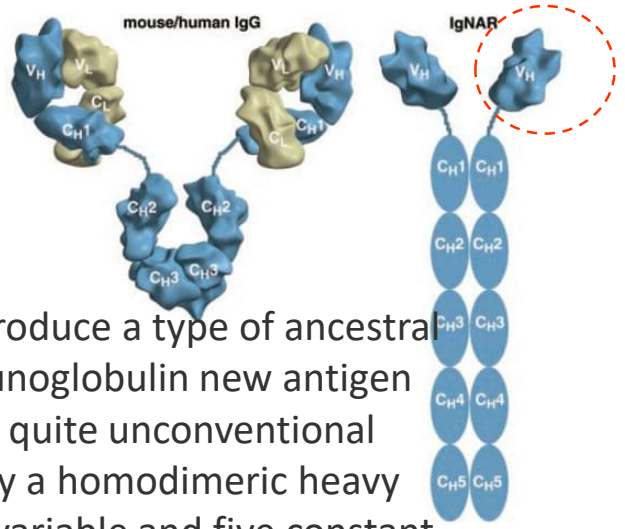
VHH 'Nanobodies'

A range of non-immunoglobulin Scaffolds

e.g. DARPins

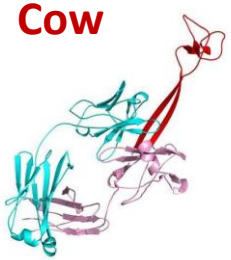
MOLECULAR partners

Shark



Cartilaginous fishes also produce a type of ancestral antibody referred to immunoglobulin new antigen receptor (IgNAR). IgNAR is quite unconventional antibody composed of only a homodimeric heavy chain which contains one variable and five constant domains for each chain https://www.creative-biolabs.com

Cow



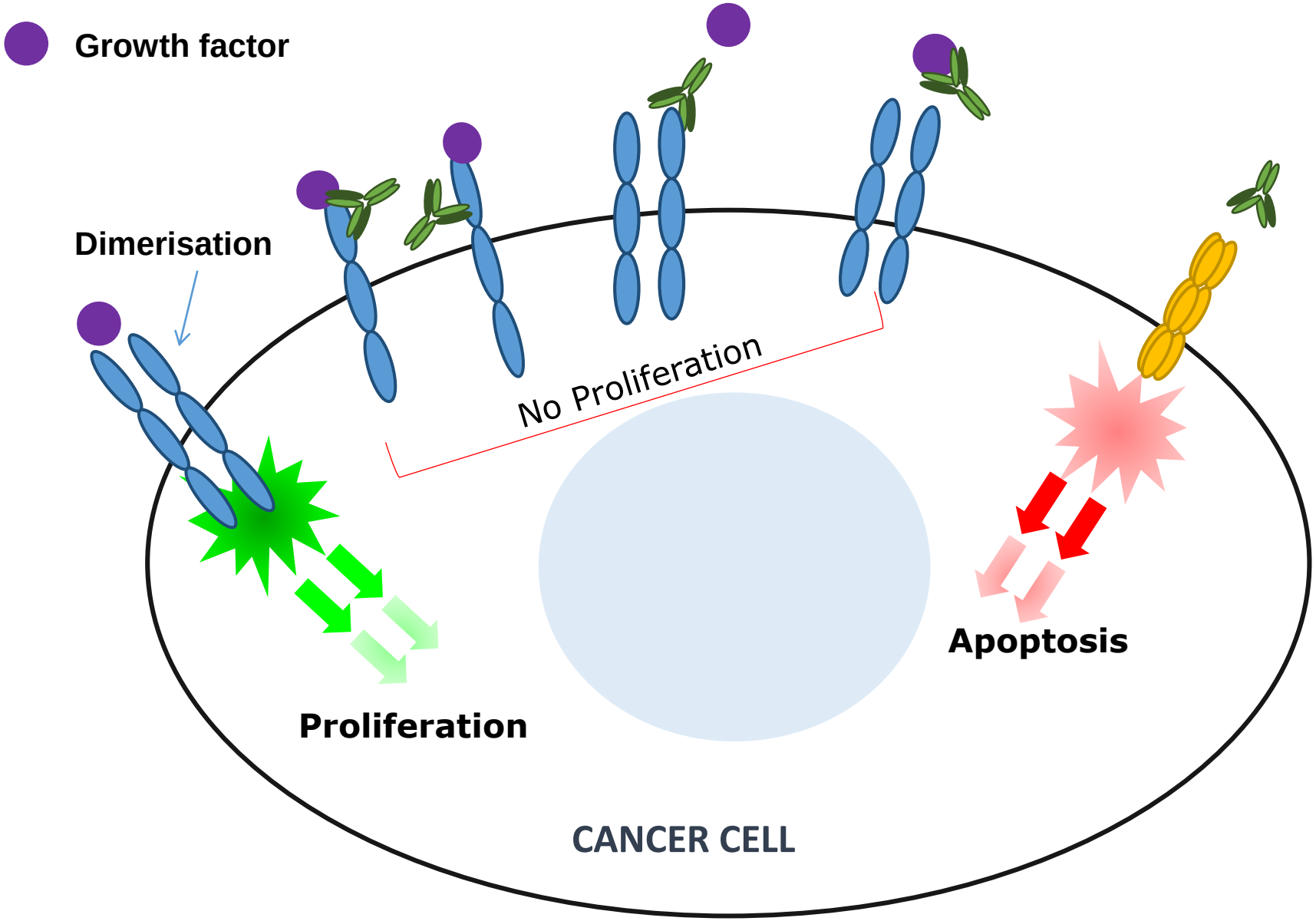
Front. Immunol., 22 March 2019 | https://doi.org/10.3389/fimmu.2019.00558
Structural Diversity of Ultralong CDRH3s in Seven Bovine Antibody Heavy Chains

How do Mabs kill cancer cells?

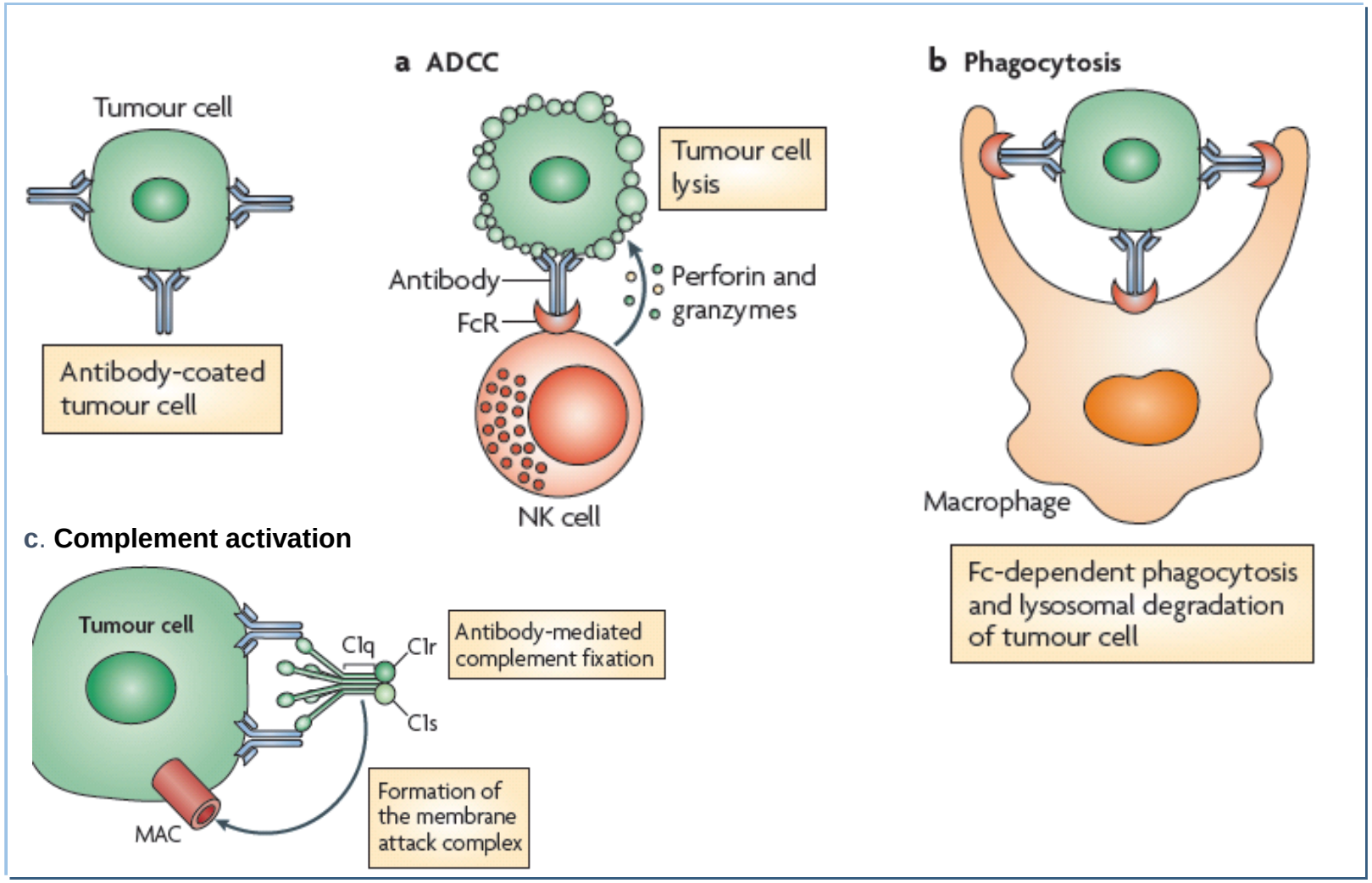
Recombinant Mabs as anti-cancer drugs

- Direct biological effects related to specificity
- Fc mediated effects
- Armed antibodies (drugs, toxins radioactivity)
- Checkpoint inhibitor antibodies (stimulate immune system)

Direct blocking and stimulating

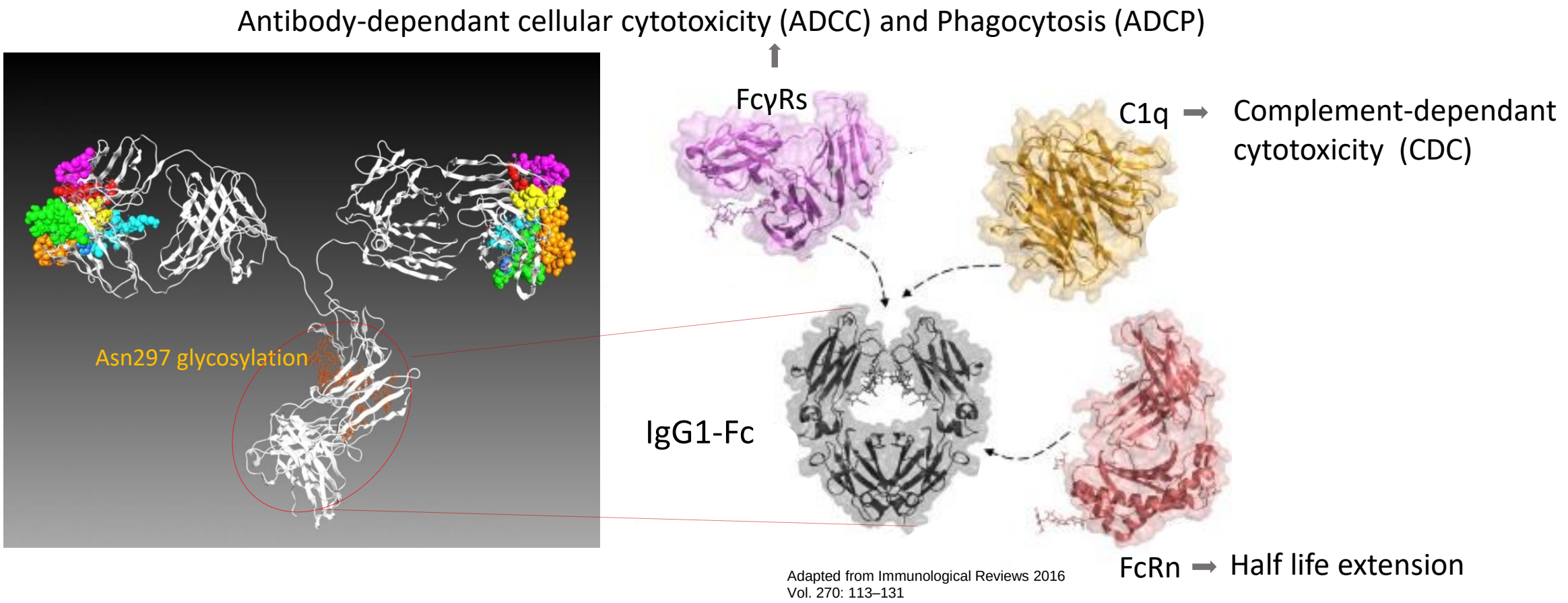


Anti-tumour mechanisms mediated by Fc



From Weiner 2010 NATURE REVIEWS | **Immunology May 2010**

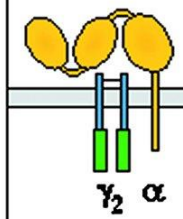
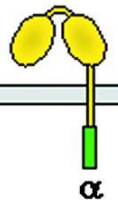
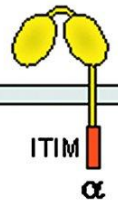
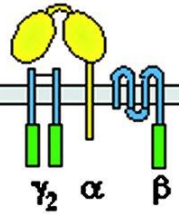
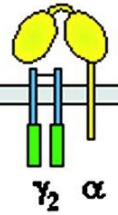
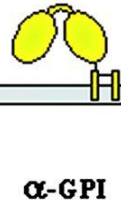
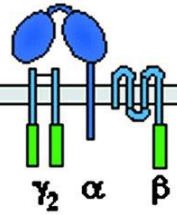
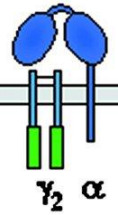
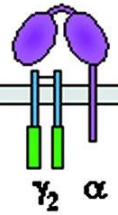
IgG and Fc binding ligands

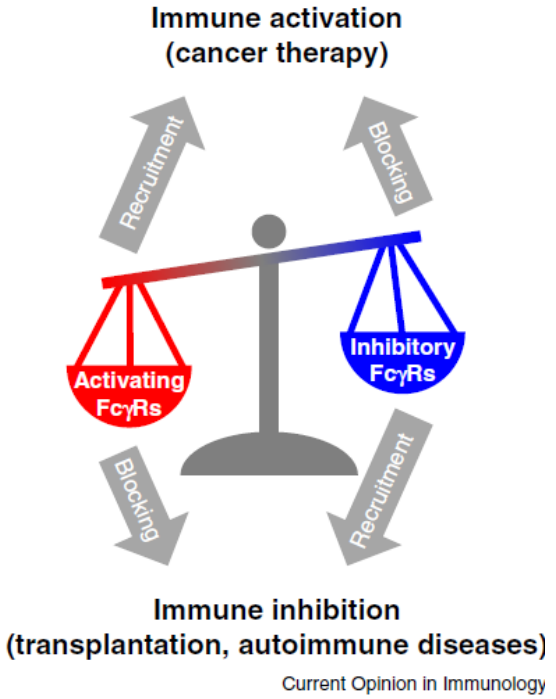


Adapted from Immunological Reviews 2016
Vol. 270: 113–131

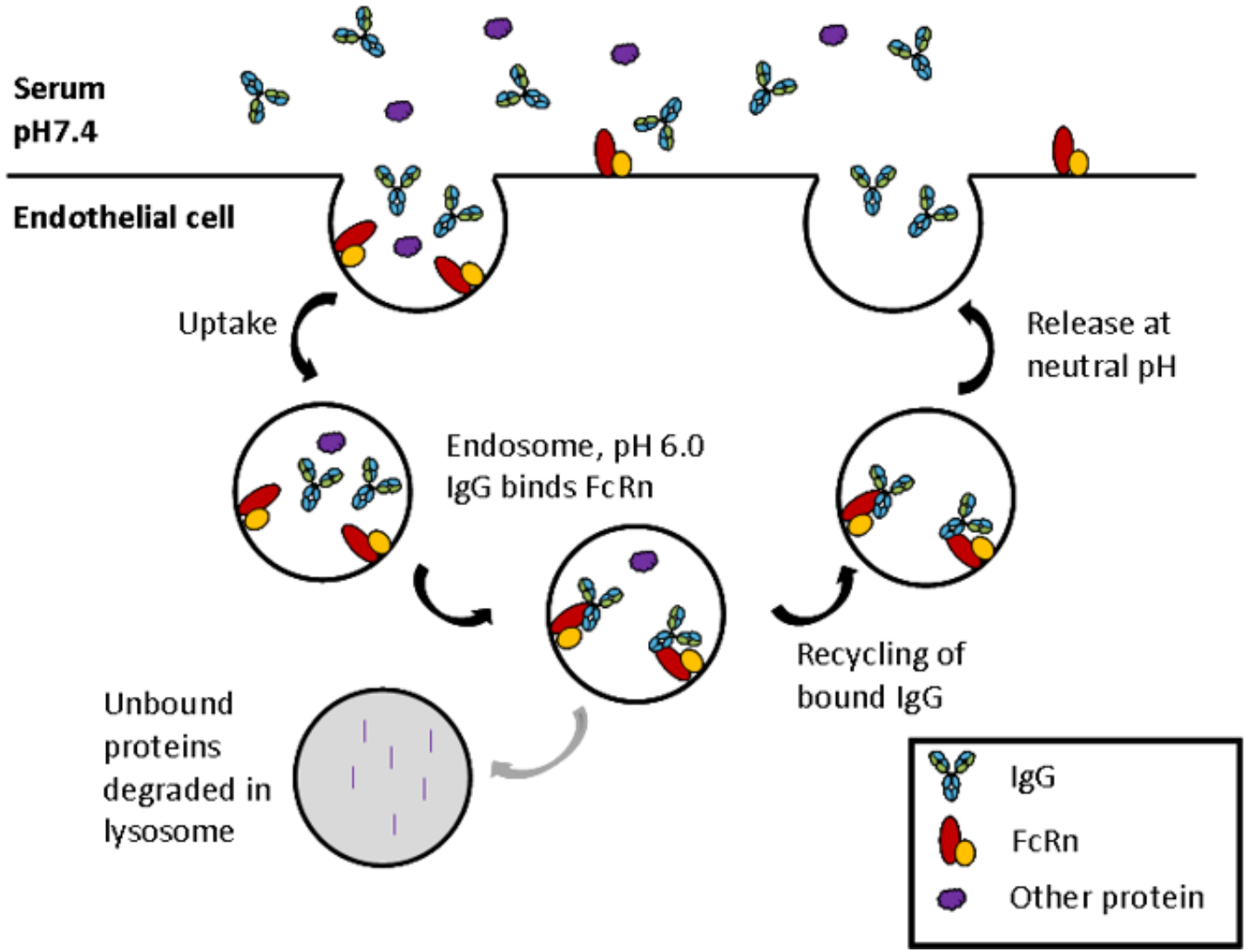
FcRn binds the Fc at a site distinct from the hinge region where FγgRs and C1q bind - so extended half-life can be coupled with other antibody enhancements.

FcR- diversity and mediated activity

	FcγRI CD64	FcγRIIA CD32	FcγRIIB CD32	FcγRIIIA CD16		FcγRIIIB CD16	FcεRI		FcαRI CD89
Structure Subunit composition									
Ka **Binding Specificity**	10^8 M^{-1} 1. IgG1=IgG3 2. IgG4 3. IgG2	$2x10^6$ M^{-1} 1. IgG1 2. IgG2=IgG3 3. IgG4	$2x10^6$ M^{-1} 1. IgG1 2. IgG2=IgG3 3. IgG4	$5x10^5$ M^{-1} 1. IgG1=IgG3	$5x10^5$ M^{-1} 1. IgG1=IgG3	$2x10^5$ M^{-1} 1. IgG1=IgG3	10^{10} M^{-1} IgE	10^{10} M^{-1} IgE	$5x10^7$ M^{-1} IgA_1=IgA_2
Expression	Macrophages Neutrophils Eosinophils Dendritic Cells	Macrophages Neutrophils Mast cells Eosinophils Platelets Dendritic Cells	Macrophages Neutrophils Mast cells Eosinophils Dendritic Cells FDC B cells	Mast cells Basophils	Macrophages Mast cells Basophils NK cells Dendritic Cells	Neutrophils	Mast cells Basophils	Mast cells Basophils Eosinophils Platelets Dendritic Cells	Macrophages Neutrophils Eosinophils
Class **Function**	Activation -Inducible by inflammatory cytokines -Enhance effector responses at inflammatory sites -IC capturing by DC	Activation -Effector cell activation by IC's, cytotoxic Ab	Inhibition -Set threshold for effector cell activation by Fc -B cell repression -Maintain tolerance	Activation -Dominant pathway for effector activation by IgG -In vivo ADCC -Arthus reaction -IC capture by DC		Decoy -Sink for IC -Focus IC to PMN -Synergize with FcγRIIA	Activation -Degranulation -Allergic reactions (Type I)	Activation -Degranulation -Allergy -Antigen caption by DC	Activation -IgA binding -IgA activation of effector cells



FcRn extends circulatory half life of antibody



absolute**antibody**.com/**antibody**-resources

Different Fc – different biological properties

Functional activity	IgM	IgD	IgG1	IgG2	IgG3	IgG4	IgA	IgE
Neutralization	+	–	++	++	++	++	++	–
Opsonization	–	–	+++	*	++	+	+	–
Sensitization for killing by NK cells	–	–	++	–	++	–	–	–
Sensitization of mast cells	–	–	+	–	+	–	–	+++
Activates complement system	+++	–	++	+	+++	–	+	–

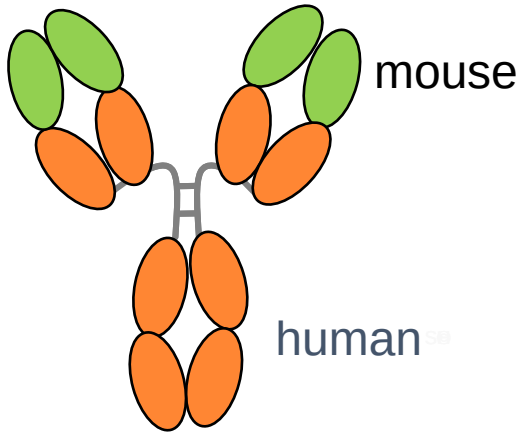
Distribution	IgM	IgD	IgG1	IgG2	IgG3	IgG4	IgA	IgE
Transport across epithelium	+	–	–	–	–	–	+++ (dimer)	–
Transport across placenta	–	–	+++	+	++	+/–	–	–
Diffusion into extravascular sites	+/–	–	+++	+++	+++	+++	++ (monomer)	+
Mean serum level (mg ml^{-1})	1.5	0.04	9	3	1	0.5	2.1	$3x10^{-5}$

Immunobiology, 5th edition Charles A Janeway,

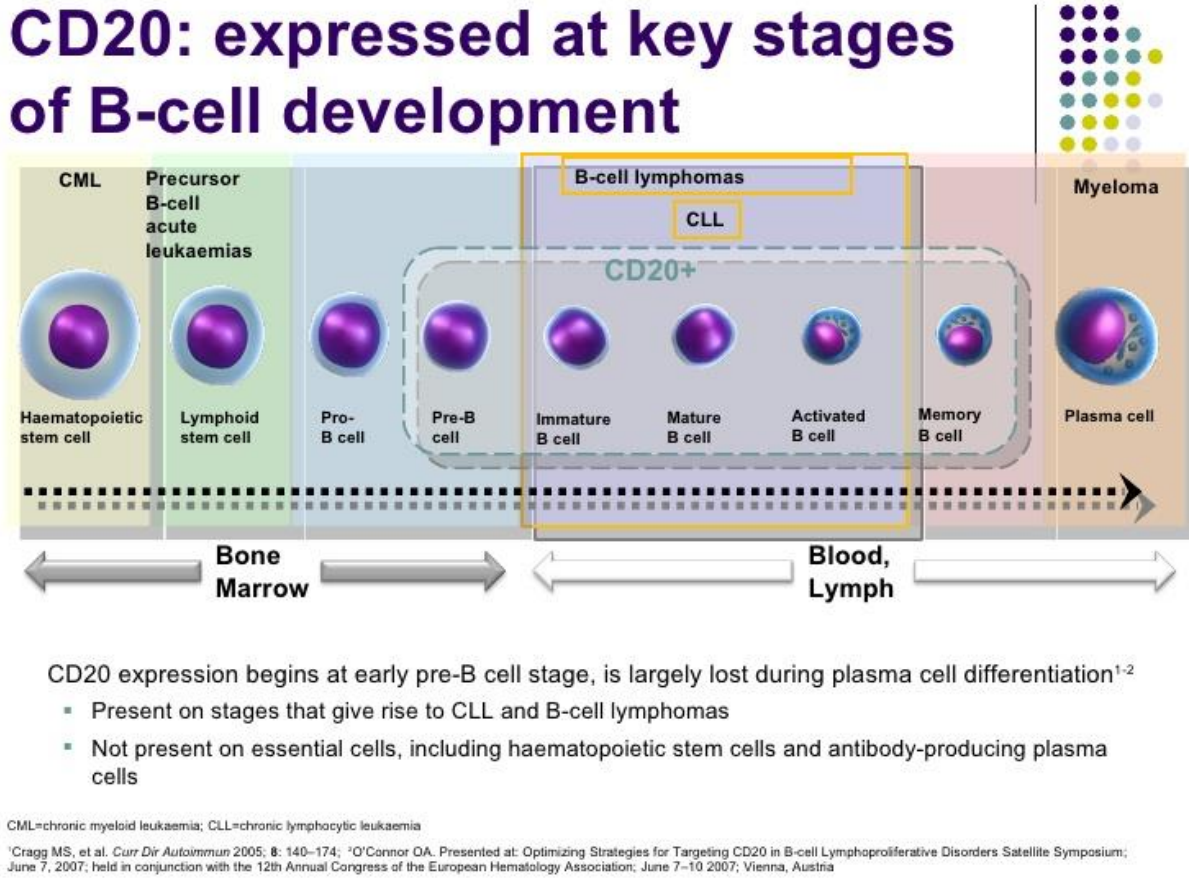
Clinical example: Rituximab

The original ®*ituximab* (Rituxan) Anti-CD 20

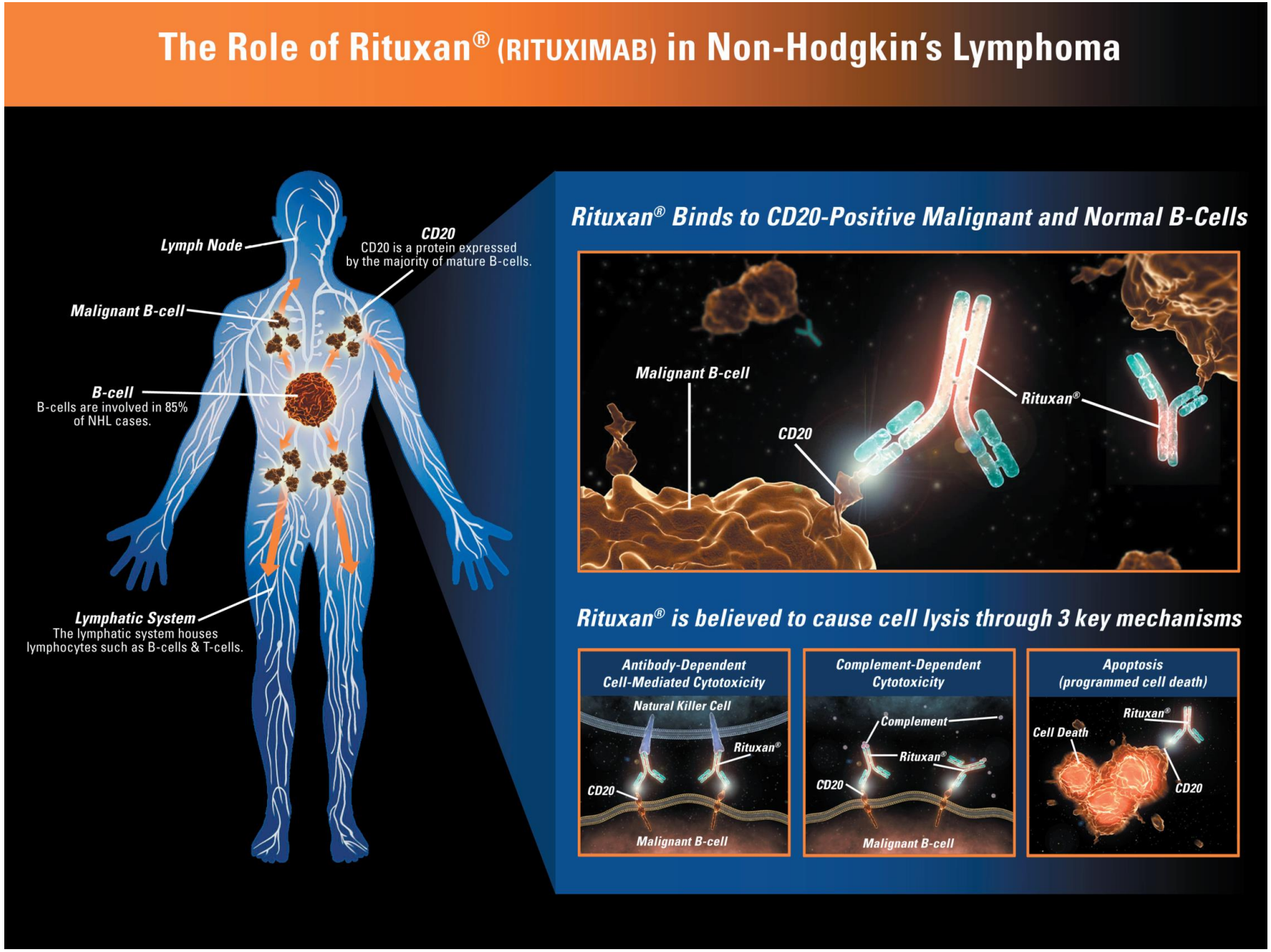
FDA approved 1997 Indication: B cell Lymphoma



Monoclonal antibody constructed from
Mouse V-regions and human C-regions



Proposed therapeutic mechanisms include: CDC, ADCC, Apoptosis



Effective therapy can target normal cells, if the effect is not harmful

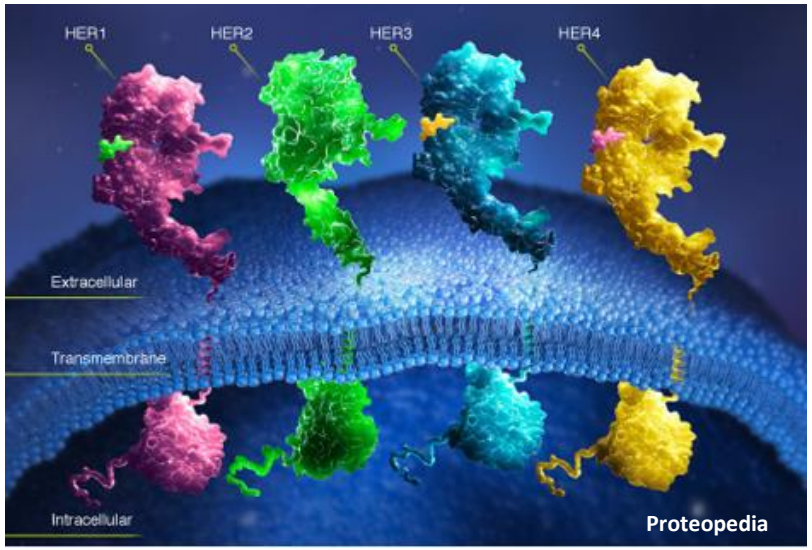
Antibody videos

Antibody action - demonstrated with rituximab

http://www.dnatube.com/video/5953/Mechanism-of-Action-Animation-for-Rituxan-a-drug-manufactur

Clinical example: Trastuzumab (Herceptin)

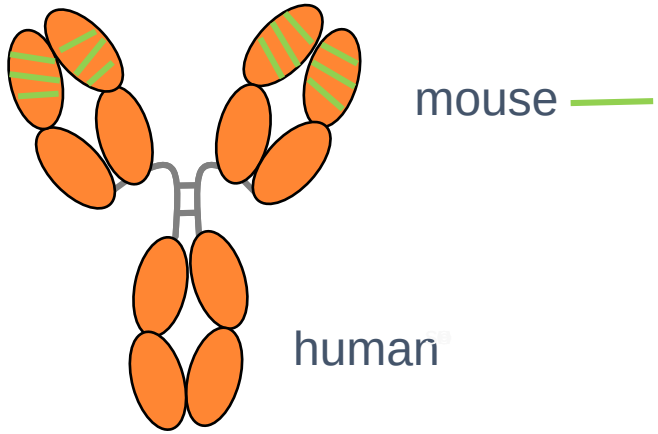
- Anti HER2 (human epidermal growth factor receptor 2)



- HER2 is a gene that helps control how cells grow, divide and repair themselves
- Patients with increased HER2 have more aggressive disease

FDA approved 1998

Indication: Breast cancer



Humanized antibody constructed with from mouse CDRs Remainder is human IgG1

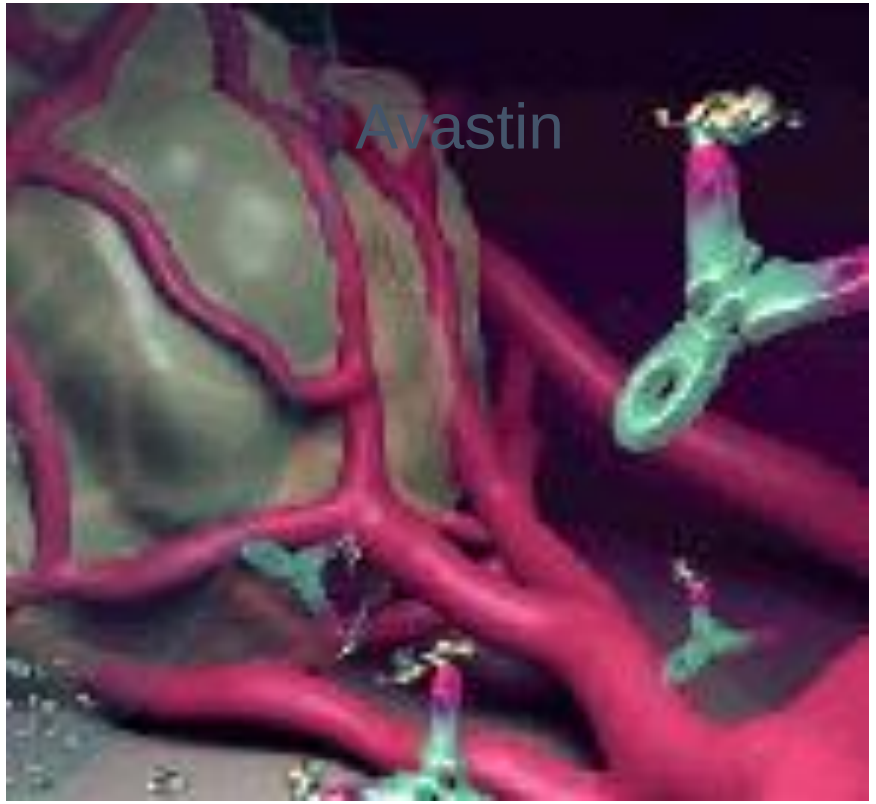
Proposed therapeutic mechanisms include

- Inhibit growth signals
- ADCC
- Enhancing chemotherapy
- Stimulating the immune system

Trastuzumab (Herceptin) was the most significant advance in clinical management of breast cancer in 4 decades

Clinical example: Bevacizumab (Avastin)

FDA approved 2004



- Anti VEGF (vascular endothelial growth factor)
- VEGF stimulates growth of tumour blood vessels
- Humanized with human IgG1 C-regions

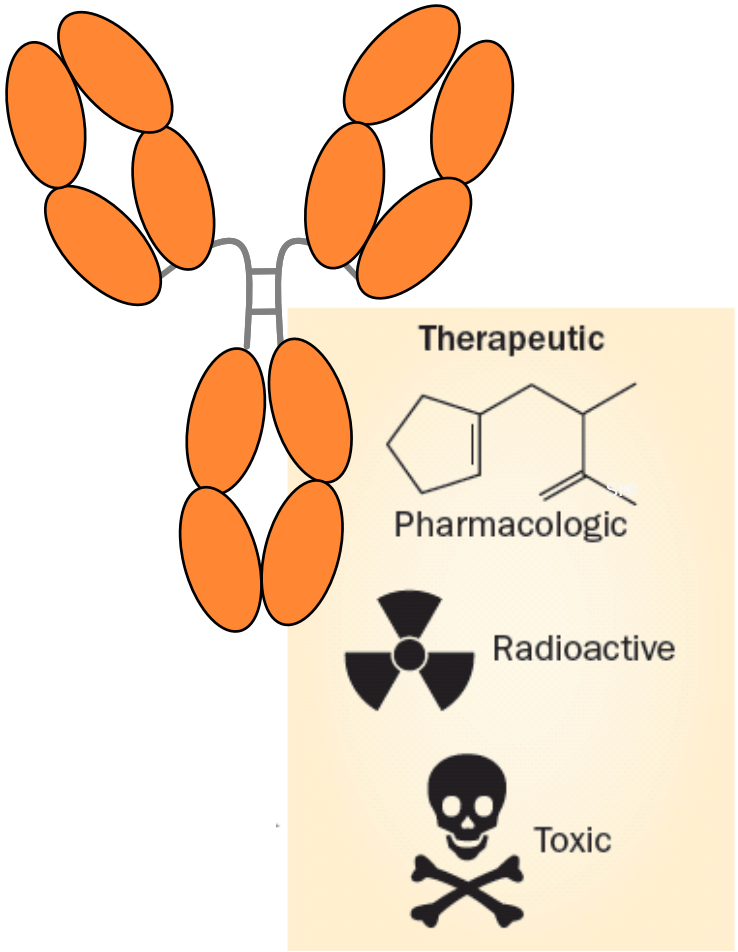
Therapeutic mechanism:

Tumor growth is dependent on angiogenesis. Angiogenesis is dependent on VEGF. Avastin directly binds to VEGF to directly inhibit angiogenesis

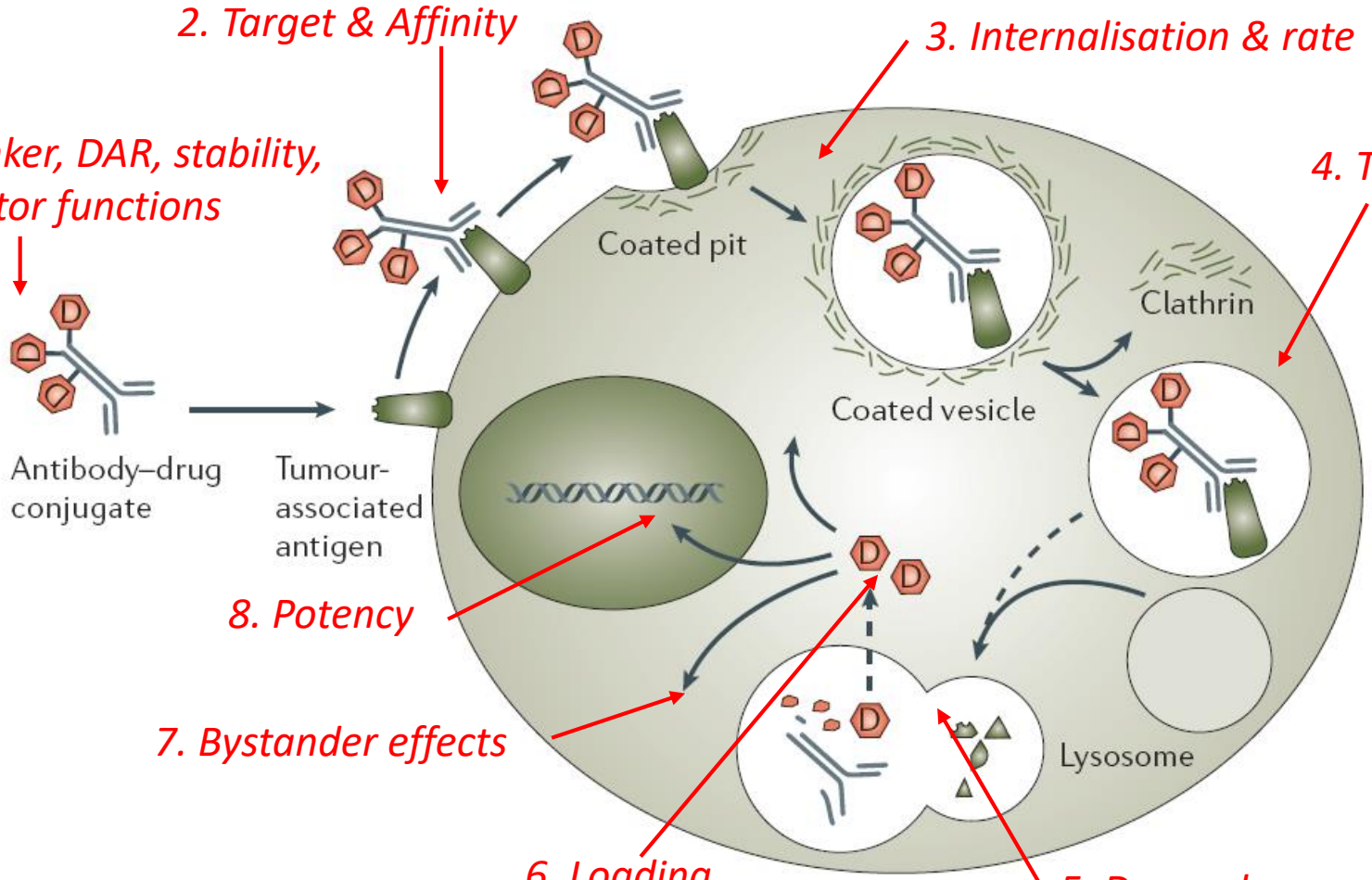
First anti-angiogenesis therapy to increase survival in cancer patients

Armed Antibodies

- Drugs
- Toxins
- Radioactivity



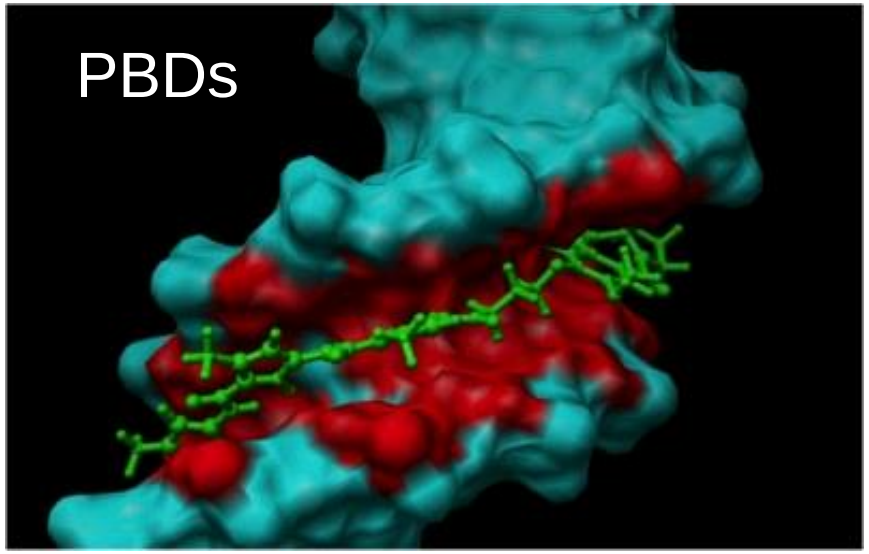
ADC Not as simple as it sounds - *many moving parts*

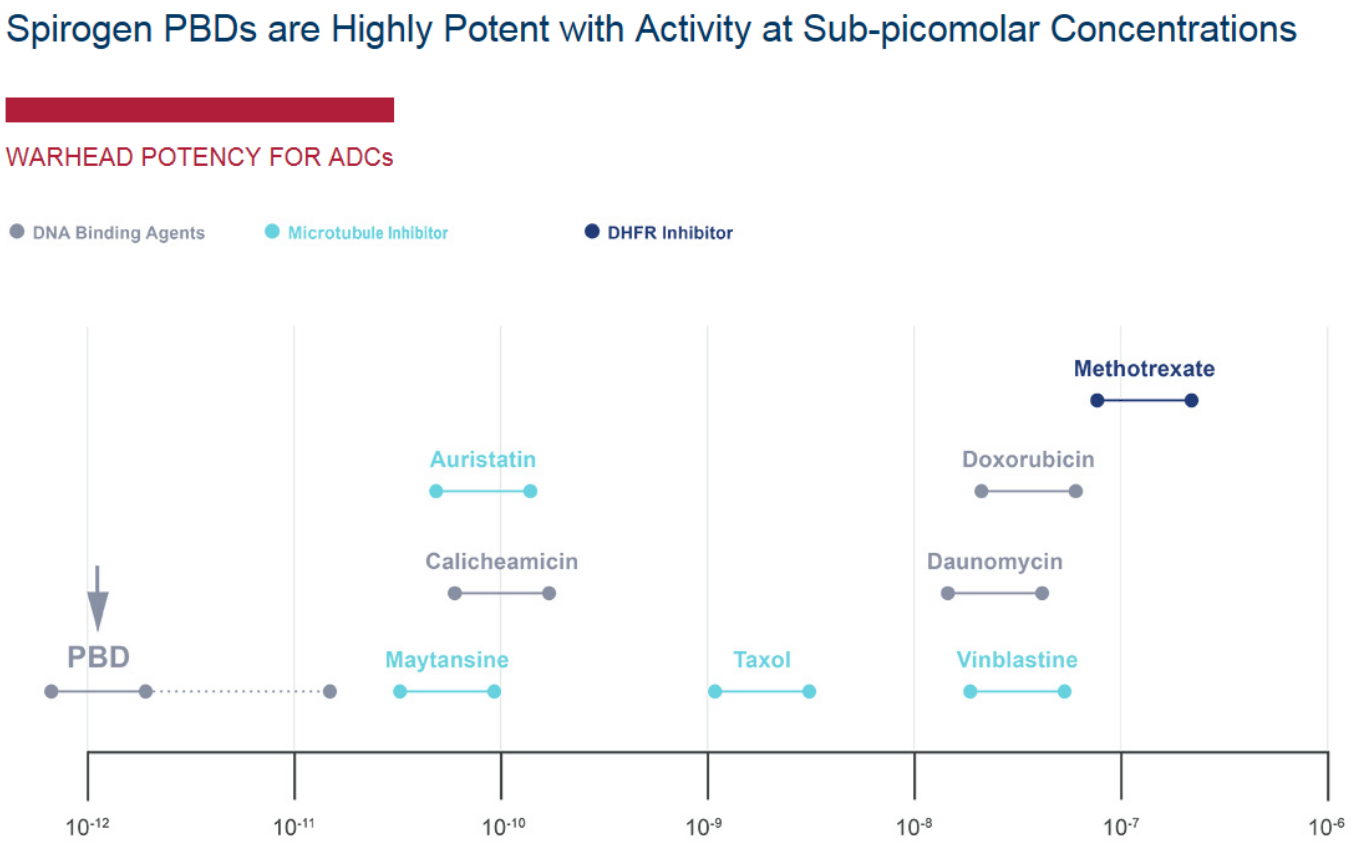


Schrama et al (2006) Nat Rev Drug Disc
Adapted by Dr Mahendra Deonarain

Choice of drug

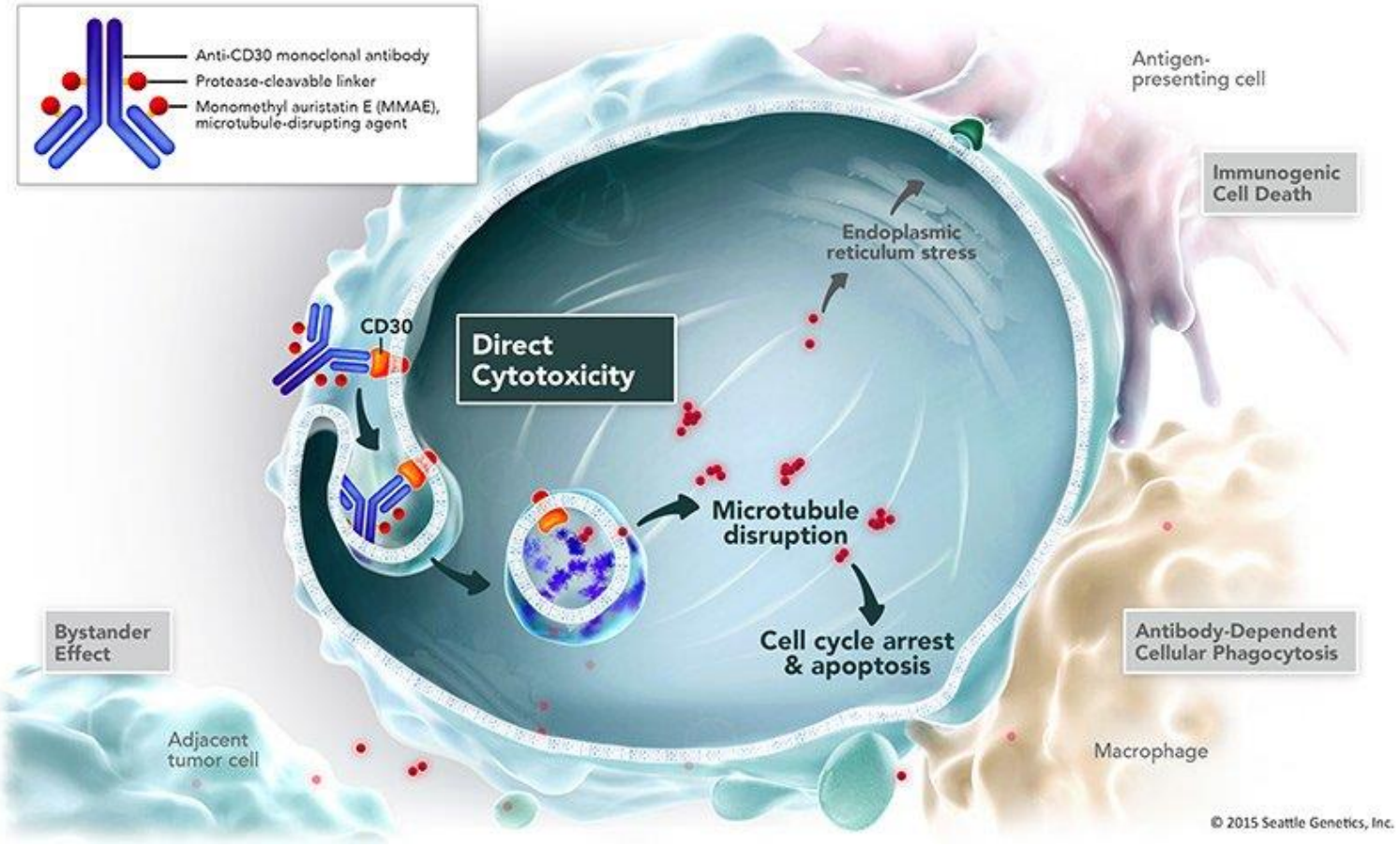
Developer	Warhead	Mode of action
Seattle Genetics	Auristatins	Tubulin polymerase inhibitor
Immunogen	Maytansines	Tubulin depolymerisation
Pfizer	Calicheamicins	DNA cleavage
Bristol-Myers Squibb	Duocarmycins	DNA minor groove alkylating agent
Syntarga (Synthon)	Duocarmycins	DNA minor groove alkylating agent
Spirogen Ltd.	PBD dimers	DNA minor groove cross-linker

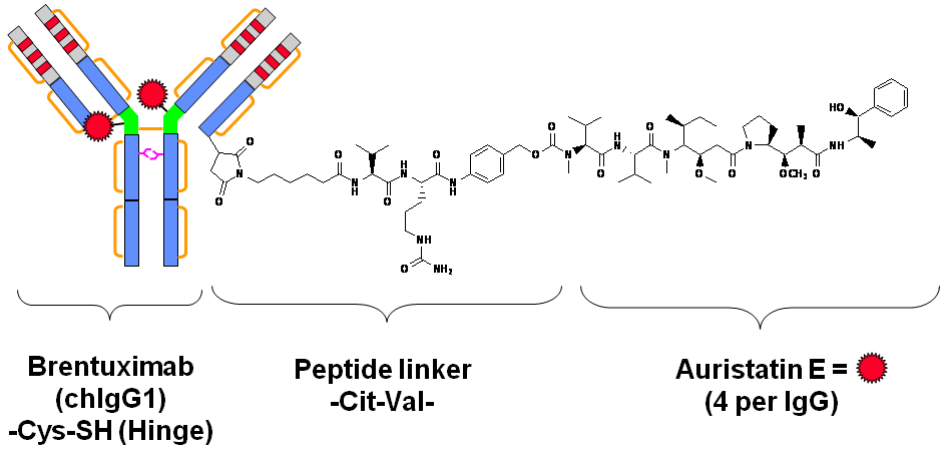


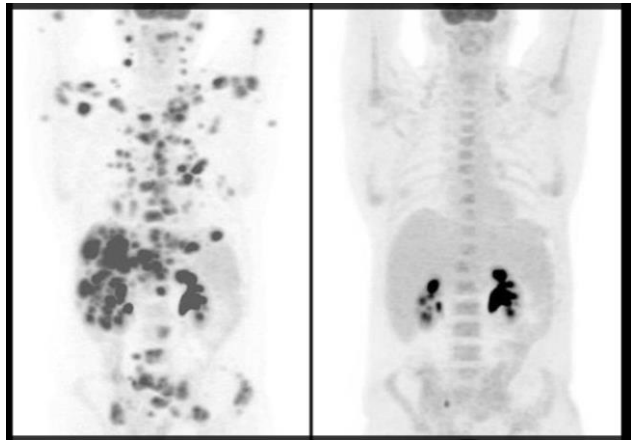


Pyrrolobenzodiazepines (PBDs), DNA minor groove binding agents, which bind and cross-link specific sites of DNA of the cancer cell. This blocks the cancer cells' division without distorting its DNA helix, thus potentially avoiding the common phenomenon of emergent drug resistance https://spirogen.com/

Clinical example: ADCETRIS® (brentuximab vedotin)

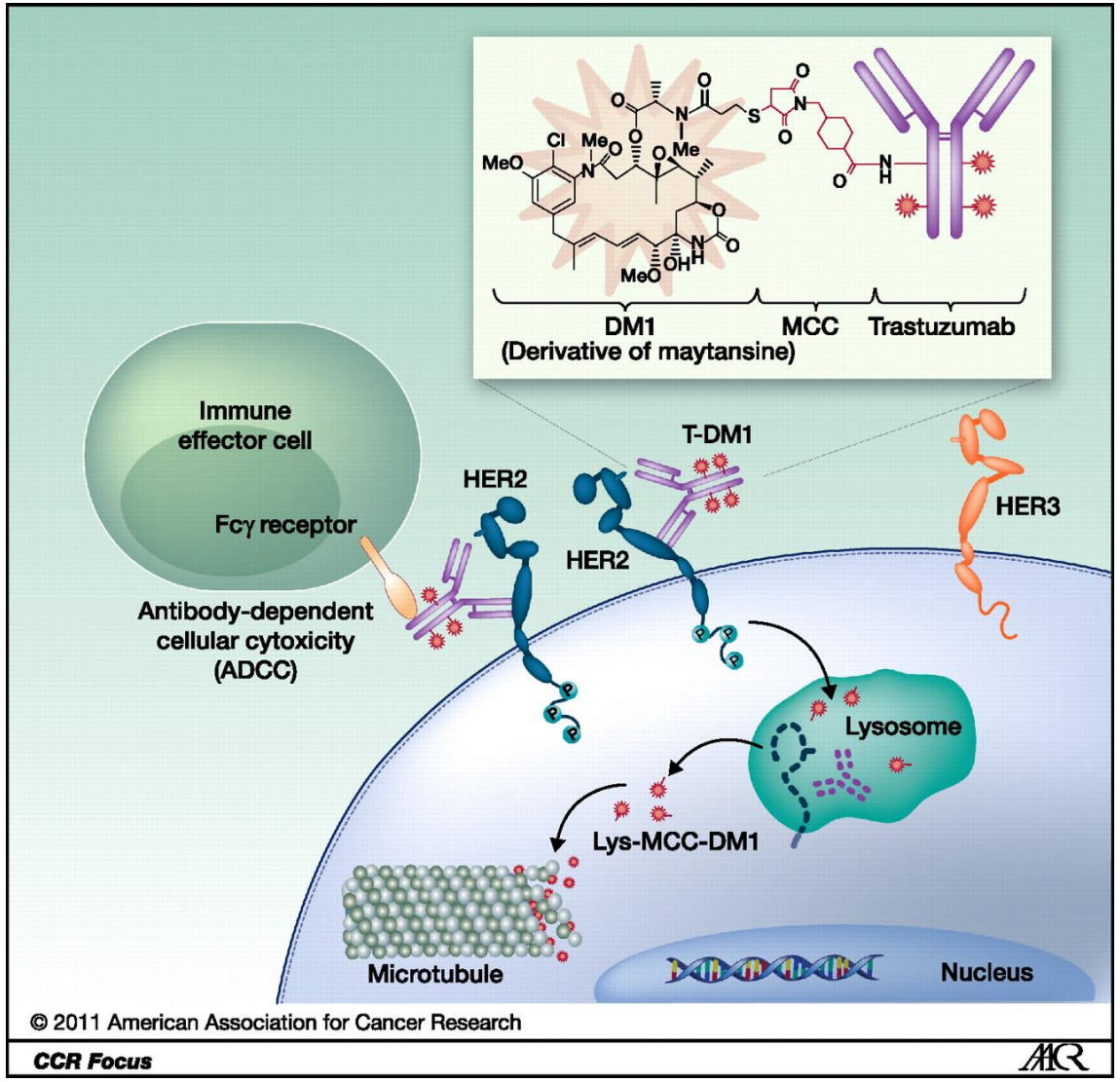






ADCETRIS is approved in more than 65 countries, including Brazil, Japan, the United States and countries in the EU.

Clinical example: Ado trastuzumab emtansine (T-DM1) Kadcyla



Patricia M. LoRusso et al. Clin Cancer Res 2011;17:6437-6447

After T-DM1 binds HER2, the HER2/T-DM1 complex internalises, followed by lysosomal degradation. This process results in the intracellular release of DM1-containing catabolites that bind to tubulin and prevent microtubule polymerization as well as suppress microtubule dynamic instability. T-DM1 has also been shown to retain mechanisms of action of trastuzumab, including disruption of the HER3/PI3K/AKT signalling pathway and Fcγ receptor–mediated engagement of immune effector cells, which leads to ADCC.

DM1 (derivative of maytansine antibiotic originally isolated from the Ethiopian shrub Maytenus serrata),

5 approved ADCs in clinic

Approved ADCs

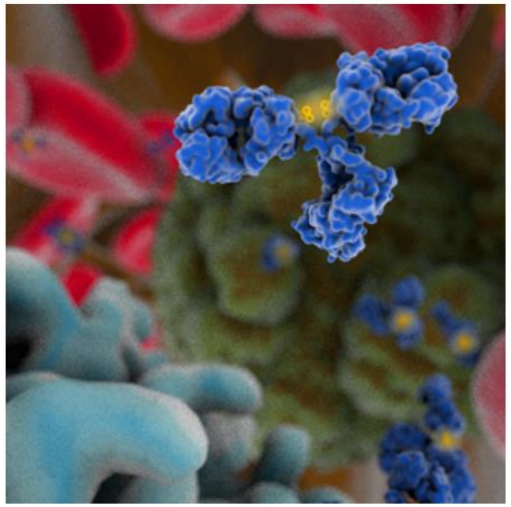
US Trade Name (generic name)	Company	Current status (US)	Target	Major Indication	Protein and conjugate
Mylotarg® (gemtuzumab ozogamicin)	Wyeth	Approved 05/17/ 2000; withdrawn 6-21-2010; re-approved 10/1/17	CD33	AML	Humanized $IgG_4κ$-calicheamycin cytotoxin conjugate
Adcetris™ (brentuximab vedotin; SGN-35)	Seattle Genetics/ Takeda	Approved 08/19/2011	CD30	Hodgkin lymphoma	Chimeric IgG-MMAE (auristatin) cytotoxin conjugate
Kadcyla® (trastuzumab-DM1)	Genentech/ Immunogen	Approved 09/04/2014	Her2	Breast cancer	Humanized IgG_1 DM1 cytotoxin conjugate
Besponza™ (Inotuzumab ozogamycin) (G5/44)	Wyeth	Approved 8/17/17	CD22	ALL	Humanized IgG_4 calicheamicin cytotoxin conjugate

Many more ADCs in pipeline

- *British Journal of Cancer (2016) 114, 362–367*
- *Alain Beck Nature reviews Drug Discovery*

New antibody-drug conjugate approved in the US

JUNE 10, 2019 BY JANICE REICHERT



On June 10, 2019, the U.S. **Food and Drug Administration (FDA) granted accelerated approval to Polivy (polatuzumab vedotin-piiq)**, in combination with the chemotherapy bendamustine and a rituximab product (BR), to treat adult patients with diffuse large B-cell lymphoma (DLBCL) that has progressed or returned after at least two prior therapies. Polivy is composed of a humanized anti-CD79b IgG1 antibody conjugated to the antimitotic agent monomethyl auristatin E (MMAE). The antibody's target is highly expressed on B cells of patients with lymphoma. The biologics license application for Polivy was granted FDA's Breakthrough Therapy and priority review designations. The drug also has European Medicines Agency (EMA)'s PRIME designation, and US and EU Orphan Drug designations for DLBCL. EMA is reviewing a marketing authorization application for Polivy.

The drug's efficacy was evaluated in a study of 80 patients with relapsed or refractory DLBCL who were randomized to receive Polivy with BR or BR alone. Efficacy was based on complete response rate and duration of response (DOR), defined as the time the disease stays in remission. At the end of treatment, the complete response rate was 40% with Polivy plus BR compared to 18% with BR alone. Of the 25 patients who achieved a partial or complete response to Polivy plus BR, 16 (64%) had a DOR of at least six months and 12 (48%) had a DOR of at least 12 months.

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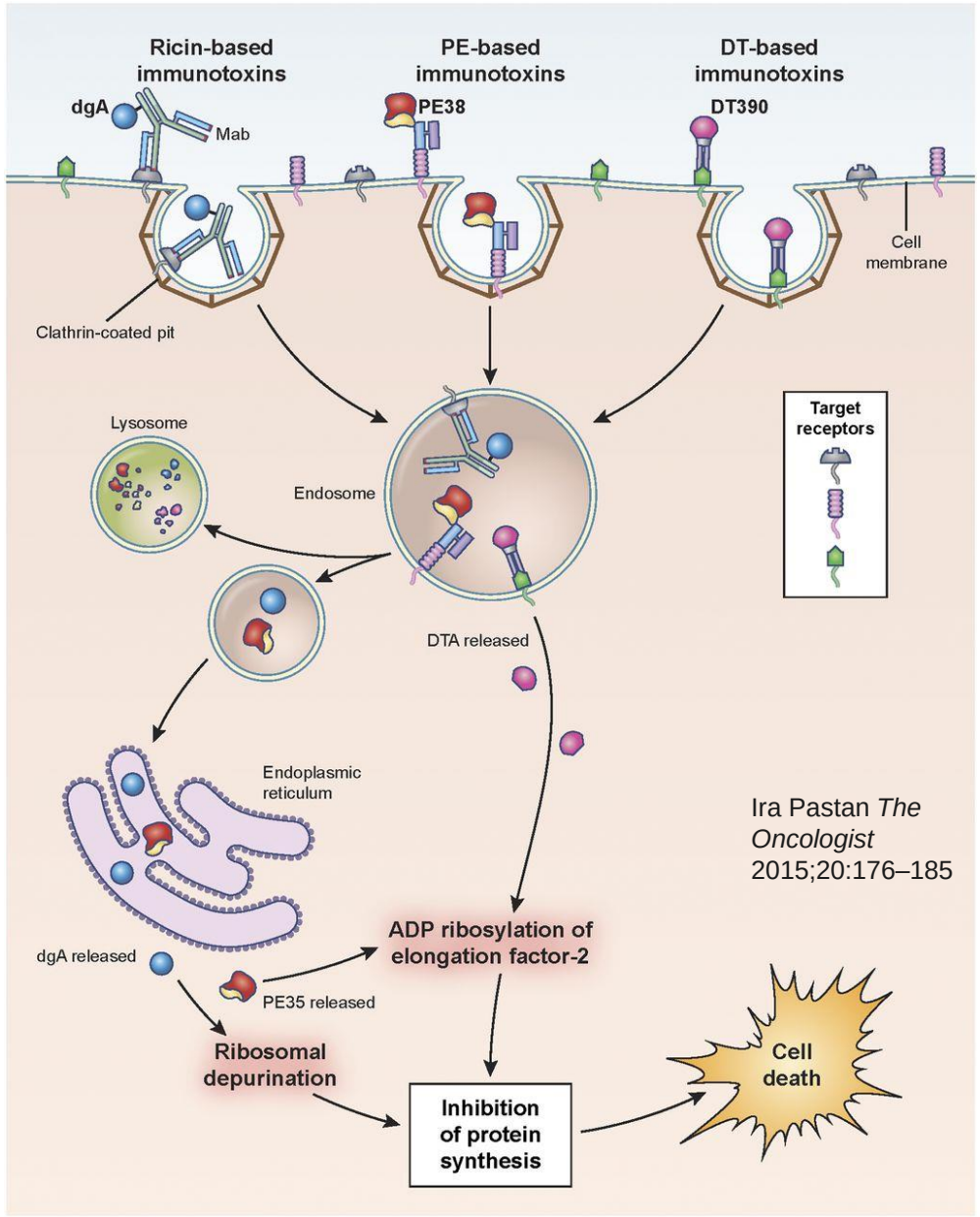
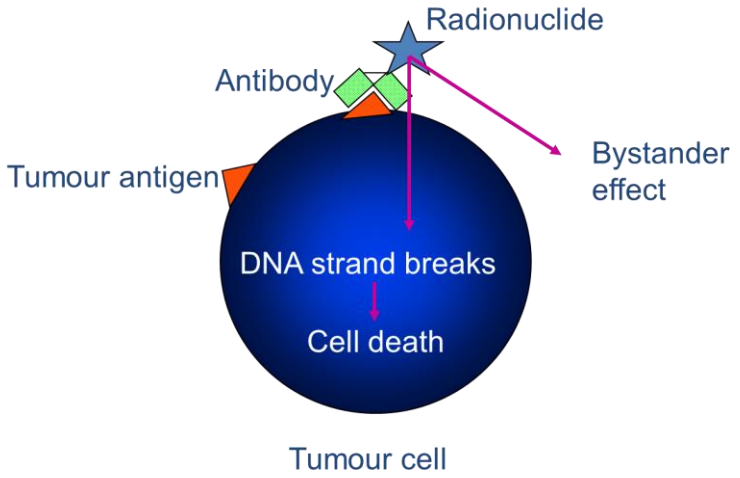
ANTIBODY SOCIETY

Immunotoxins (IT)



Radioimmunotherapy (RIT)

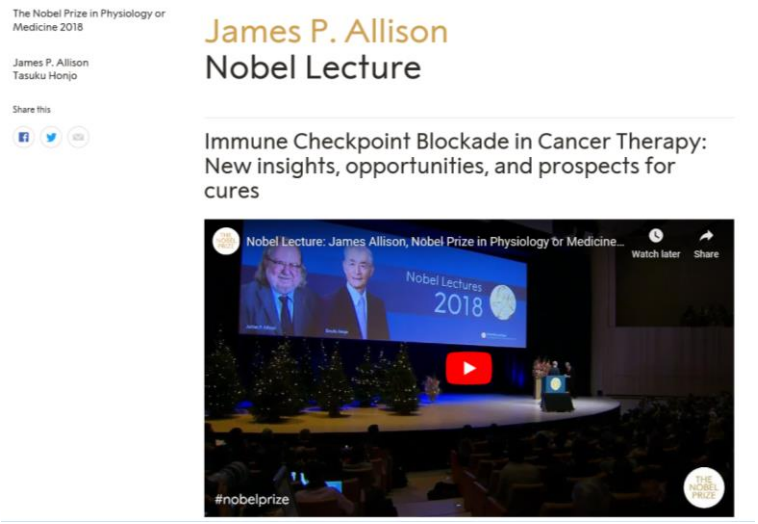


RIT and IT not been developed as much as ADCs

Recent success

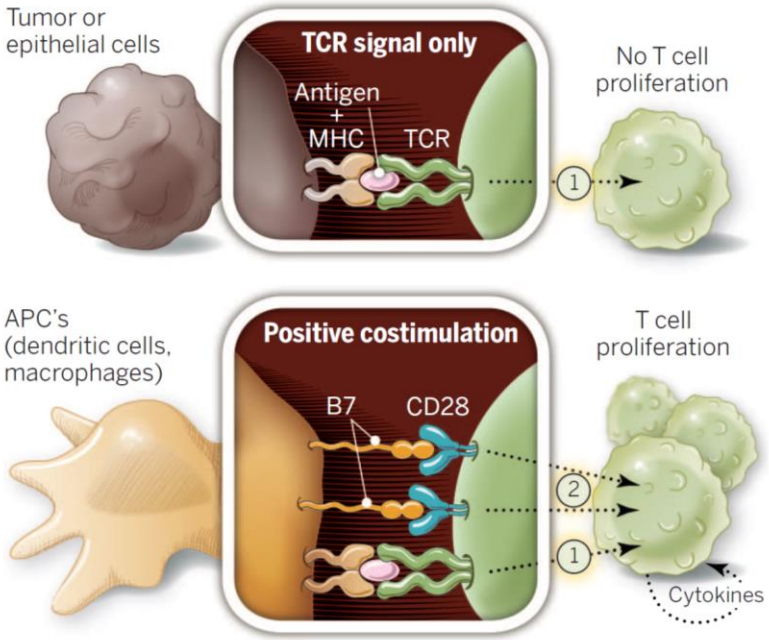
in 14 September 2018 FDA approved Lumoxiti (moxetumomab pasudotox-tdfk) as first-in-class treatment for certain patients with relapsed or refractory hairy cell leukaemia. Moxetumomab pasudotox is a fusion protein of a CD22-targeting antibody and *Pseudomonas* exotoxin

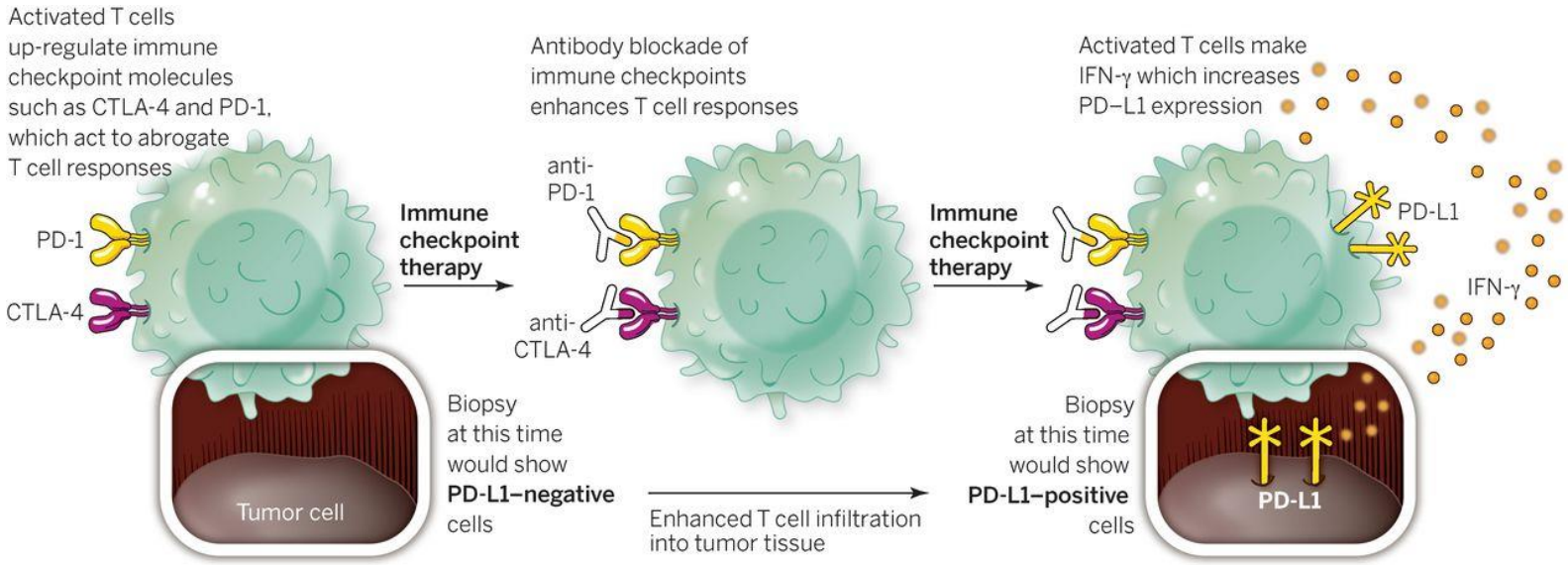
Immune-checkpoint blockade



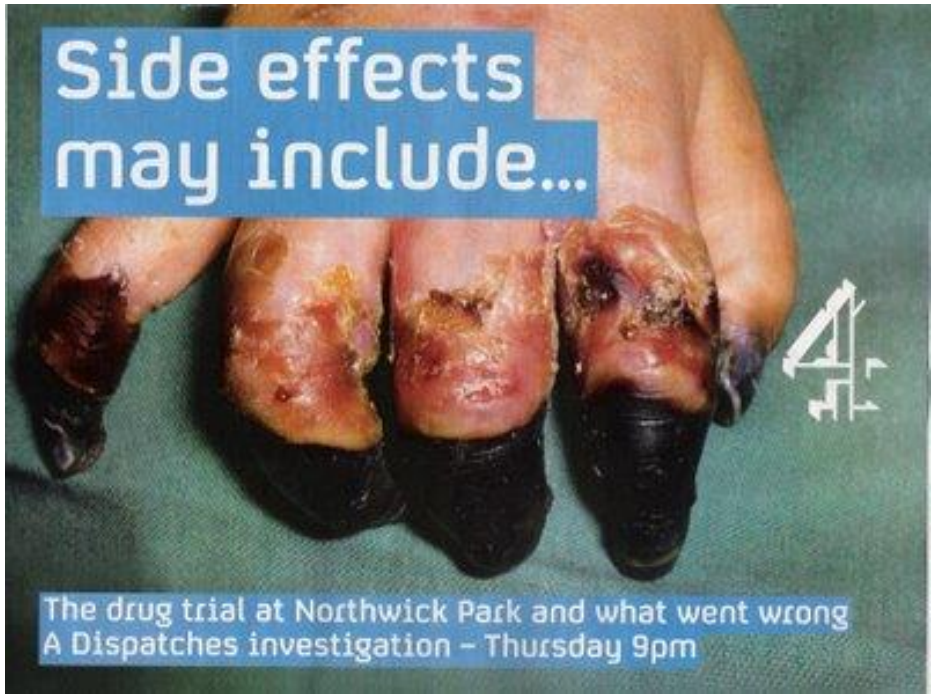
- T cell activation occurs only after interaction between T- cell receptor (TCR) and antigen in the context of MHC (signal 1) plus CD28 co-stimulation (signal 2). **Jim Allison – Nobel prize**
- After T-cell activation, T- cells express immune checkpoints such as CTLA-4 and PD-1 that act as brakes to supress activity
- T-cell activation can be suppressed in the tumour microenvironment
- Antibodies can interfere to take off the “brakes” and re-energise T cells these antibodies are called “checkpoint inhibitors”

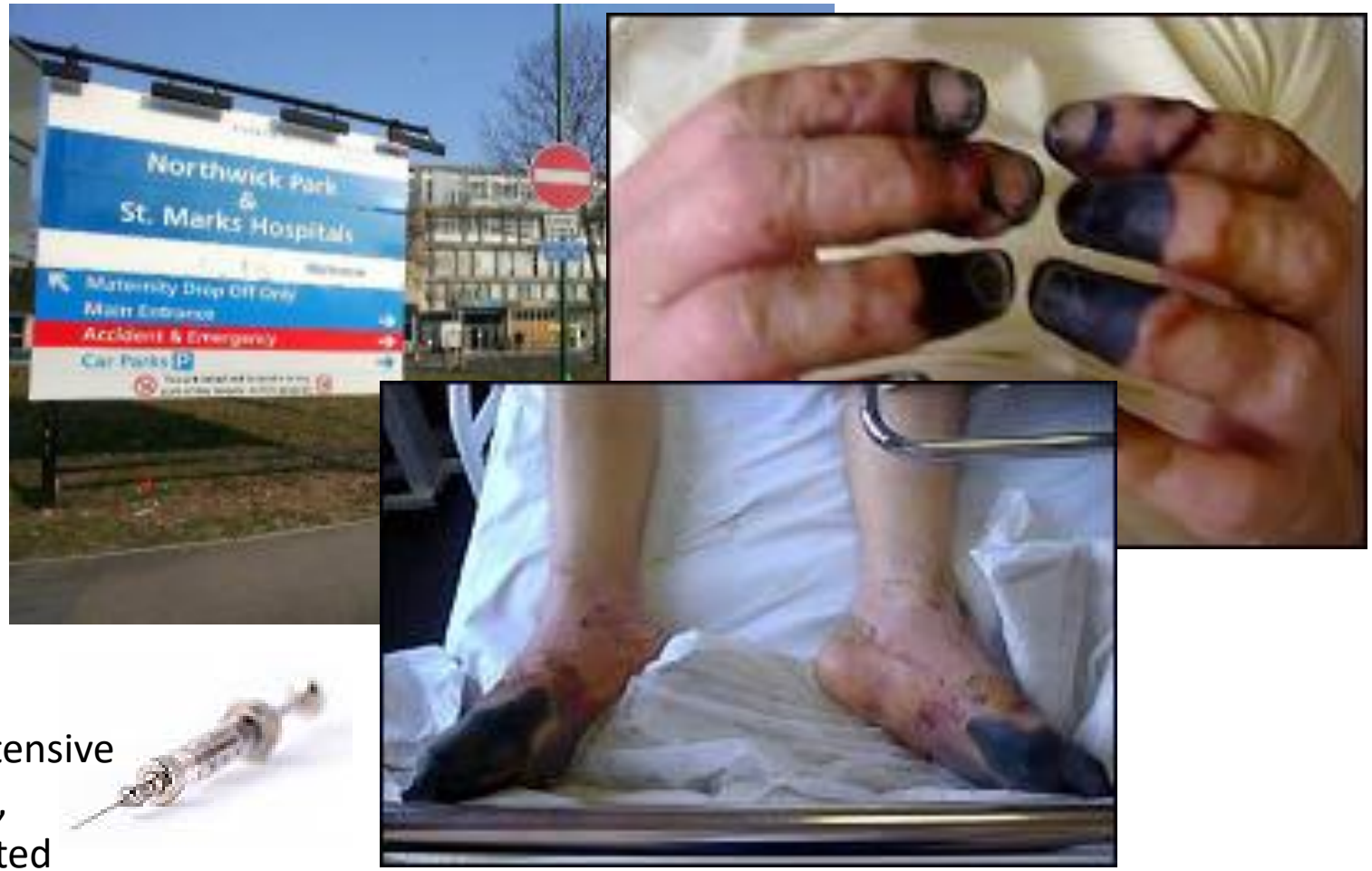
SCIENCE sciencemag.org 3 APRIL 2015 • VOL 348 ISSUE 6230





Stimulating the immune system can be dangerous





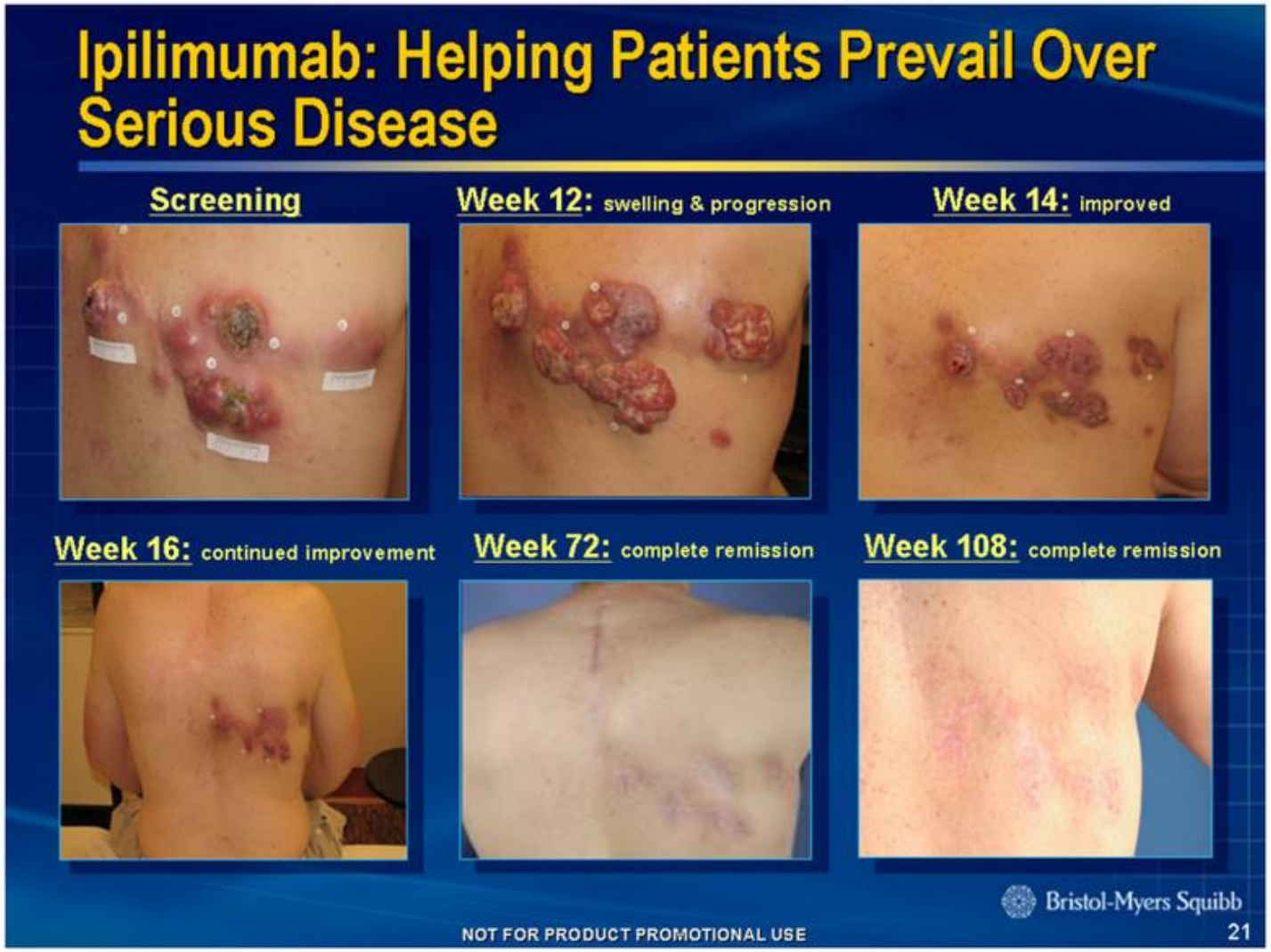
13 March 2006: New drug trial puts six men in intensive care The novel drug, TGN1412 (CD28-SuperMAB), caused multiple organ failure in the six men injected with it at a Northwick Park Hospital, London, UK,

TGN1412 is a Humanised IgG4 Monoclonal Antibody

TGN1412 is a T cell 'super agonist'. It stimulated the immune system and caused cytokine release

http://www.bbc.co.uk/iplayer/episode/b08g8np3/the-drug-trial-emergency-at-the-hospital

First immune checkpoint Mab in the clinic: anti-CTLA4



In 2001, Ipilimumab (Yervoy) A “human” antibody from TG mice set a new standard of care for patients with Melanoma
Nils Lonberg!

Many more checkpoint inhibitor antibodies particularly to PD1/PDL1 are now licenced or in clinical studies

http://www.antibodysociety.org/?s=checkpoint

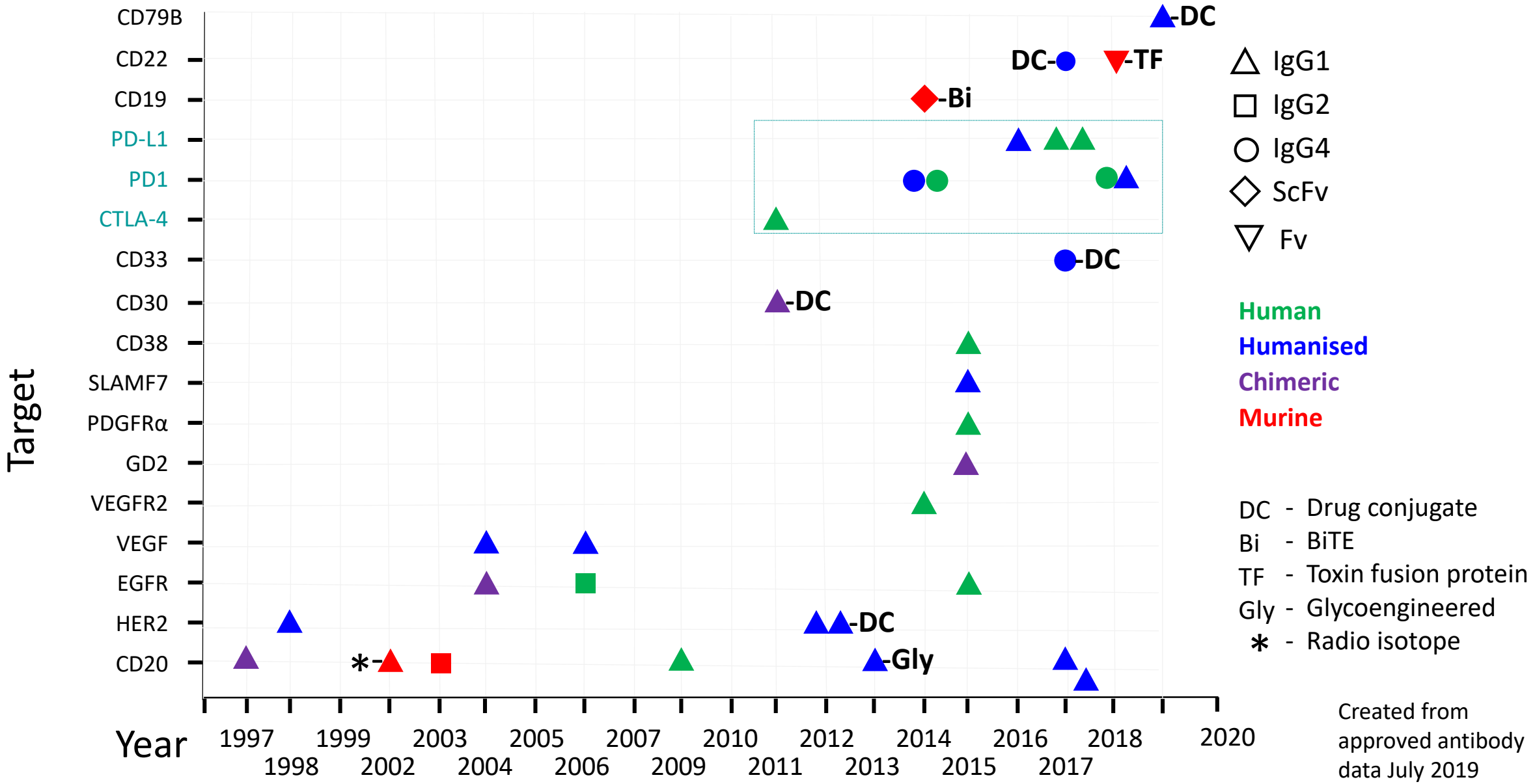
July 2019

Antibodies approved for cancer treatment

http://www.antibodysociety.org

International non-proprietary name	Brand name	Target; Format	Indication first approved or reviewed	First EU approval	First US approval
Polatuzumab vedotin	Polivy	CD79B; humanized IgG1; ADC	Diffuse large B-cell lymphoma	In review	2019
Moxetumomab pasudotox	Lumoxiti	CD22; Murine IgG1 dsFv immunotoxin	Hairy cell leukemia	N/A	2018
Cemiplimab	Libtayo	PD-1; Human mAb	Cutaneous squamous cell carcinoma	Decision pending	2018
Durvalumab	IMFINZI	PD-L1; Human IgG1	Bladder cancer	Decision pending	2017
Mogamulizumab	Poteligeo	CCR4; Humanized IgG1	Mycosis fungoides or Sézary syndrome	2018	2018
Gemtuzumab ozogamicin	Mylotarg	CD33; Humanized IgG4; ADC	Acute myeloid leukemia	2018	2017
Atezolizumab	Tecentriq	PD-L1; Humanized IgG1	Bladder cancer	2017	2016
Avelumab	Bavencio	PD-L1; Human IgG1	Merkel cell carcinoma	2017	2017
Inotuzumab ozogamicin	BESPONSA	CD22; Humanized IgG4; ADC	Acute lymphoblastic leukaemia	2017	2017
Daratumumab	Darzalex	CD38; Human IgG1	Multiple myeloma	2016	2015
Elotuzumab	Empliciti	SLAMF7; Humanized IgG1	Multiple myeloma	2016	2015
Olaratumab	Lartruvo	PDGFRα; Human IgG1	Soft tissue sarcoma	2016	2016
Blinatumomab	Blincyto	CD19, CD3; Murine bispecific tandem scFv	Acute lymphoblastic leukaemia	2015	2014
Nivolumab	Opdivo	PD1; Human IgG4	Melanoma, non-small cell lung cancer	2015	2014
Pembrolizumab	Keytruda	PD1; Humanized IgG4	Melanoma	2015	2014
Dinutuximab	Unituxin	GD2; Chimeric IgG1	Neuroblastoma	2015	2015
Necitumumab	Portrazza	EGFR; Human IgG1	Non-small cell lung cancer	2015	2015
Obinutuzumab	Gazyva, Gazyvaro	CD20; Humanized IgG1 Glycoengineered	Chronic lymphocytic leukaemia	2014	2013
Ramucirumab	Cyramza	VEGFR2; Human IgG1	Gastric cancer	2014	2014
Ado-trastuzumab emtansine	Kadcyla	HER2; humanized IgG1; ADC	Breast cancer	2013	2012
Pertuzumab	Perjeta	HER2; humanized IgG1	Breast Cancer	2013	2012
Brentuximab vedotin	Adcetris	CD30; Chimeric IgG1; ADC	Hodgkin lymphoma, T-cell lymphoma	2012	2011
Ipilimumab	Yervoy	CTLA-4; Human IgG1	Metastatic melanoma	2011	2011
Ofatumumab	Arzerra	CD20; Human IgG1	Chronic lymphocytic leukaemia	2010	2009
Panitumumab	Vectibix	EGFR; Human IgG2	Colorectal cancer	2007	2006
Bevacizumab	Avastin	VEGF; Humanized IgG1	Colorectal cancer	2005	2004
Ibritumomab tiuxetan	Zevalin	CD20; Murine IgG1	Non-Hodgkin lymphoma	2004	2002
Cetuximab	Erbitux	EGFR; Chimeric IgG1	Colorectal cancer	2004	2004
Trastuzumab	Herceptin	HER2; Humanized IgG1	Breast cancer	2000	1998
Rituximab	MabThera, Rituxan	CD20; Chimeric IgG1	Non-Hodgkin lymphoma	1998	1997

Antibodies approved for cancer treatment



Created from approved antibody data July 2019

http://www.antibodysociety.org

The Antibody Society

Founded in 2007, The Antibody Society (TAbS) is an international, non-profit association representing individuals and organizations involved in antibody-related research and development. ~ 1,700 members and fantastic sponsors. Antibody news, latest information, education, travel awards for junior scientists, reduced conference fees to major meetings and much more….

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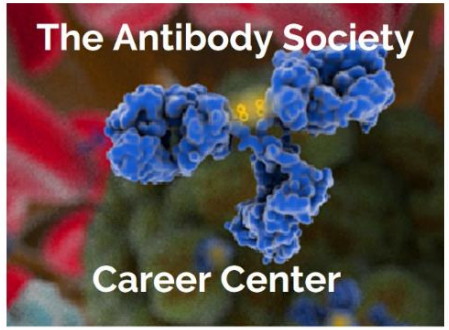


The Antibody Society's new Career Center is a premier resource to connect highly qualified talent with matching career opportunities. Visit our Career Center to find details on over 800 jobs!

Jobs

Membership is free for students, post-docs and employees of corporate sponsors

Why not check it out?

www.antibodysociety.org

Fashion

Summary – Part 1

Recombinant monoclonal antibodies are:

- Powerful biological tools for use as anti-cancer drugs
- High value commercial products

Biological effects of antibody related to specificity include:

- Neutralizing growth factors (eg VEGF)
- Blocking receptors
- Stimulating apoptotic signaling

Fc Mediated effects include:

- Recruitment of natural effectors (ADCC, CDC, phagocytes)
- Prolonging circulation

Armed antibodies deliver war heads:

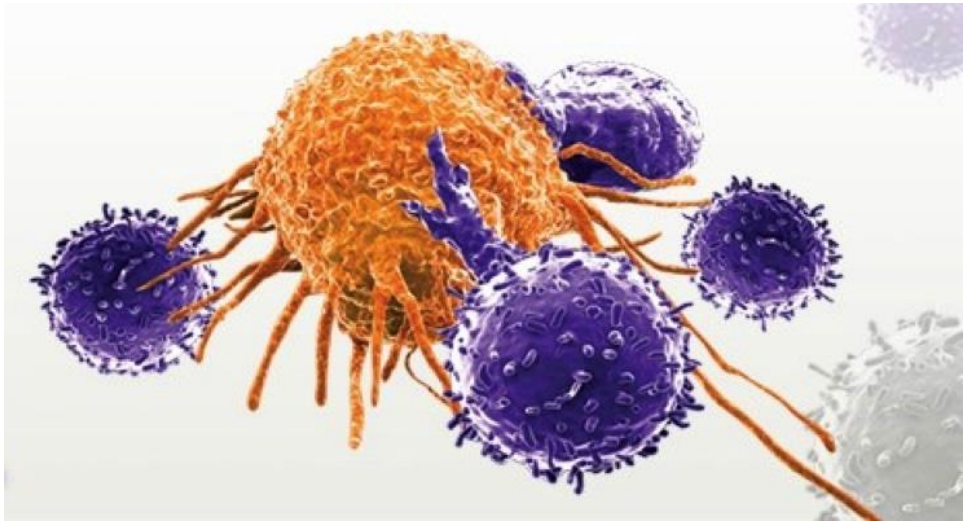
- Drugs
- Toxins
- Radioactivity

Checkpoint inhibitor antibodies:

- A growing class of drug
- Stimulate immune system by blocking natural brakes
- Help address the immunosuppressive tumour microenvironment

UCL CANCER INSTITUTE
Paul O'Gorman Building

Recombinant Antibodies for Cancer Therapy: 2



Kerry Chester

Antibodies: Sequence, Structure and Designing Therapeutics 4th - 5th July 2019

Talk Outline

PART I

Why engineered antibodies are such powerful tools

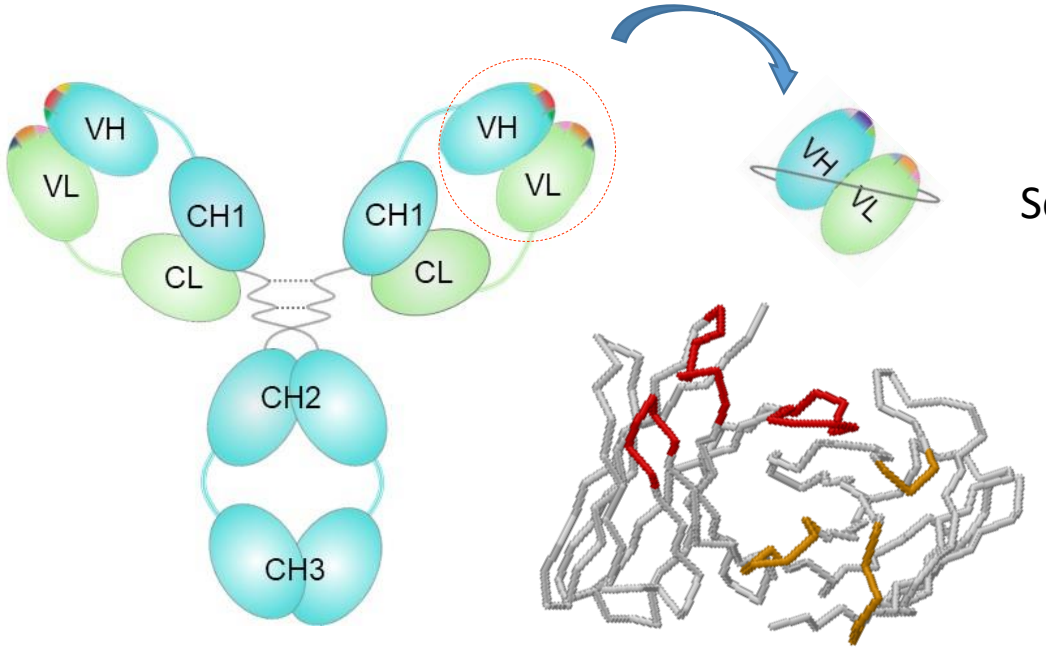
Main ways that (usually whole) engineered antibodies are used as anti-cancer drugs

PART II

Wider scope

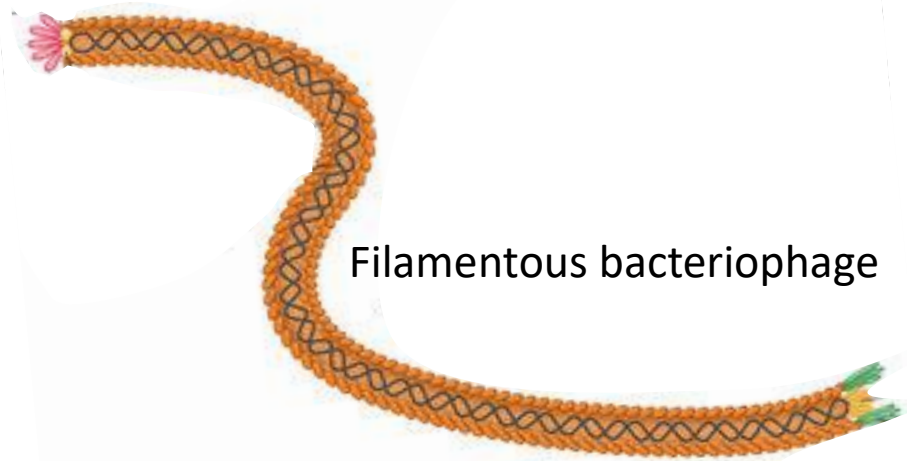
- Engineered Single chain Fv fragments (ScFv)
- Phage technology
- ScFv for T cell therapy (CAR-T)
- Bi-specifics and Bi-specific T-cell engagers (BiTEs)

The single chain Fv (scFv)

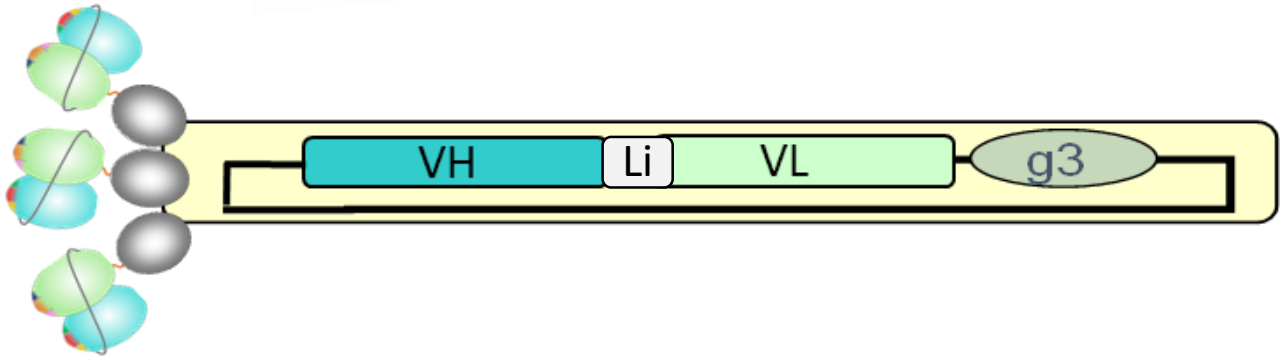


ScFv

MFE-23 (PDB 1QOK)



Filamentous bacteriophage



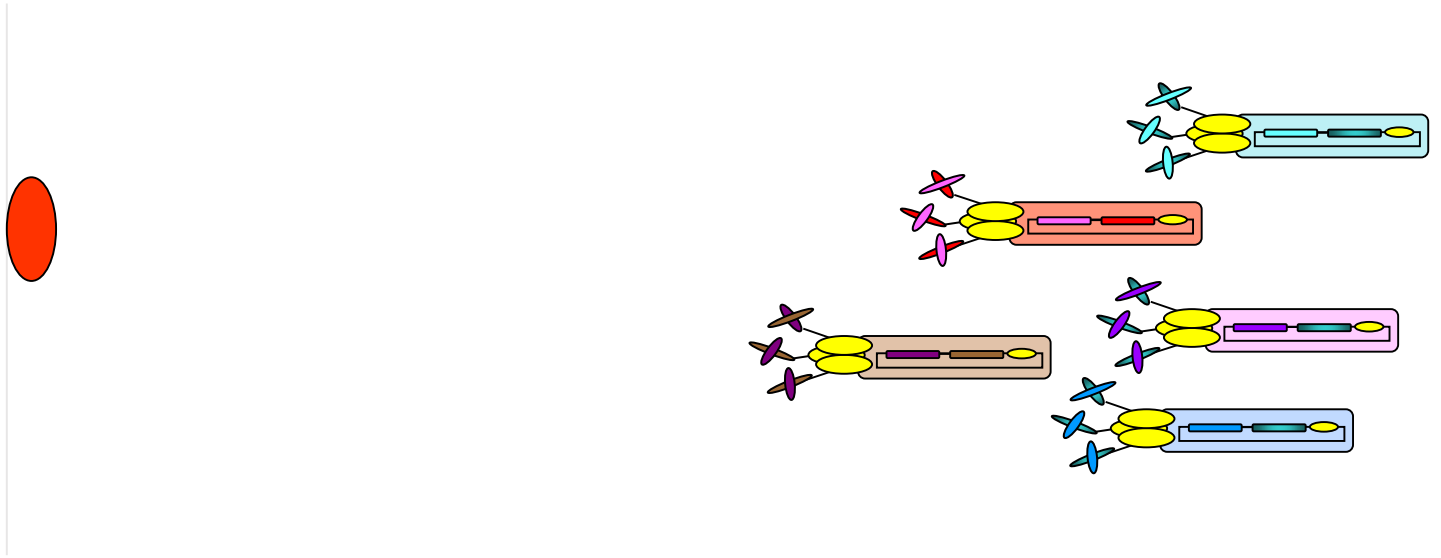
- Elegant
- Patentably distinct from Mabs
- A key building block for future therapies – particularly as fusion proteins

James Huston *PNAS* August 1998, Robert Bird *Science* October 1998

Power of phage display

Rapid section of leads from millions of candidates

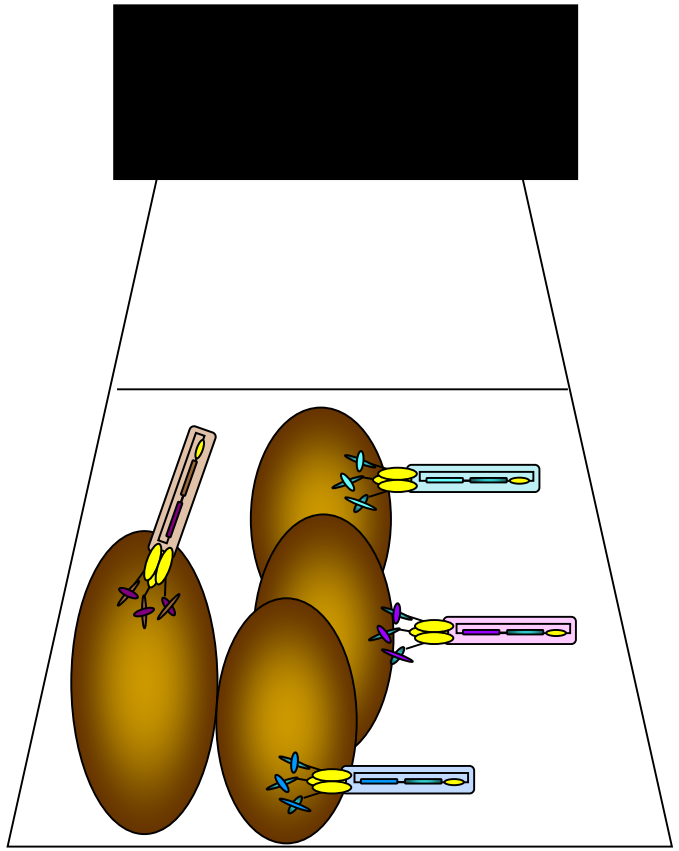
No. Independent clones in phage scFv library	10 – 1000 million
No. People in UK	60.6 million (2006)



Target antigen

(protein on plastic, cell, human tissue)

Amplification of selected phage



Elute phage - infect bacteria – obtain cloned scFvs

Phage display libraries

The Nobel Prize in Chemistry 2018

Frances H. Arnold
George P. Smith
Sir Gregory P. Winter

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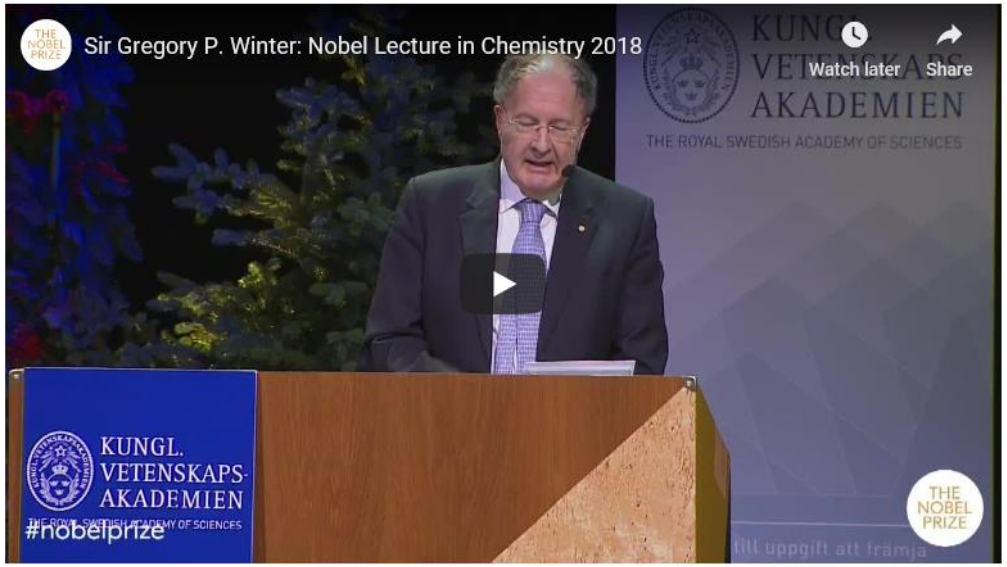
Sir Gregory P. Winter
Nobel Lecture

Harnessing Evolution to Make Medicines



Sir Gregory P. Winter delivered his Nobel Lecture on 8 December 2018 at the Aula Magna, Stockholm University.

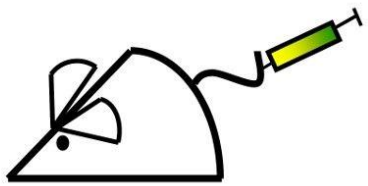
Nature. 1990 Dec 6;348(6301):552-4.
Phage antibodies: filamentous phage displaying antibody variable domains.
McCafferty J[1], Griffiths AD, Winter G, Chiswell DJ.

- ScFv can be displayed on the surface of fd bacteriophage,
- Phage bind specifically to antigen
- Rare phage (one in a million) can be isolated

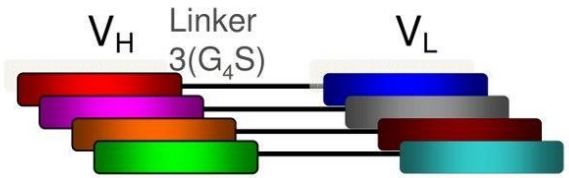
https://www.iontas.co.uk/
Bespoke human scFv libraries

The first scFv used in the clinic

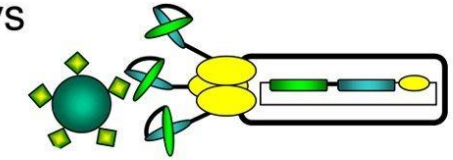
Antibody V regions
CEA-immunised mouse



PCR & link to
scFv format

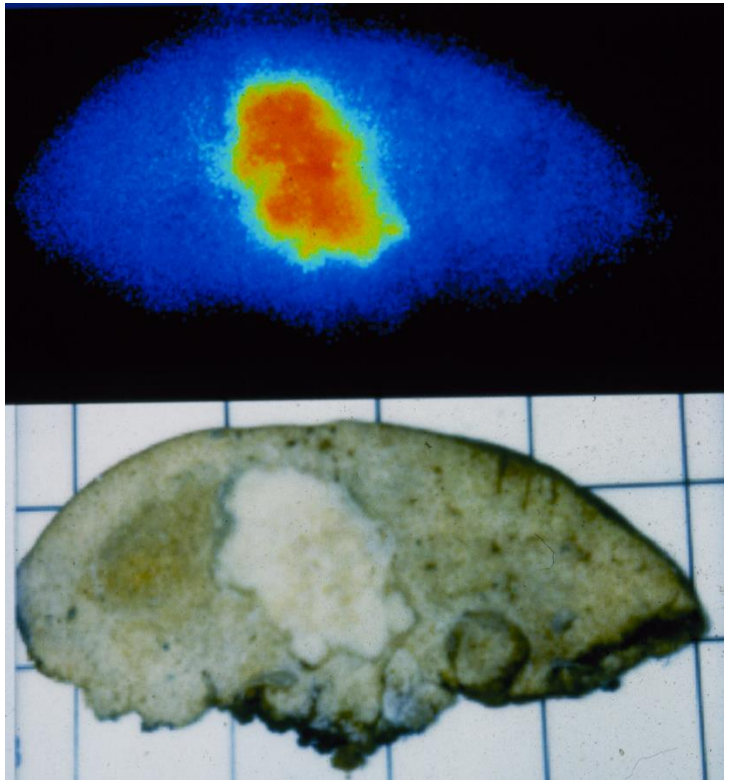


Clone into phage
library of 10^7 scFvs



Select the best
CEA binder (MFE-23)

Express MFE-23 as
soluble scFv purify by
IMAC via his tag

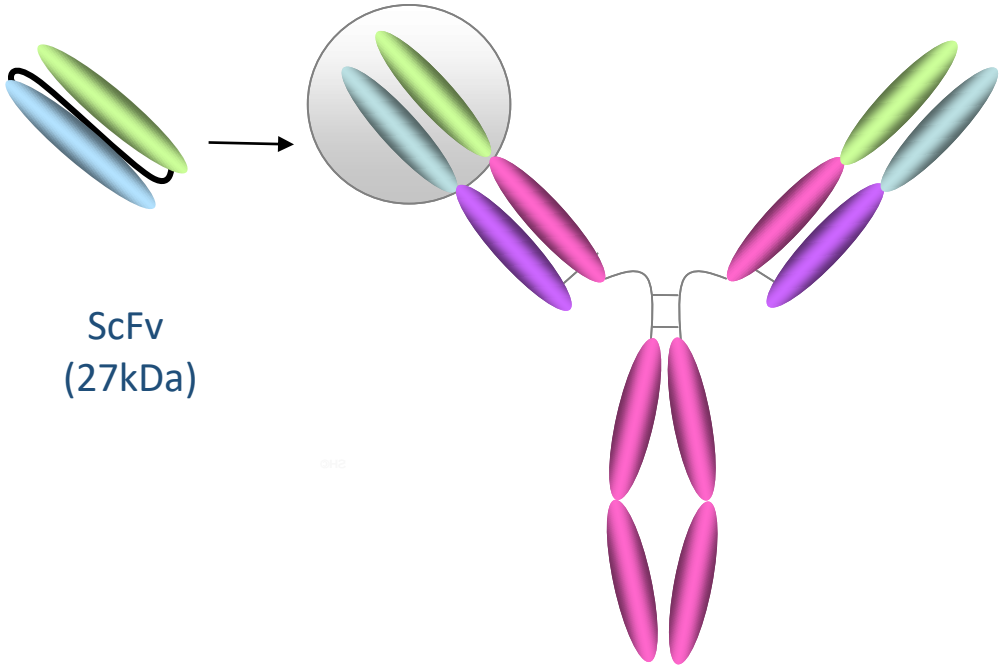


MFE-23 (phage-derived) anti-CEA

Chester et al 1994 Lancet, Begent et al 1996 Nat Med. Mayer et al 2000 Clin Cancer Res

For therapy scFvs can used in many ways – examples

ScFv can be reformatted as IgG



ScFv
(27kDa)

IgG
Antibody
(150kDa)

ScFv can be used to recruit T-cells

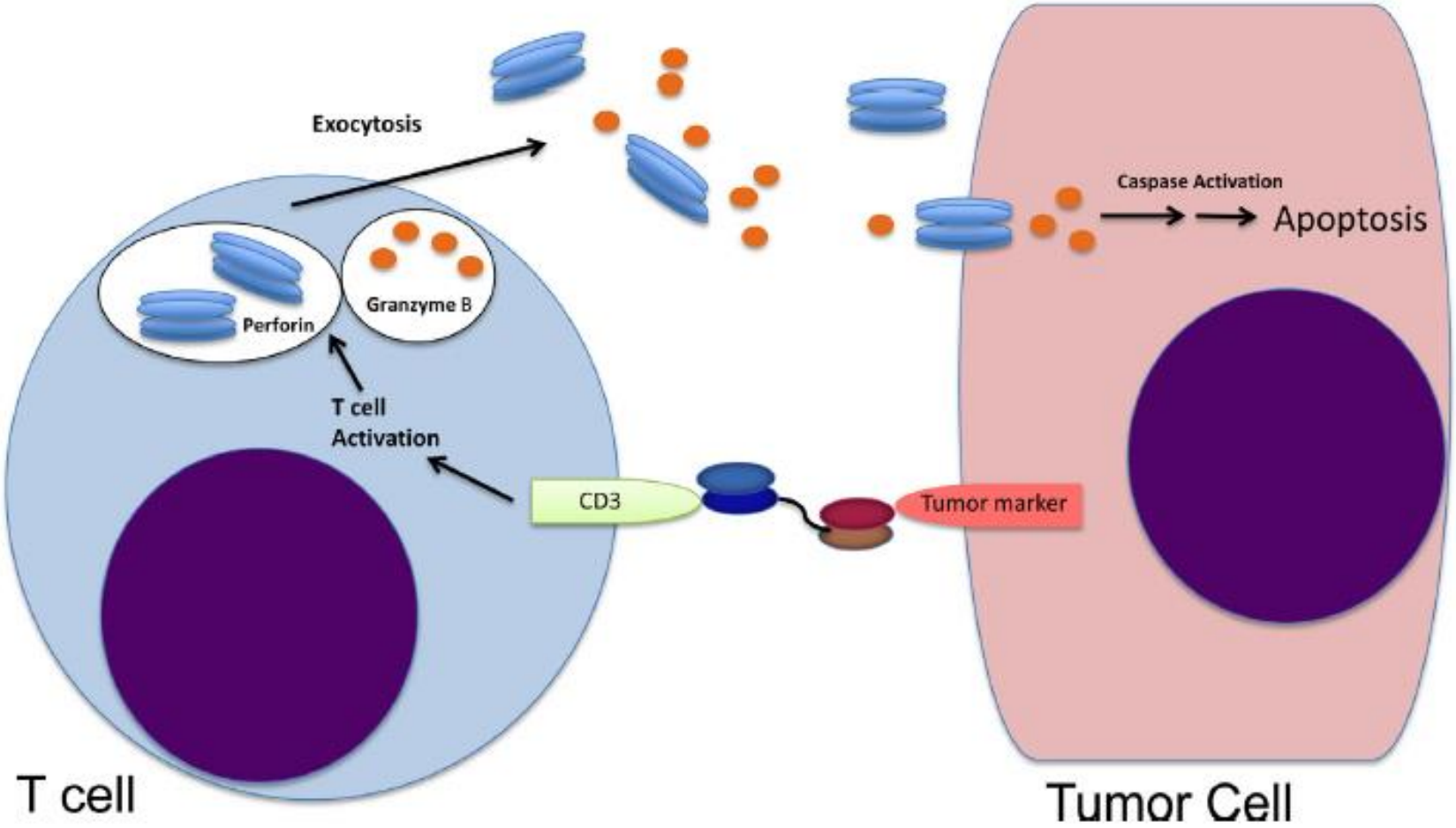
How ?
Why is this so exciting?

Cytotoxic T-cells are serial killers

https://www.youtube.com/watch?v=ntk8XsxVDi0

Bi-specific T-cell engagers (BiTEs)

https://www.youtube.com/watch?v=P_2C3tXoxRw

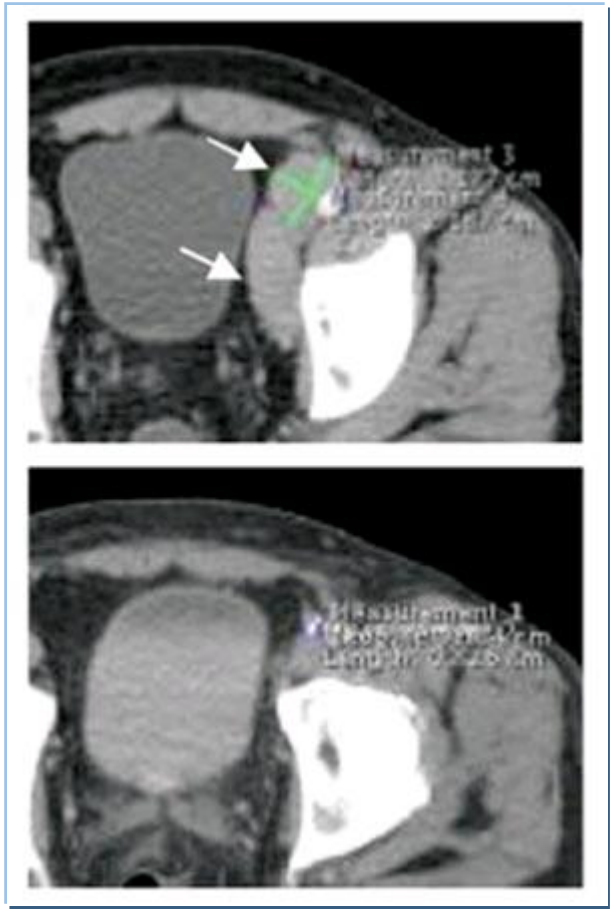


Bi-specific scFvs - a powerful means to recruit cytotoxic effector cells

From review in Molecular Immunology 67 (2015) 28–45

Clinical example: Blinatumomab BiTE

Blinatumomab specifically targets CD19 antigen present on B cells



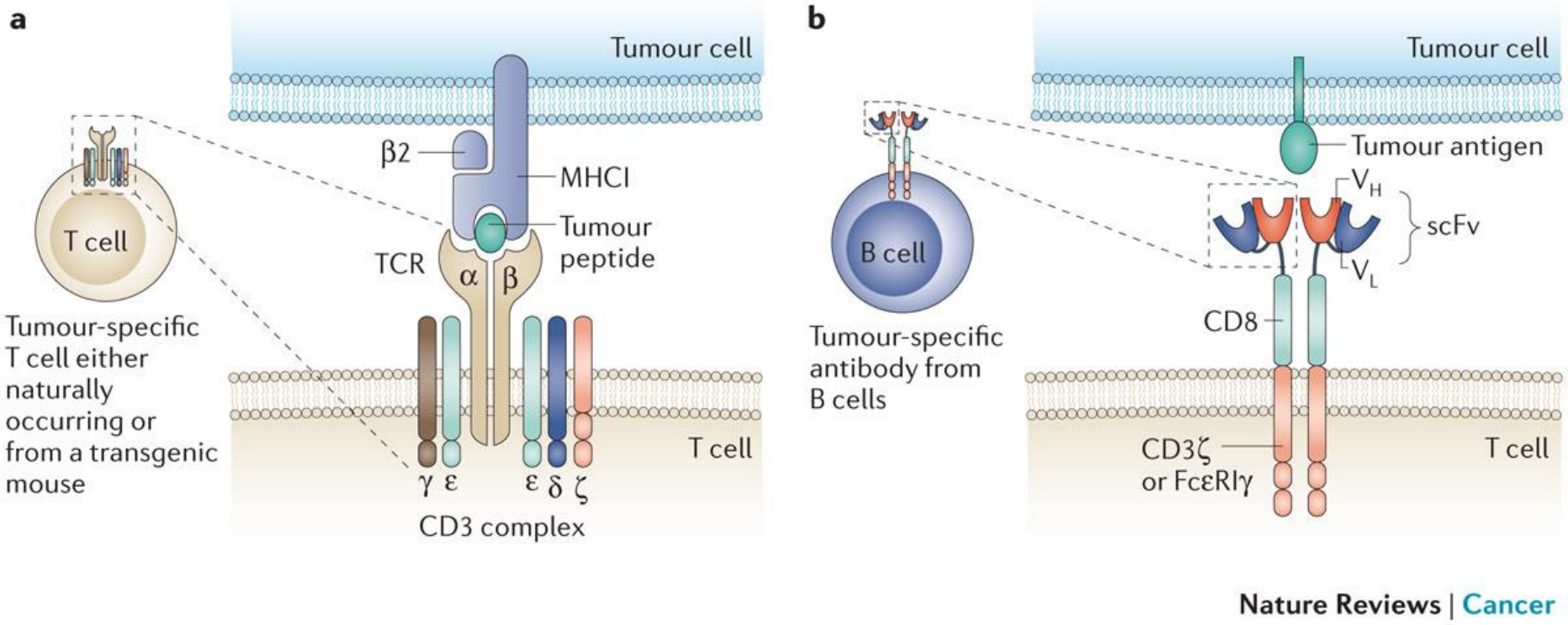
In a phase 1 clinical study with blinatumomab, patients with non-Hodgkin's lymphoma showed tumour regression, and in some cases complete remission.

Licenced FDA - December 2014 for B cell precursor acute lymphoblastic leukaemia

Science 15 August 2008: 974-977
Tumor Regression in Cancer Patients by Very Low Doses of a T Cell–Engaging Antibody

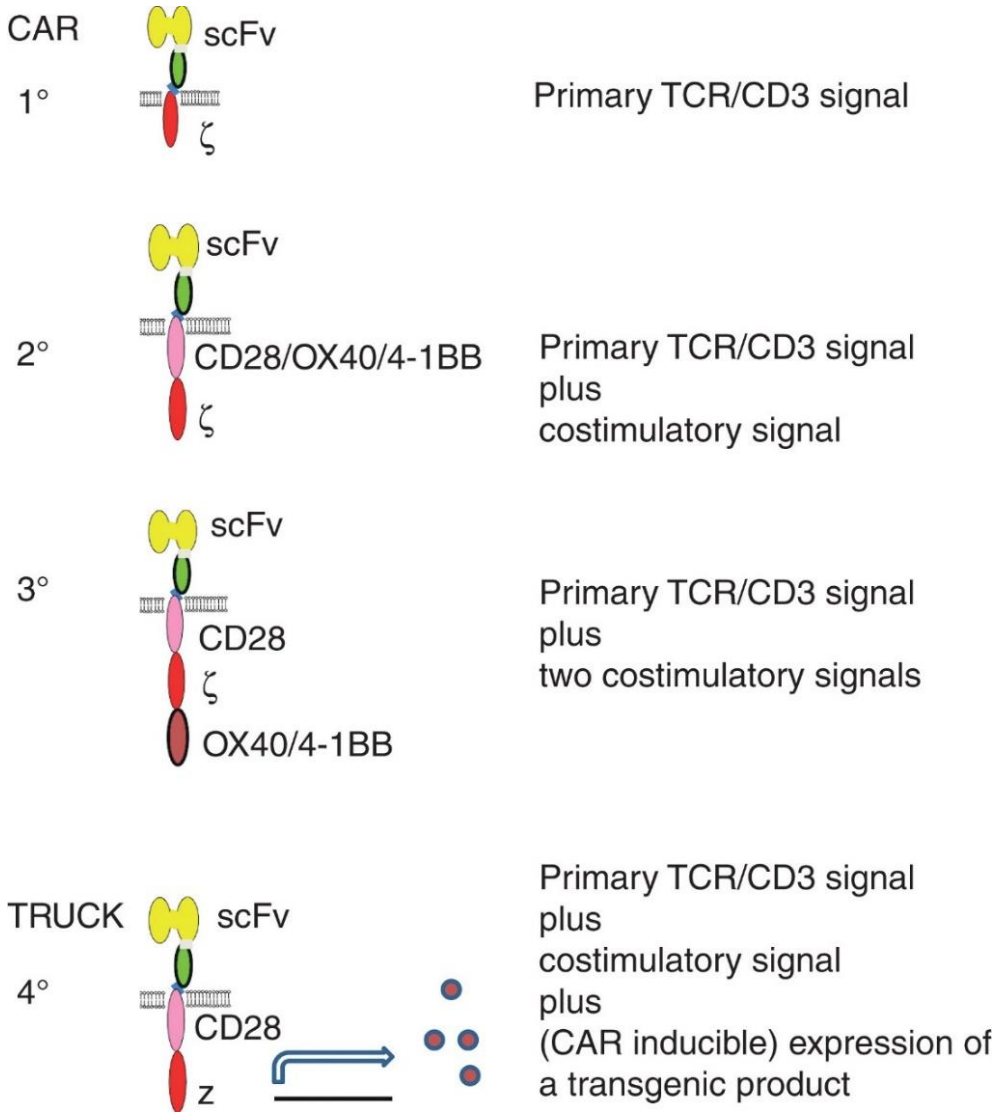
Blinatumomab: A historical perspective Pharmacology & Therapeutics Volume 136, Issue 3 2012

Chimeric antigen receptor (CAR)



CARs are composed of an scFv linked through hinge and transmembrane domains to a T-cell cytoplasmic signalling region

The evolution of chimeric antigen receptors

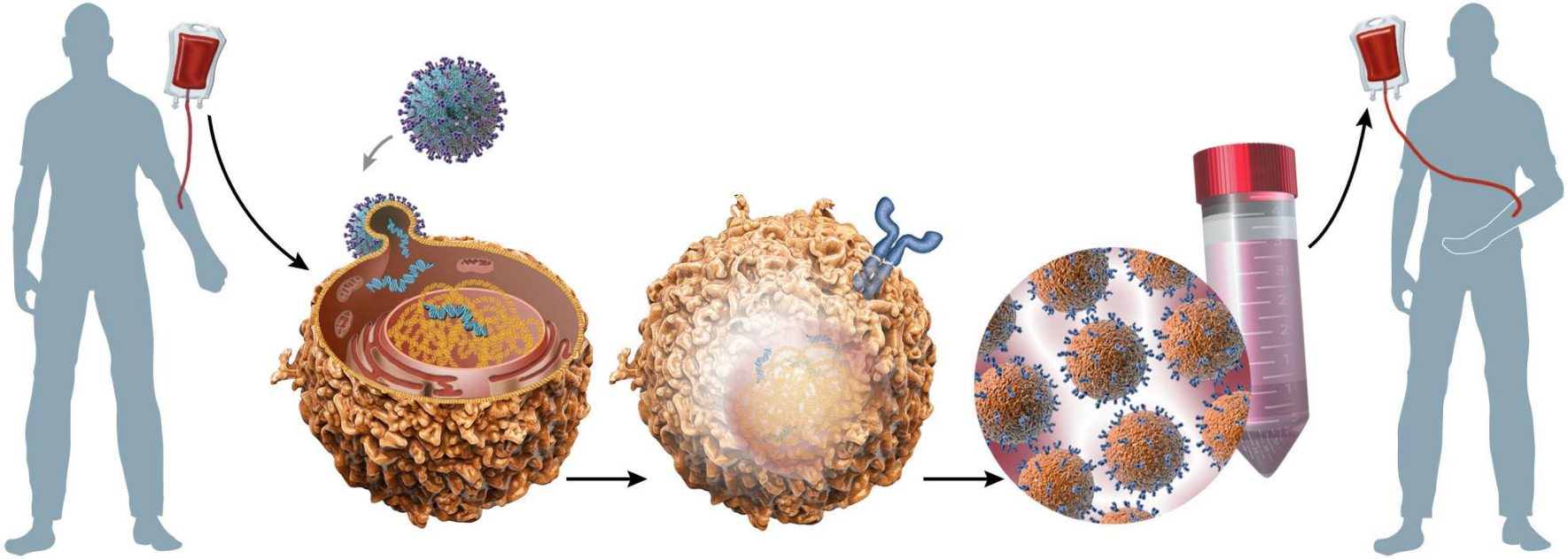


- CARs of the first generation consist of a single chain fragment of variable region (scFv) antibody for target binding linked by a spacer domain to the transmembrane and intracellular signaling domain of CD3ζ derived from the TCR.
- Addition of a costimulatory domain, mostly of the CD28 family, makes up a second-generation CAR.
- Third-generation CARs contain two costimulatory domains.
- Fourth-generation CAR T cells which are additionally modified with a constitutive or inducible expression cassette for a transgenic protein, for instance a cytokine, which is released by the CAR T cell to modulate the T-cell response.
- CAR T cell immunotherapy is a highly effective, with high remission rates in patients with B cell acute lymphoblastic leukaemia or large B cell lymphoma, leading to FDA approvals.
- Many innovative CAR T therapies are in developemnt

Markus Chmielewski & Hinrich Abken
https://doi.org/10.1517/14712598.2015.1046430

Engineered T cells: the promise and challenges of cancer immunotherapy Nature Reviews Cancer 16, 566–581 (2016)

Chimeric antigen receptor (CAR) – A personalized therapy



HaemaLogiX

1. T cells are collected from the patient.A machine removes the desired cells from th eblood, then returns the rest back to the patient.

2. A modified virus (blue) is used to transfer DNA to the patient's T cells so they will produce CAR proteins.

3. CARs have two ends: a binding site (blue) specific to the tumor cells, and a signaling engine that activates the T cell to kill the tumor it binds to.

4. Once designed, millions of engine-ered CAR T cells are grown in the laboratory.

5. The expanded popula-tion of CAR T cells is infused into the patient through a standard blood transfusion

Complete remission (CR) rates as high as 90% have been reported in children and adults with relapsed and refractory ALL treated with CAR-modified T cells targeting the B-cell–specific antigen CD19. *(Short review - Blood. 2015;125(26): 4017-4023)*

UCL Spin out (Martin Pule)

Autolus

https://www.youtube.com/watch?v=w7mMamTRvko

http://www.autolus.com/

Clinical example: CAR T-cells gain FDA approval

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CAR T-Cell Therapy Approved for Some Children and Young Adults with Leukemia

Subscribe

September 11, 2017, by NCI Staff

On August 30, the Food and Drug Administration (FDA) approved a type of immunotherapy called CAR T-cell therapy for certain children and young adults with a form of acute lymphoblastic leukemia (ALL). The treatment, tisagenlecleucel (Kymriah™), is the first CAR T-cell therapy to receive FDA approval.

Acute lymphoblastic leukemia is the most common cancer among children in the United States. Intensive chemotherapy cures more than 80% of children with ALL that arises in B cells, which is the predominant type of pediatric ALL. But there are few treatment options for patients whose cancers do not respond to treatment or go into remission and later relapse.

FDA approved tisagenlecleucel, which is manufactured by Novartis, for patients up to 25 years of age with B-cell ALL that does not respond to treatment or has relapsed two or more times.

Manufactured CAR T cells ready for infusion into a patient
Credit: Penn Medicine

#HEALTH NEWS NOVEMBER 6, 2017 / 6:46 AM / 16 DAYS AGO

Novartis seeks European approval for cell therapy Kymriah

Reuters Staff 2 MIN READ

ZURICH (Reuters) - Swiss drugmaker Novartis has submitted its cell therapy Kymriah for European approval in two forms of blood cancer, seeking to expand the use of a new treatment it hopes will eventually become a $1 billion-a-year seller.

KYMRIAH is a CD19-directed genetically-modified autologous T cell immunotherapy

https://www.multivu.com/players/English/8163751-novartis-kymriah-fda-approval/

Future

Trends indicate more anticancer antibodies in more innovative formats:

- Bispecific
- ADCs
- Immune modulators
- CAR-T

Many ways of making bi-specific antibodies

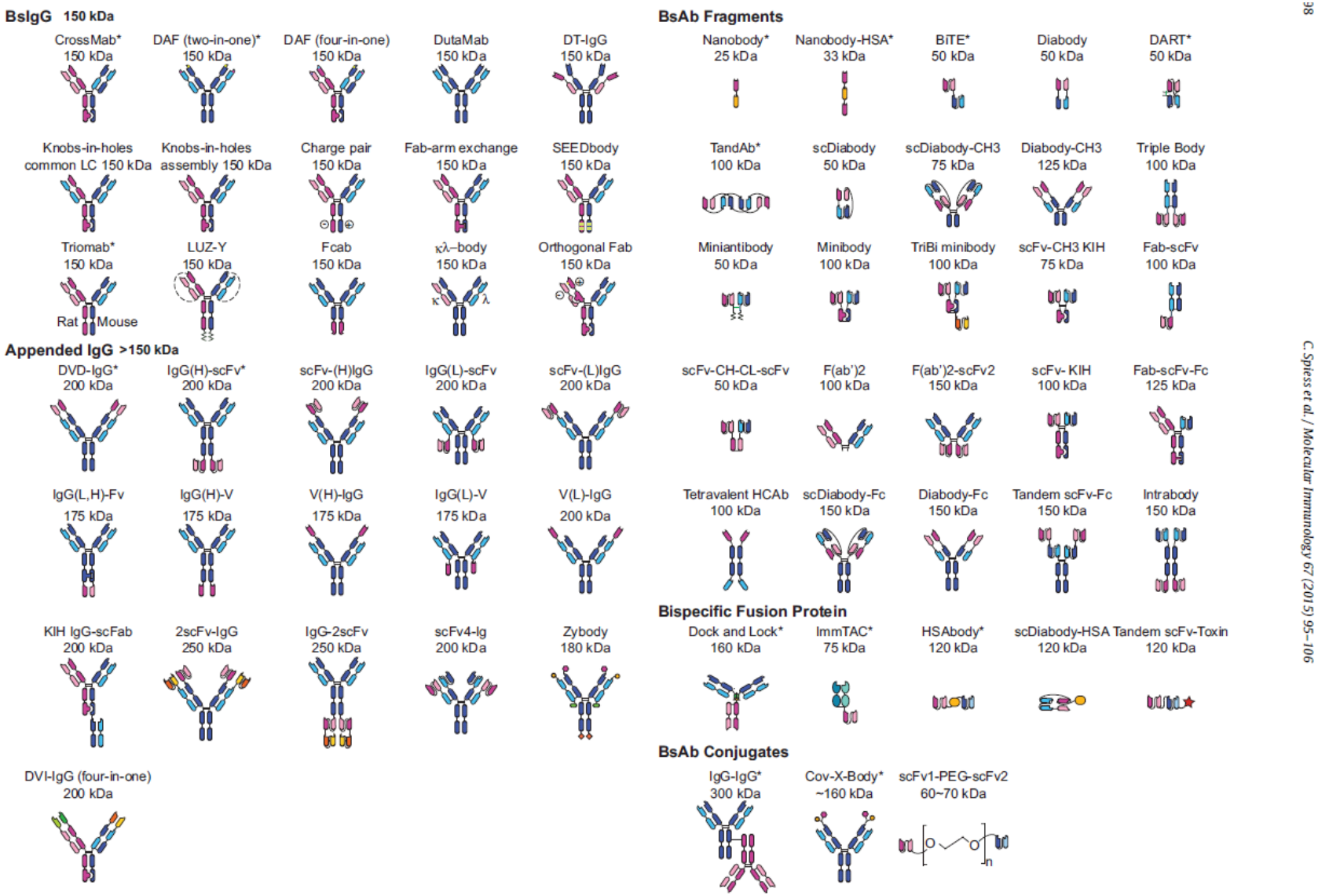
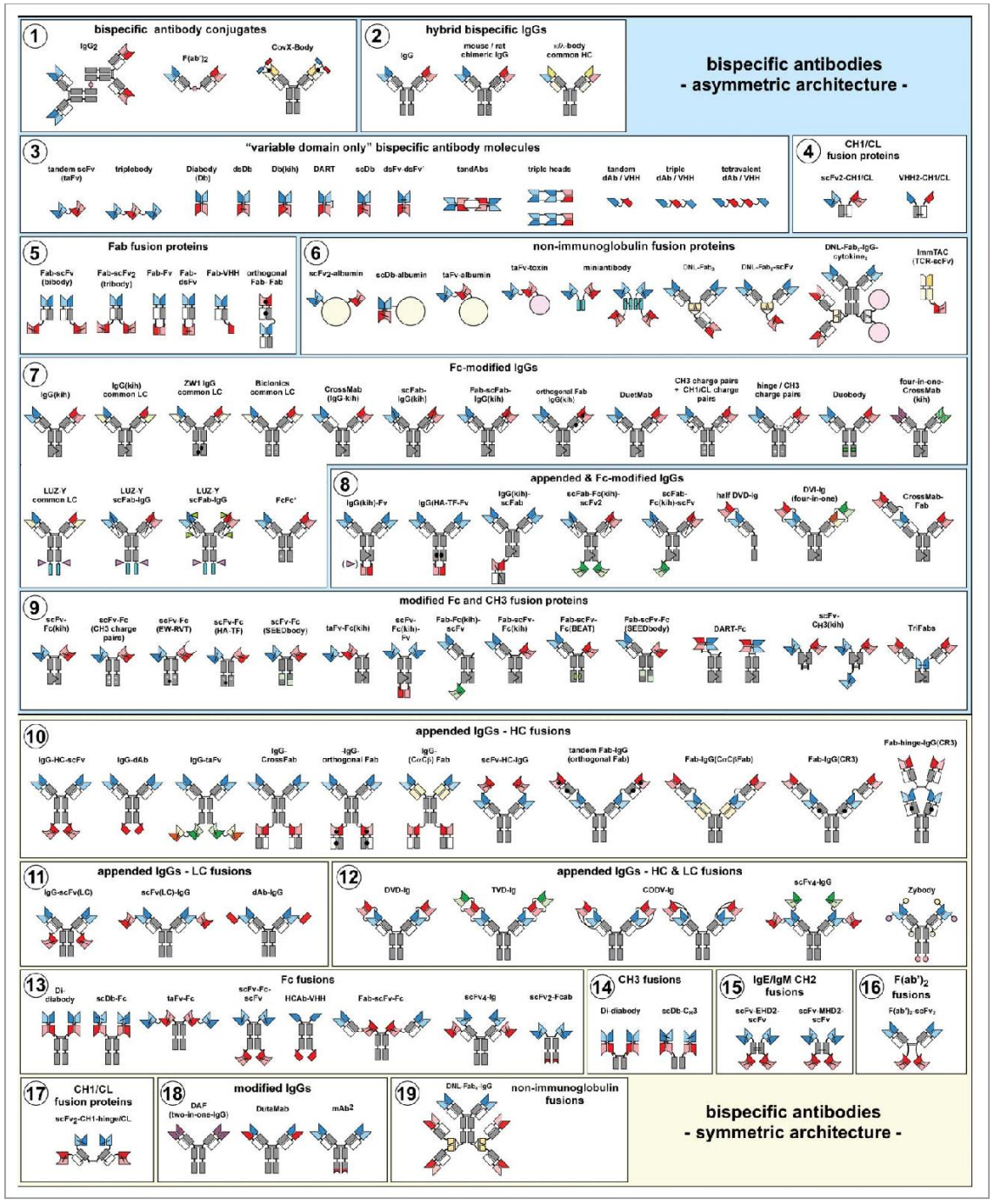


Fig. 1. Alternative formats for bispecific antibodies and other bispecific immunotherapeutics subdivided into five major classes: BsIgG, appended IgG, BsAb fragments, bispecific fusion proteins and BsAb conjugates. Heavy chains are shown in dark blue, dark pink and dark green and corresponding light chains are in lighter shades of the same colors. Connecting peptide linkers are shown by thin black lines and engineered disulfide bonds by thin green lines. Approximate molecular weights are shown assuming ~12.5 kDa per immunoglobulin domain. BsAb formats that have advanced into clinical testing are highlighted (*). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

Paul Carter Molecular Immunology 67 (2015) 95–106



The making of bispecific antibodies

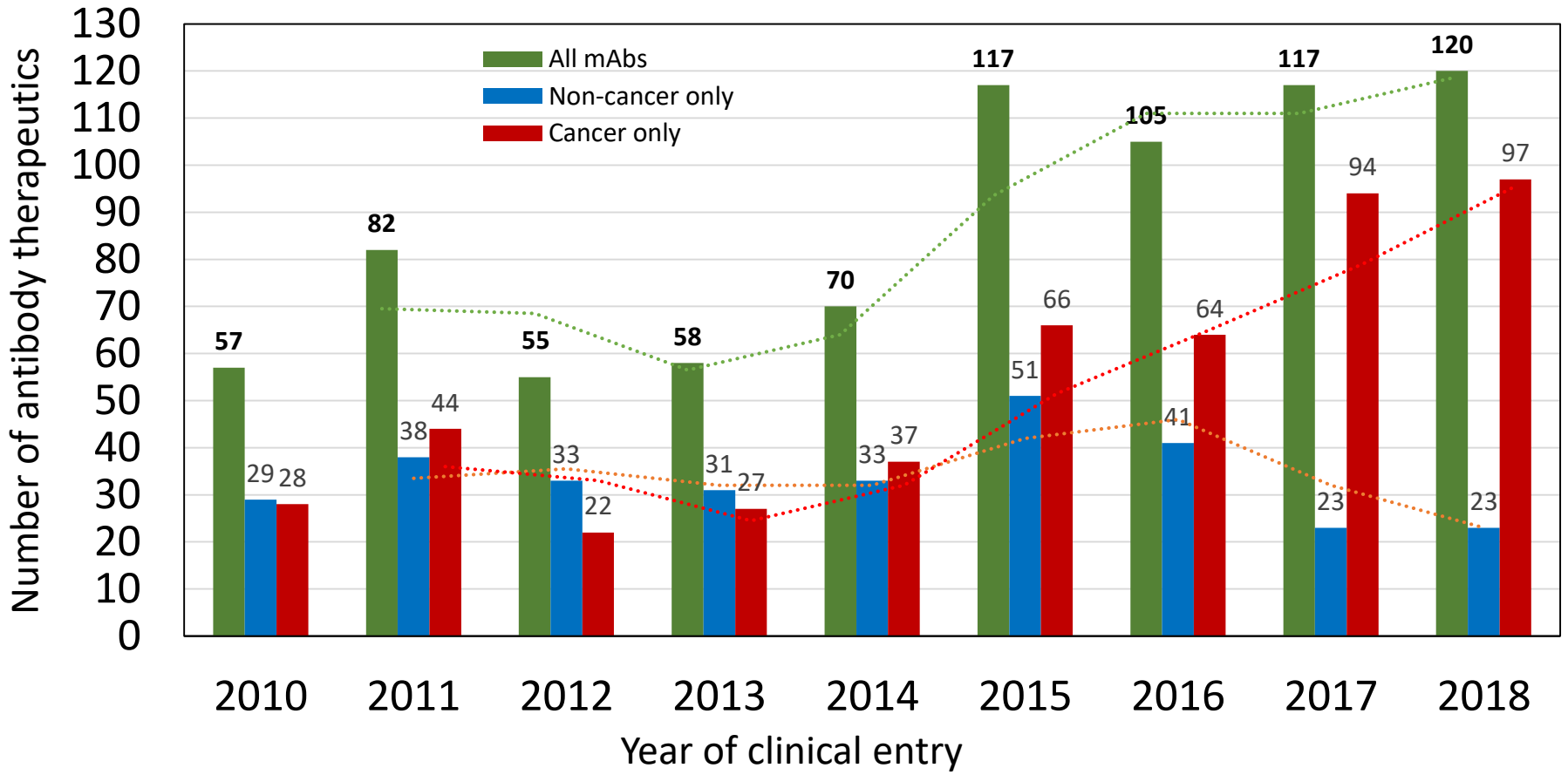
Ulrich Brinkmann, Roland E Kontermann

Published in mAbs 2017
•DOI:10.1080/19420862.2016.1268307

During the past two decades we have seen a phenomenal evolution of bispecific antibodies for therapeutic applications. The 'zoo' of bispecific antibodies is populated by many different species, comprising around 100 different formats, including small molecules composed solely of the antigen-binding sites of two antibodies, molecules with an IgG structure, and large complex molecules composed of different antigen-binding moieties often combined with dimerization modules. The application of sophisticated molecular design and genetic engineering has solved many of the technical problems associated with the formation of bispecific antibodies such as stability, solubility and other parameters that confer drug properties.

Figure 2. The zoo of bispecific antibody formats
Overview of bispecific antibody formats reduced to practice, grouped into molecules with symmetric or asymmetric architecture.

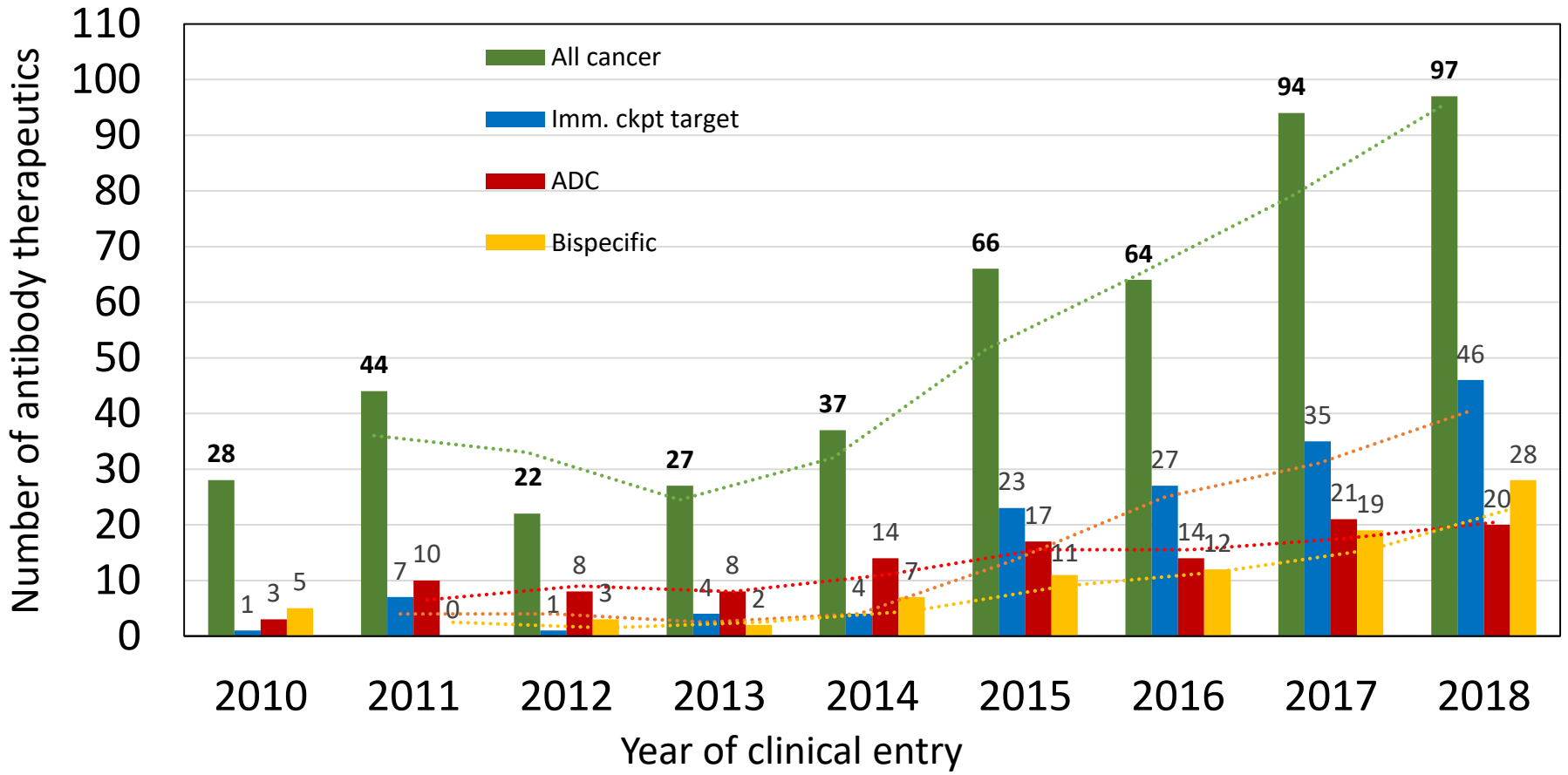
Start of clinical studies: All mAbs



Totals include mAbs sponsored by commercial firms only; dotted lines are 2-yr moving ave.

Source: The Antibody Society. Graph based on data available as of March 2019 and 'Antibodies to watch'

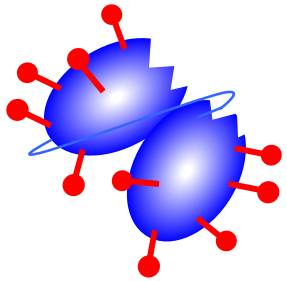
Start of clinical studies: Cancer only



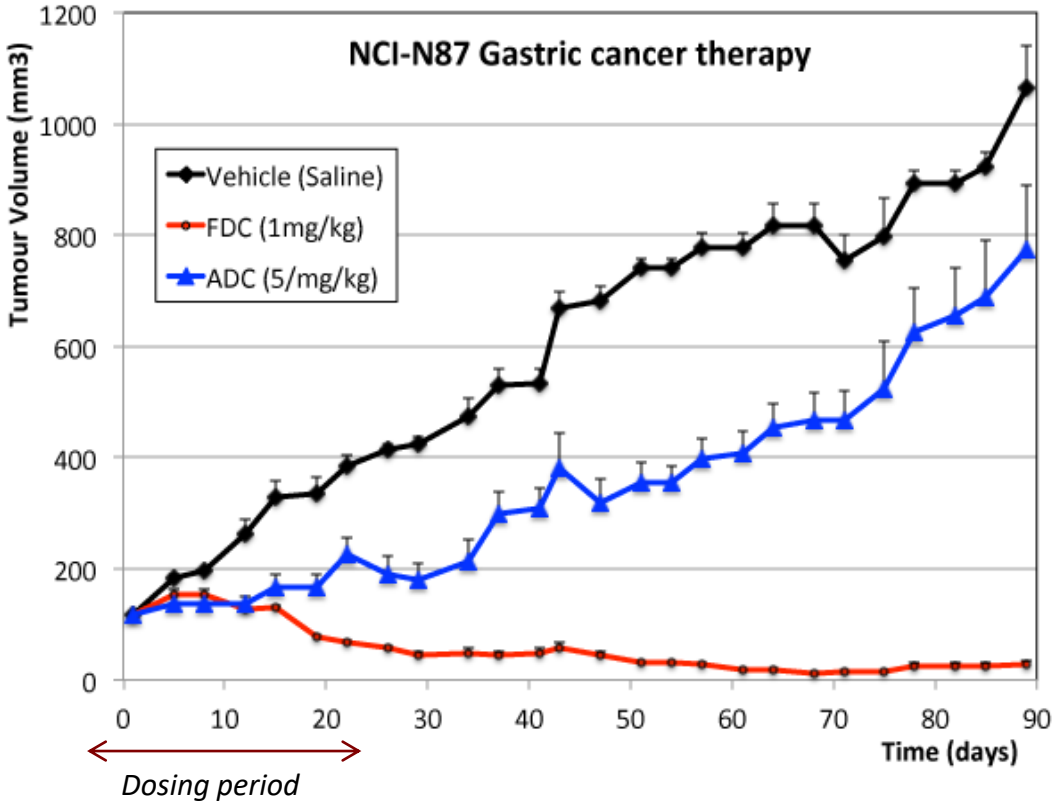
Totals include mAbs sponsored by commercial firms only; dotted lines are 2-yr moving ave.

Source: The Antibody Society. Graph based on data available as of March 2019

Fragment Drug Conjugates (FDCs)



Antikor's FDC – OptiLink™ technology
Antibody fragments optimized for high payload loading whilst retaining key benefits



OptiLinked anti-HER2 FDC with releasable MMAE payload DAR = 8

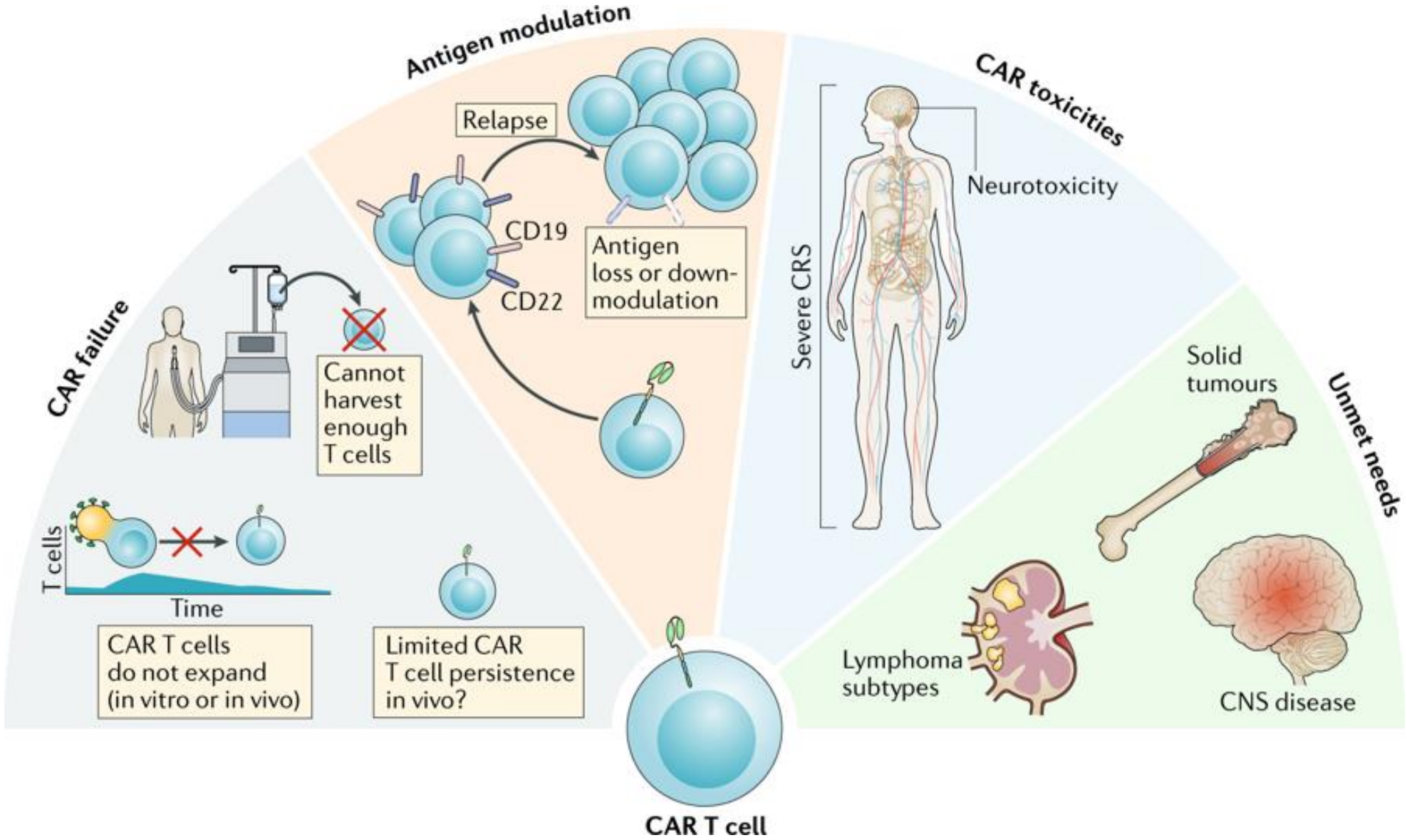
Human gastric cancer xenograft (NCI-N87) model

- Antikor FDC (1mg/kg=0.2mg/kg payload) 8 doses outperforms
- Trastuzumab ADC (5mg/kg=0.1mg/kg payload), 4 doses
- 6/8 cured at day 65
- 4-times more payload systemically delivered in total
- ~Same amount of payload exposed to tumour
- FDC is better tolerated than ADC

Antikor are completing an investment deal and partnership agreement with a listed Chinese pharma company to develop products for solid tumours.

Details will be announced in late April 2019

Meeting more challenges of CAR T cell therapy



Nature Reviews | Clinical Oncology Mechanisms of resistance to CAR T cell therapy Published: 05 March 2019

Summary – Part 2

- ScFvs are recombinant antibody fragments that can be used as building blocks of many antibody-based therapies
- ScFvs are used to recrurit T-cells as BiTes and to target CAR T-cells as CARs.
- BiTEs and CAR-Ts have been approved as a licensed drugs
- There many ways of making bi-specific antibodies and there are many applications for this class of drug
- Antibodies are enormously powerful tools and effective anti-cancer drugs
- An exciting future is envisaged
- Check out the Antibody Society http://www.antibodysociety.org

Questions?