

Antibodies as therapeutic drugs

June 30, 2011
Winfried Wels

Antibodies as drugs

Antibodies are not good drugs, at least not in any conventional sense.

They are biological molecules and hence intrinsically complicated.

No sane medicinal chemist would touch them.

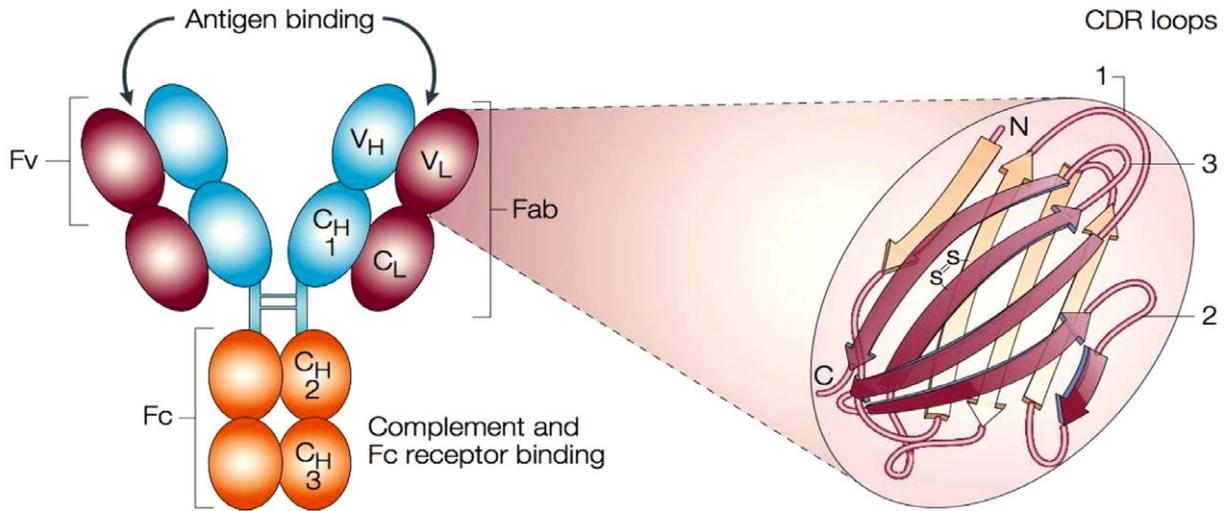
It takes about twice the time and it costs twice as much to work up antibodies to clinical-grade material comparing with small molecules.

In short, antibodies are large and clunky, difficult to make and difficult to use.

Antibodies have one saving grace that overrides all their deficiencies.

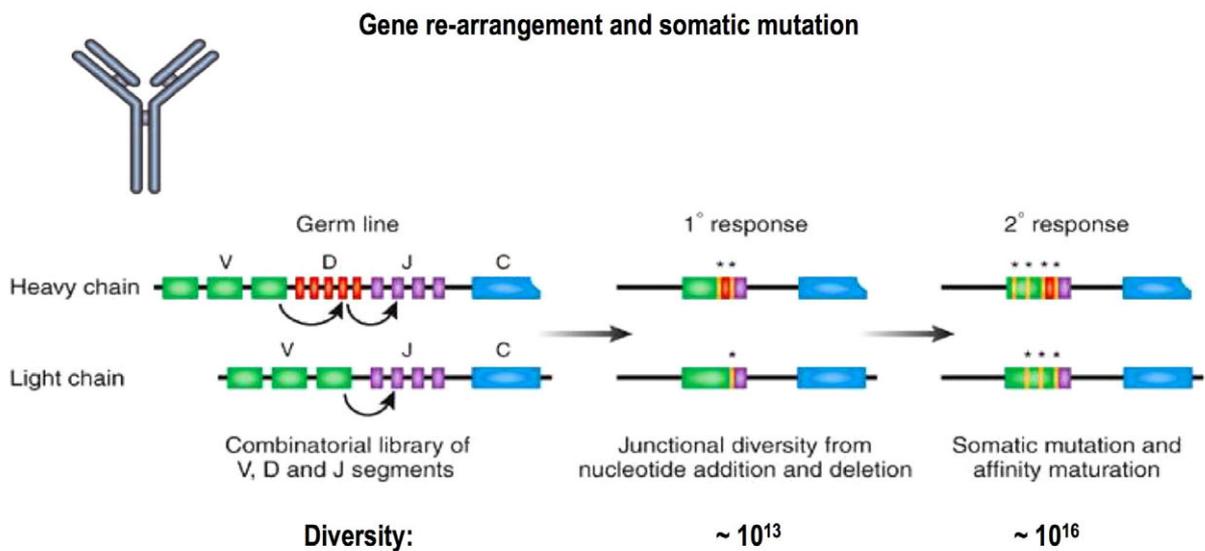
It is their exquisite specificity, their ability to home in on a particular target.

The modular structure of immunoglobulins

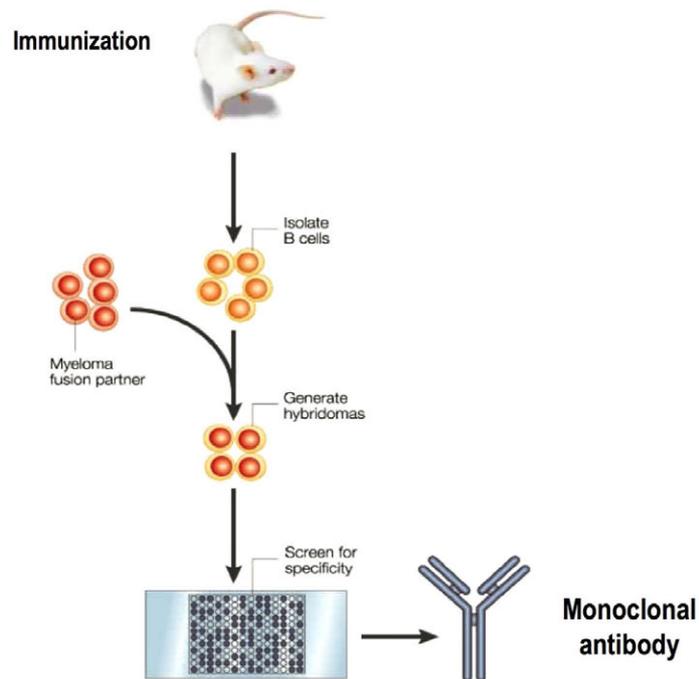


Brekke & Sandlie, 2003

The molecular basis of antibody diversity



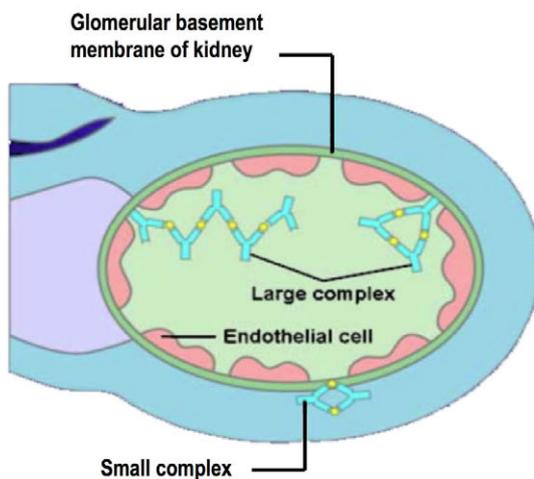
Generation of monoclonal antibodies with hybridoma technology



Köhler & Milstein, 1975

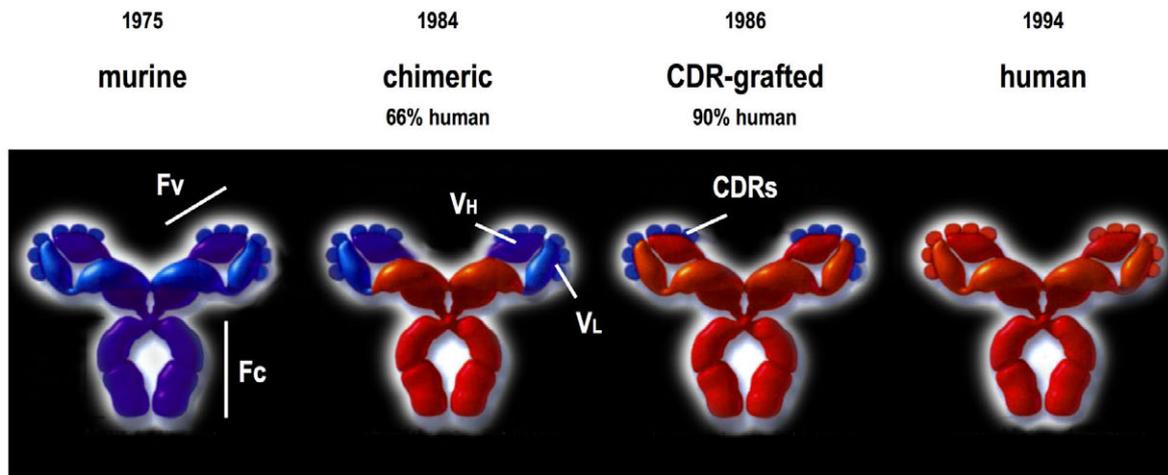
Murine antibodies induce HAMA and can cause serum sickness

Formation of immune complexes due to human anti-mouse antibodies



Antibody complexes are deposited into tissues, activating complement, attracting inflammatory cells and provoking damage.

Humanization as a prerequisite for the success of monoclonal antibodies in the clinic



-omab, -ximab, -zumab, -umab How to decrypt the names of antibody therapeutics

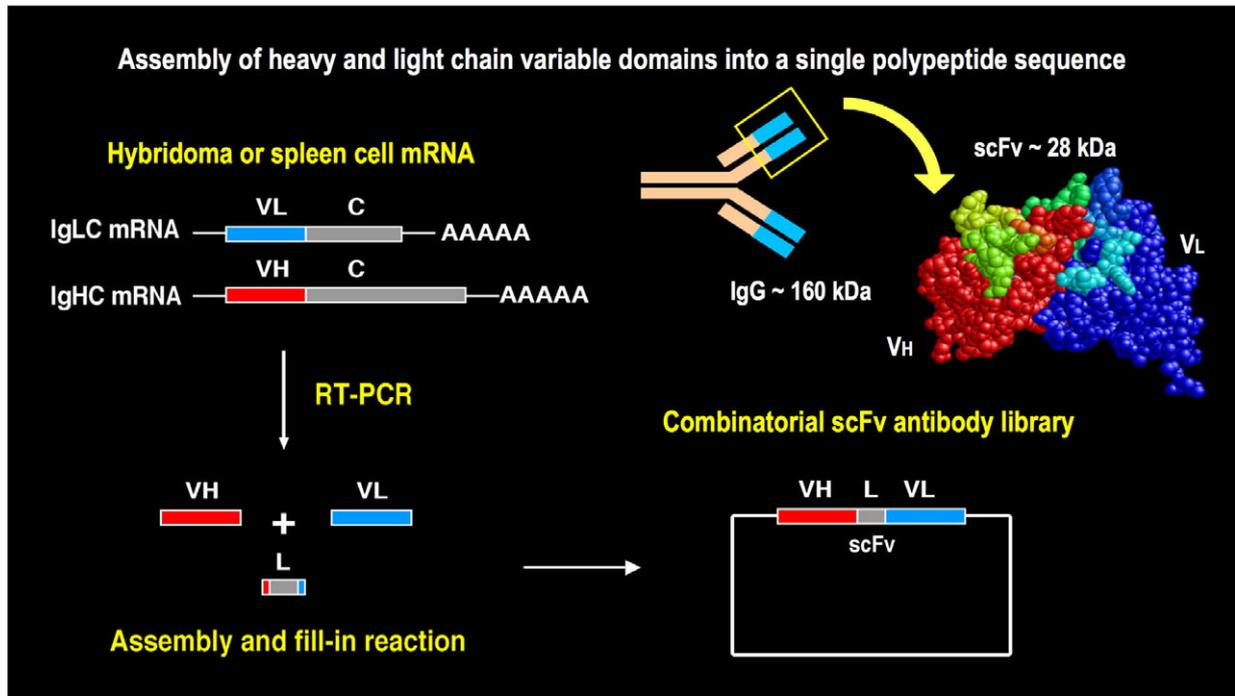
<i>Rituximab</i>	Ri-	tu-	xi-	mab
<i>Trastuzumab</i>	Tras-	tu-	zu-	mab
<i>Panitumumab</i>	Pani-	tum-	u-	mab

<i>Unique identifier</i>	<i>Disease/target</i>	<i>Source</i>	<i>Drug class</i>
tum-	tumor	o- mouse	monoclonal antibody
lim-	immune	xi- chimeric	or antibody fragment
cir-	cardiovascular	zu- humanized	
ner-	neurological	xizu- humanized/chimeric	
vir-	viral	u- human	

More on antibody nomenclature:

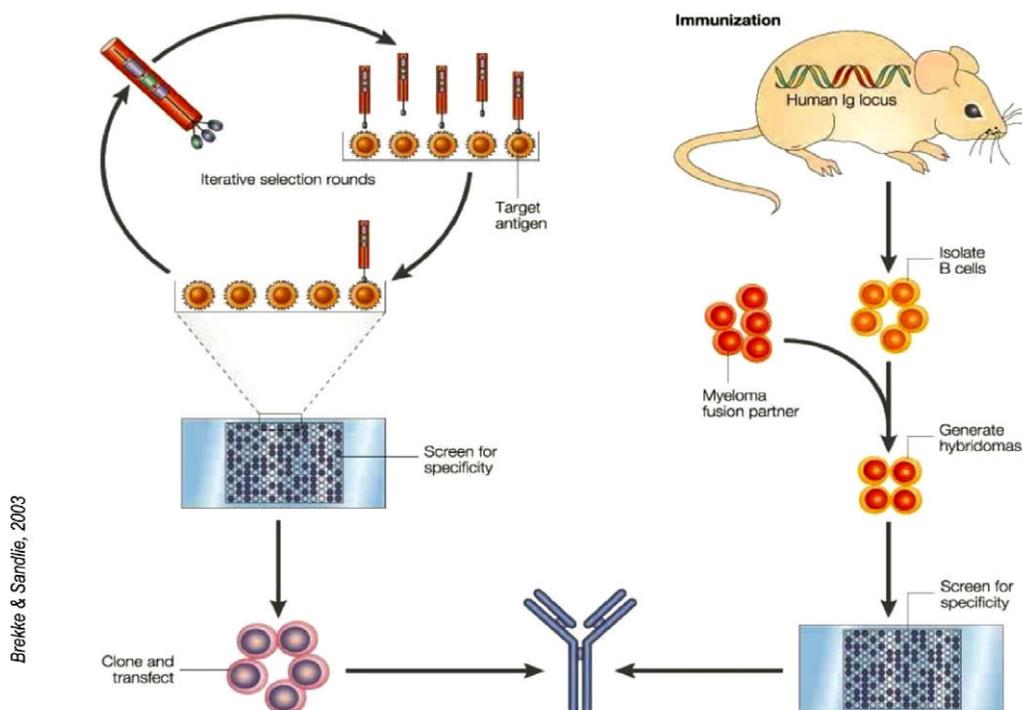
www.ama-assn.org/ama/pub/physician-resources/medical-science/united-states-adopted-names-council/naming-guidelines/naming-biologics/monoclonal-antibodies.page

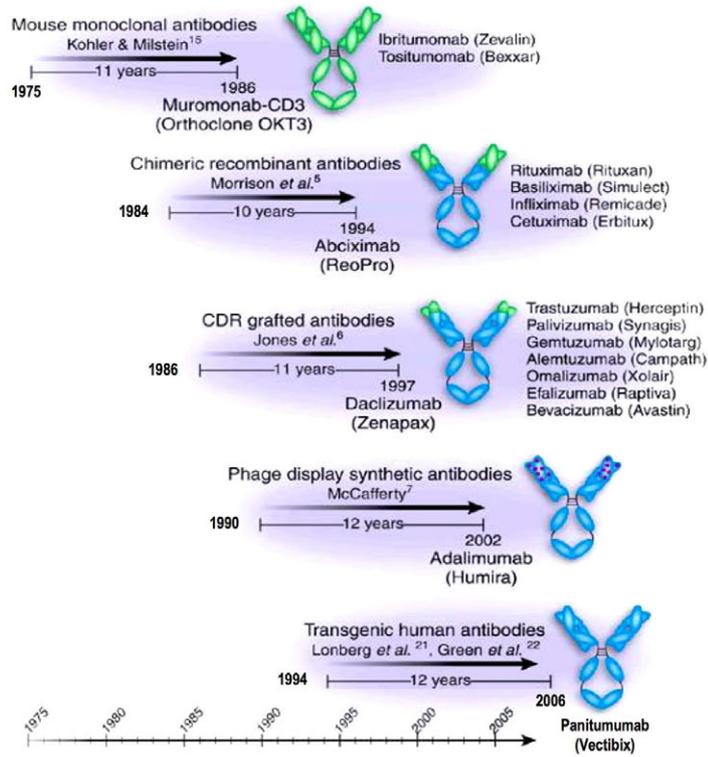
Construction of scFv antibody fragments



Human antibody library

Human IgG transgenic mouse





Lonberg, 2005

The market for monoclonal antibodies

Generic Name Target	Brand ® FDA Approval	Companies	Indication	Sales \$ billion		
				2007	2008	2009
Infliximab c TNFα	Remicade 1998	J&J, Merck	CD, UC, AS RA, Ps, PsA	5.04	6.5	6.91
Bevacizumab VEGF hz	Avastin 2004	Roche	Colon mCRC, Lung NSCLC Breast mBC, mRCC Glioblastoma	3.93	4.7	5.92
Rituximab c CD20	Rituxan 1997	Roche	Leukemia, CLL Lymphoma, RA	5.01	5.6	5.8
Adalimumab h TNFα	Humira 2002	Abbott	RA, JIA, PsA, Ps, AS, CD	3.06	4.4	5.48
Trastuzumab hz HER2	Herceptin 1998	Roche	Breast Cancer	4.4	4.8	5.02
Cetuximab c EGFR	Erbix 2004	Bristol Myers Squibb Merck KgA	Colon Cancer HNC	1.35	2.0	2.57
Ranibizumab hz VEGF	Lucentis 2006	Novartis, Roche	Macular Degeneration	1.2	1.5	2.43

kno1.google.com/monoclonal-antibody-market-2009

AS ankylosing spondylitis, CD Crohn's disease, Ps psoriasis, PsA psoriatic arthritis, RA rheumatoid arthritis, UC ulcerative colitis.

m metastases

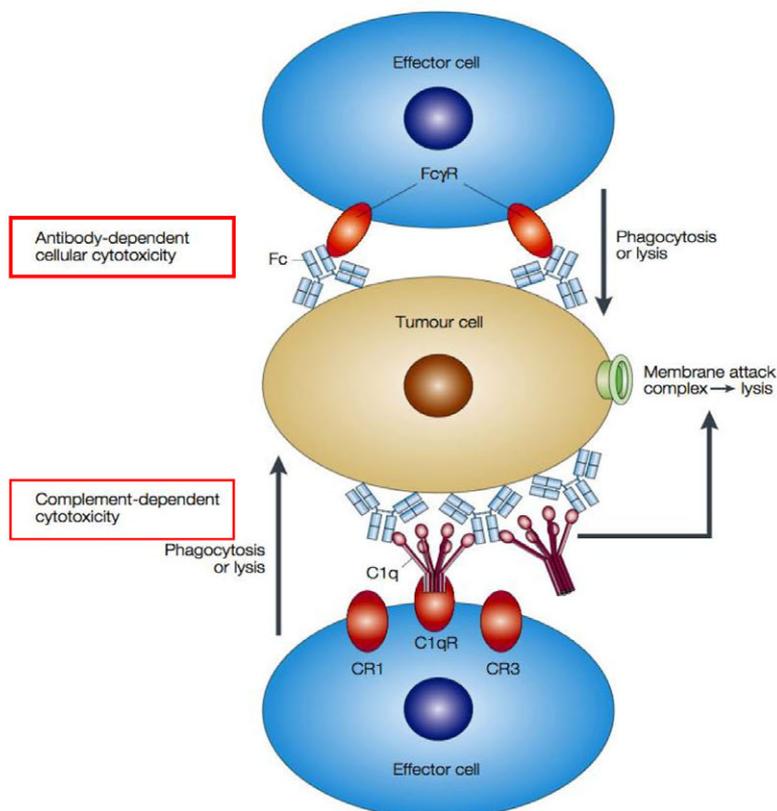
RCC renal cell carcinoma, NSCLC Non small cell lung cancer, BC breast cancer, CRC Colorectal cancer, CLL Chronic Lymphocytic Lymphoma, HNC Head and Neck cancer

RSV Respiratory syncytial virus

Examples of antibodies in clinical use for cancer therapy

Antibody	Type	Target	Cancer * (and other indications)
Rituxan (rituximab)	ch IgG1	CD20	non-Hodgkin's lymphoma (rheumatoid arthritis) first FDA approval 1997
Herceptin (trastuzumab)	hu IgG1	ErbB2/HER2	breast cancer first FDA approval 1998
Erbix (cetuximab)	ch IgG1	EGFR	colorectal cancer head and neck cancer first FDA approval 2004
Avastin (bevacizumab)	hu IgG1	VEGF	colorectal cancer lung cancer first FDA approval 2005

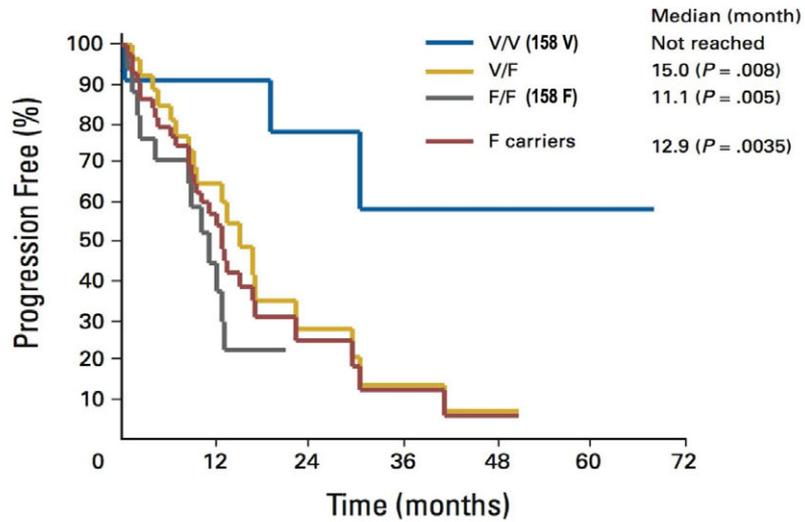
* in combination with chemotherapy.



Antibody-dependent effector mechanisms

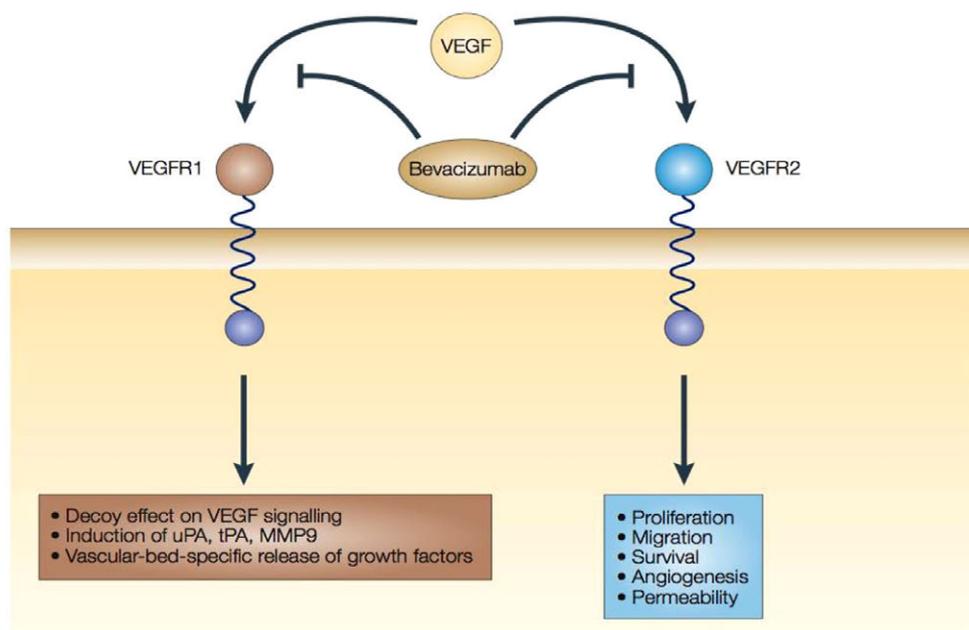
FcγRIII polymorphism and clinical responses to trastuzumab

Progression-free survival of breast cancer patients after trastuzumab treatment

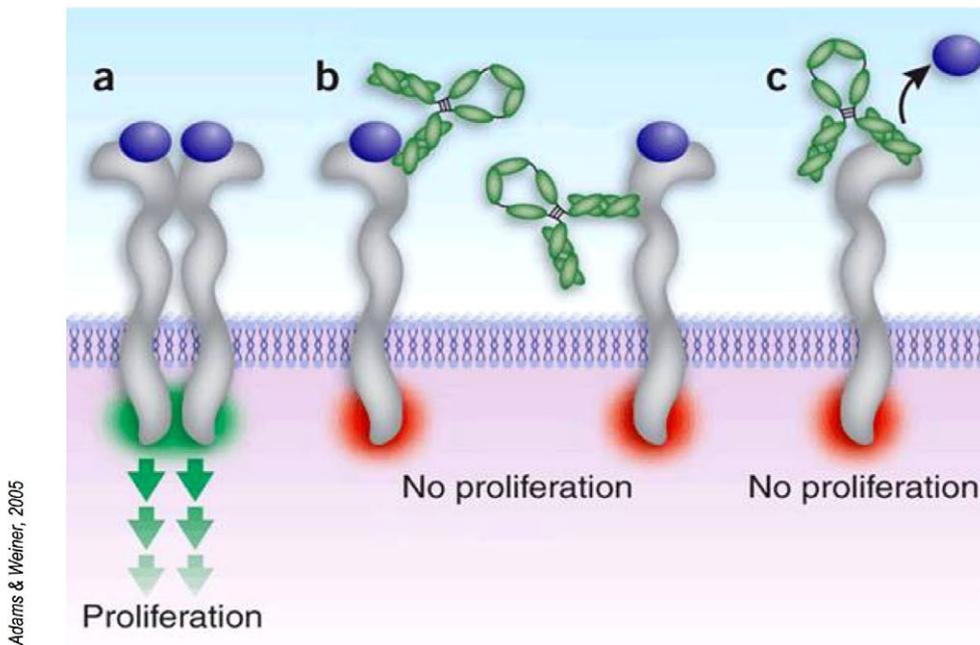


Musolino et al., 2008

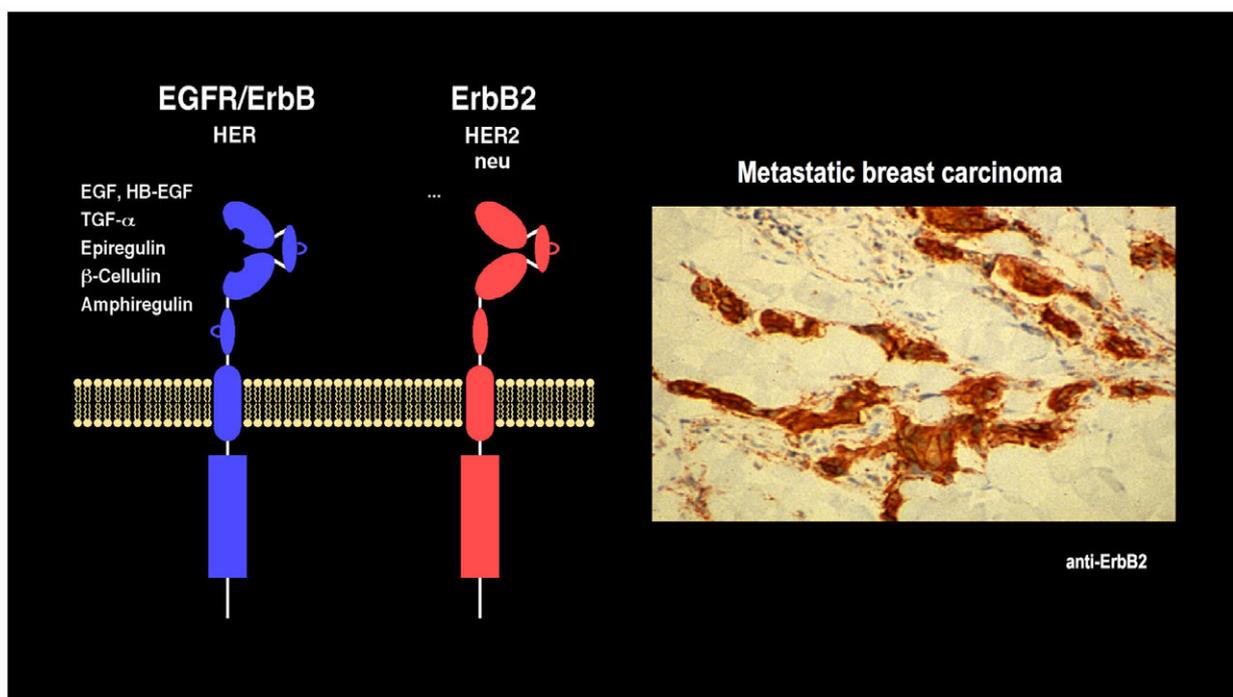
Blockade of VEGF by antibody binding



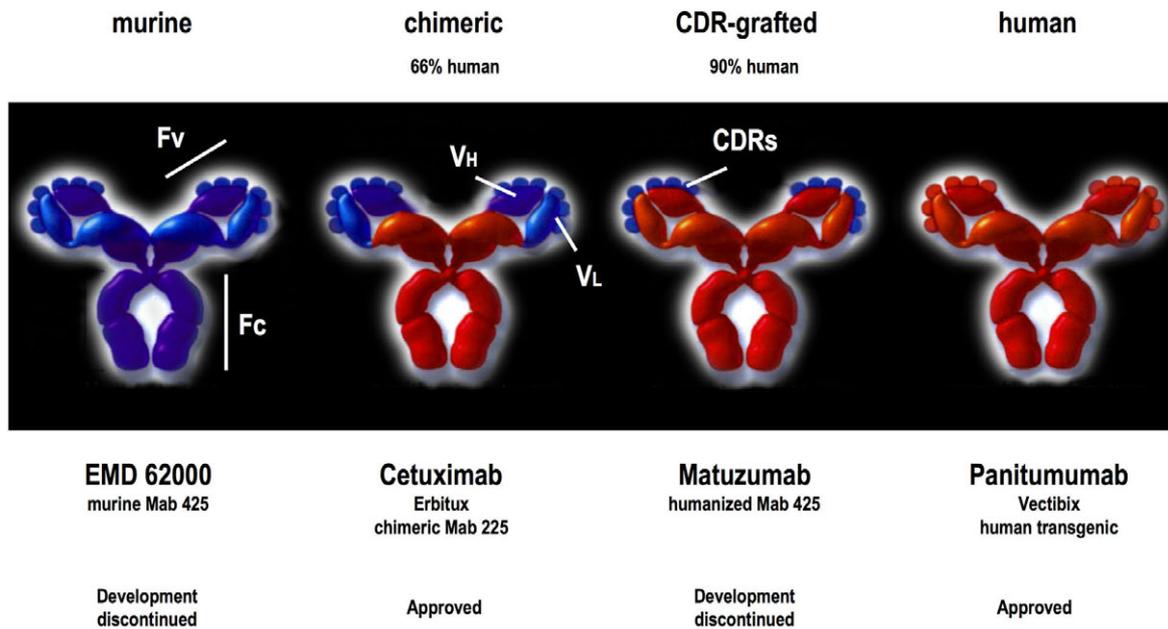
Antibody-mediated signaling inhibition



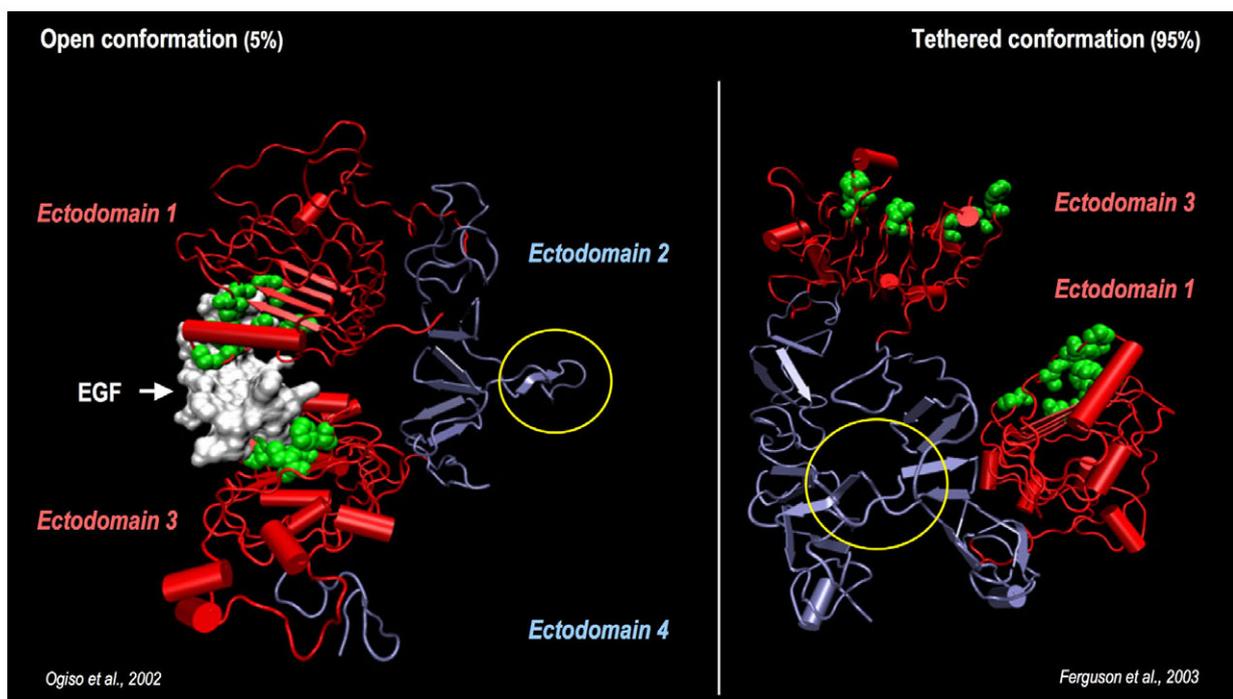
The ErbB family of receptor tyrosine kinases



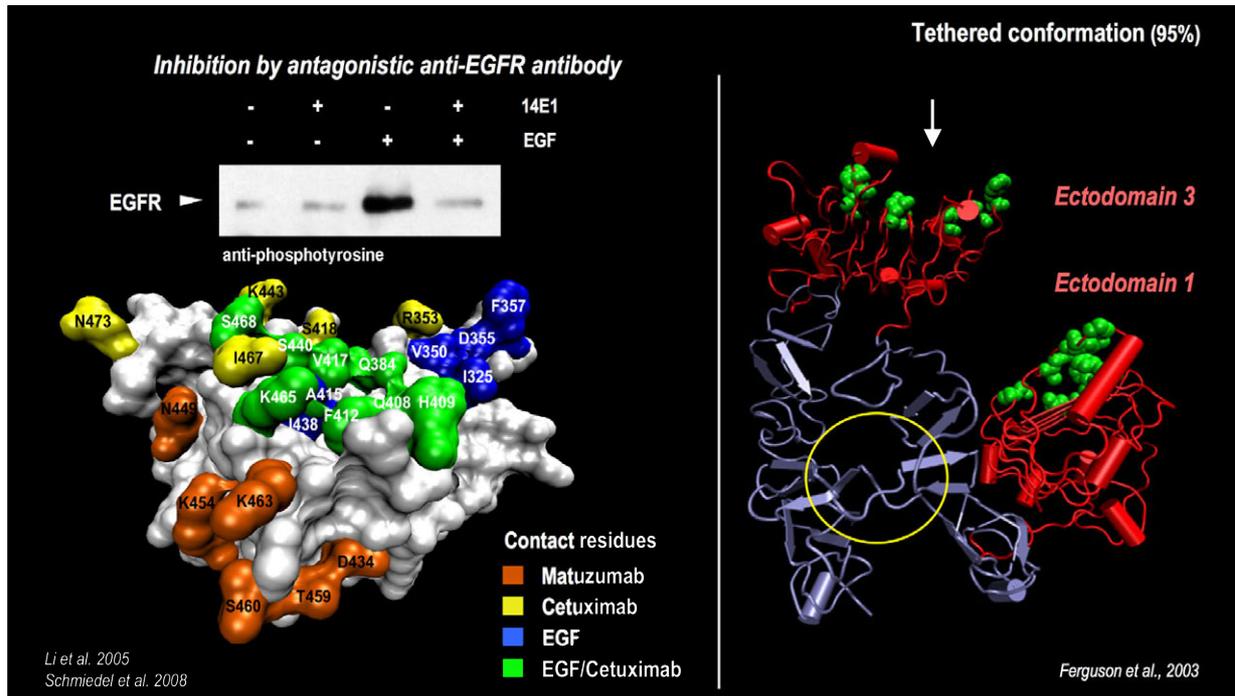
Anti-EGFR antibodies in the clinic



Structure of EGFR extracellular domain and inhibition of ligand binding by antibodies



Structure of EGFR extracellular domain and inhibition of ligand binding by antibodies

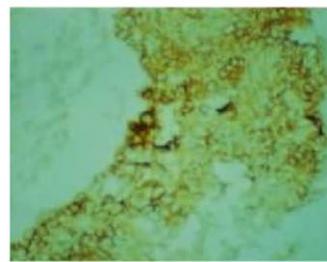


Treatment of cancer patients with EGFR-specific antibody Erbitux (cetuximab) in combination with cisplatin

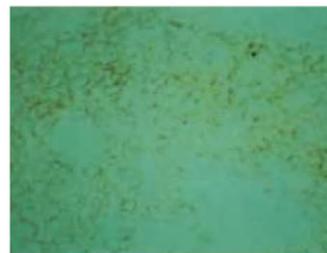
before therapy



EGFR expression

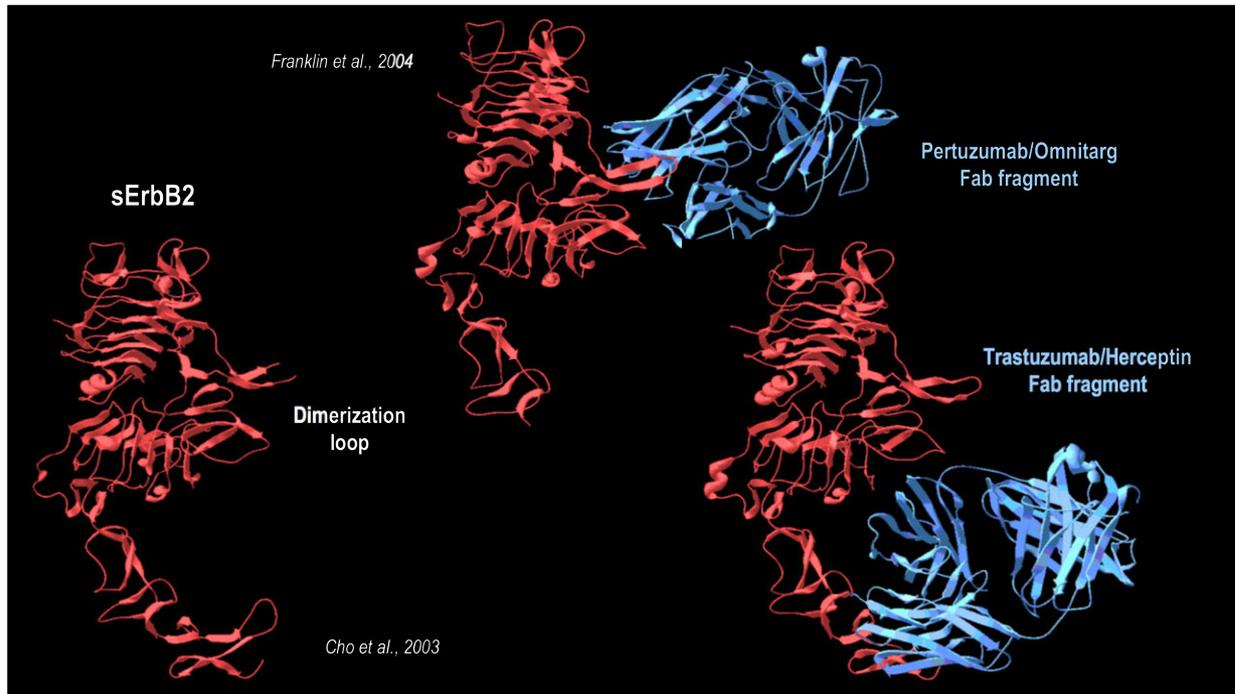


after therapy



Mendelsohn, 2002

Binding sites of ErbB2-specific antibodies



Large-scale production of monoclonal antibodies

Critical issues:

- Product safety
- Product heterogeneity
- Glycosylation
- Manufacturing capacity
(for licensed product up to hundreds of kilograms needed)
- Costs

Production systems:

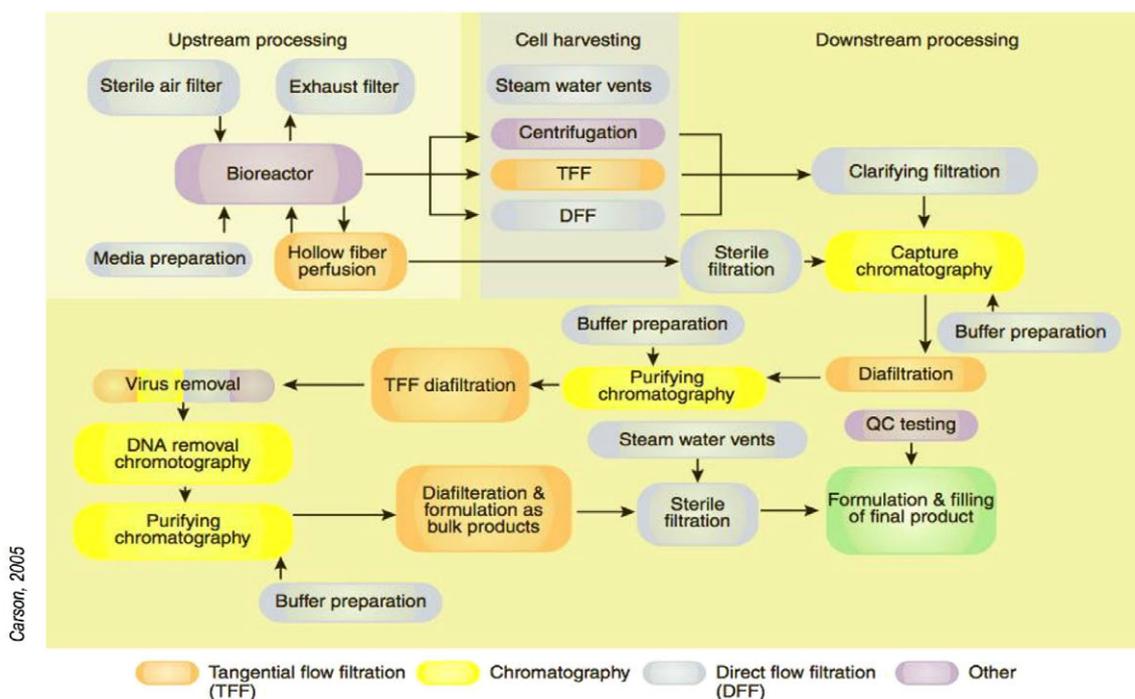
- Hybridoma cells
- Transfected cell lines (chinese hamster ovary cells, CHO)
- Transgenic plants
- Transgenic animals (secreted antibodies in milk; chicken eggs)
- Bacteria
- Yeast

Large-scale production of monoclonal antibodies

Minimally required chromatography purification steps:

- **Capture chromatography**
(Protein A affinity chromatography)
- **Removal of host cell proteins, aggregates and leached protein A**
(hydrophobic interaction; hydroxyapatite; cation exchange chromatography)
- **Removal of endotoxins, DNA, retroviruses**
(anion exchange chromatography)

Large-scale production of monoclonal antibodies



Potential toxicities of monoclonal antibodies

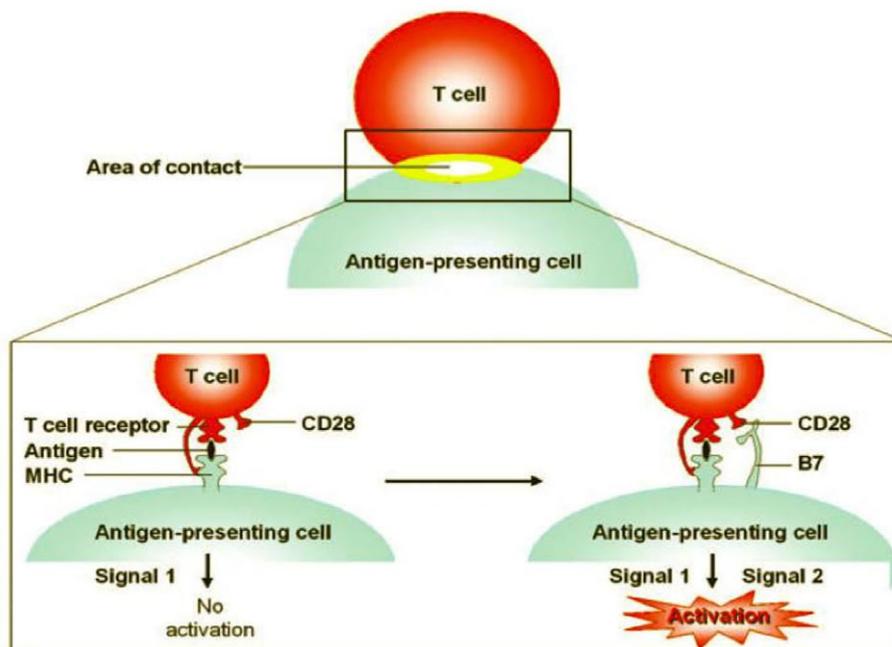
General problems:

- **Anti-antibody immune responses**
(rare for chimeric and humanized antibodies)
- **First injection hypersensitivity reactions**
(frequent; usually minor)

Examples for specific side effects:

- **Acne-like skin reactions**
(Erbix, anti-EGFR)
- **Cardiac toxicity**
(rare; Herceptin, anti-HER2/ErbB2)
- **Reactivation of dormant JC polyoma virus**
(rare; Tysabri, anti- $\alpha 4\beta 1$ integrin)
- **Cytokine storm after T-cell activation**
(TGN1412, anti-CD28)

The TGN1412 disaster



Treatment of melanoma patients with anti-CTLA-4 antibody

Clinical Responses and Immune-Related Adverse Events (irAEs) in Trials of Ipilimumab and Tremelimumab Melanoma

Regimen	% CR (No.)	% PR (No.)	% SD (No.)	Most common grade 3/4 or serious irAEs
Tremelimumab, first/ pretreated/adjuvant	5.9 (2 of 34)	5.9 (2 of 34)	11.8 (4 of 34)	Dermatitis*, diarrhea*
Tremelimumab, pretreated	3.3 (3 of 90)	4.4 (4 of 90)	28.9 (26 of 90)	Diarrhea
Ipilimumab, first-line	0	5.4 (2 of 37)	10.8 (4 of 37)	N/A
Ipilimumab+DTIC, first-line	5.7 (2 of 35)	11.4 (4 of 35)	11.4 (4 of 35)	
Ipilimumab+vaccine, pretreated	3.6 (2 of 56)	8.9 (5 of 56)	N/A	Colitis, dermatitis
Ipilimumab+vaccine, pretreated	14.3 (2 of 14)	7.1 (1 of 14)	0	Dermatitis, colitis/enterocolitis
Ipilimumab+IL-2	8.3 (3 of 36)	13.9 (5 of 36)	N/A	Enterocolitis

CR indicates complete response; PR, partial response; SD, stable disease; IL-2, interleukin-2; DTIC, dacarbazine; N/A, neither available nor reported.

* Dose-limiting toxicity.

O'Day et al., 2007

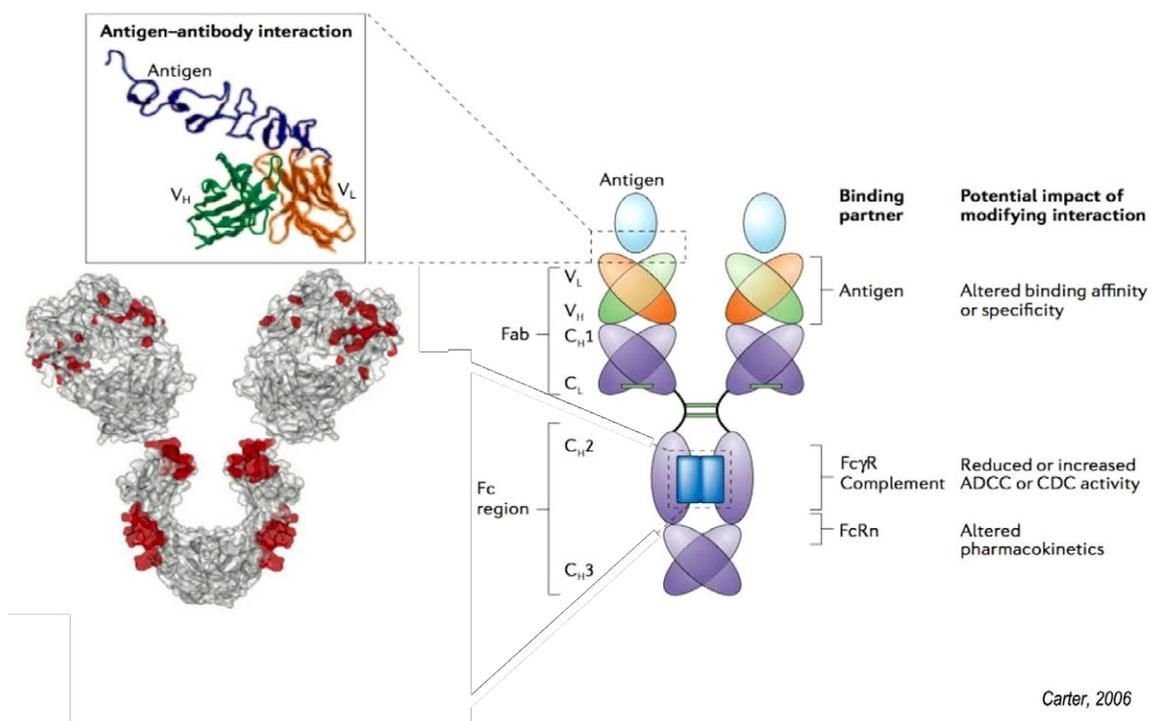
Monoclonal antibodies: Achievements and challenges ahead

- **Now an established and rapidly growing drug class**
- **Basic problems solved; well-tolerated human molecules**
- **High success rate**
(22-25% from first use in humans to approval; ~ 11% for small-molecule drugs)
- **Seldom, if ever, curative**
- **Costs** (dose; manufacture; intellectual property)
- **Contribution of effector functions**
- **Number of validated targets**
- **Interference with intracellular targets**
- **Alternative antibody formats** (antibody fragments; isotypes; conjugates)

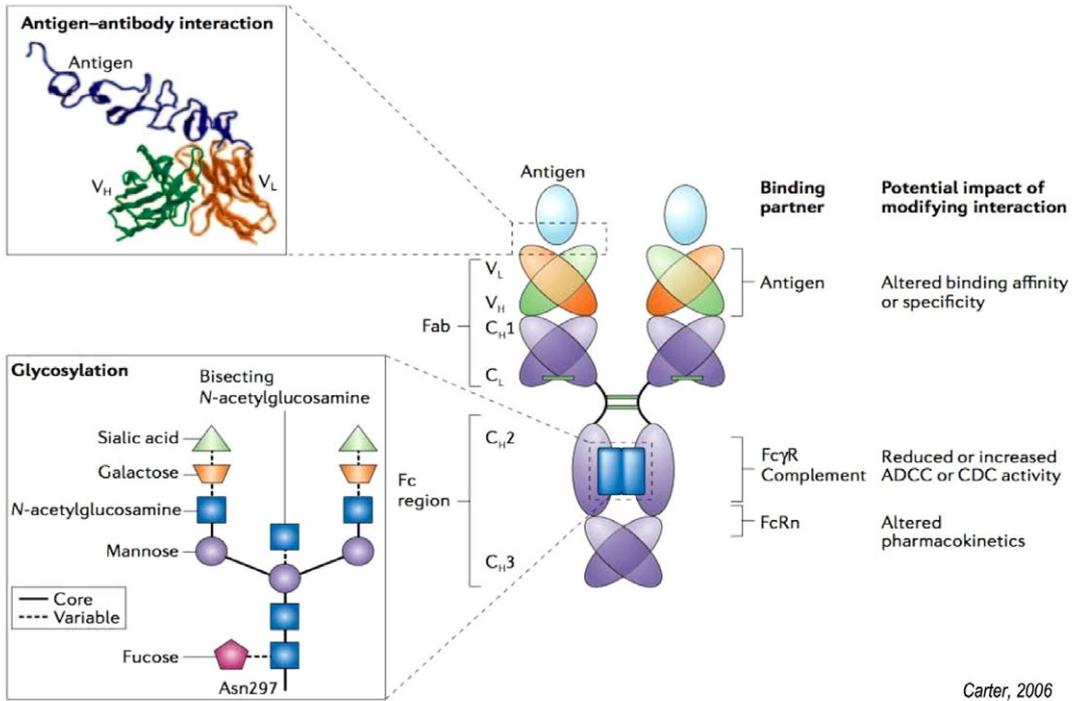
Antibodies as therapeutic drugs

Additional slides

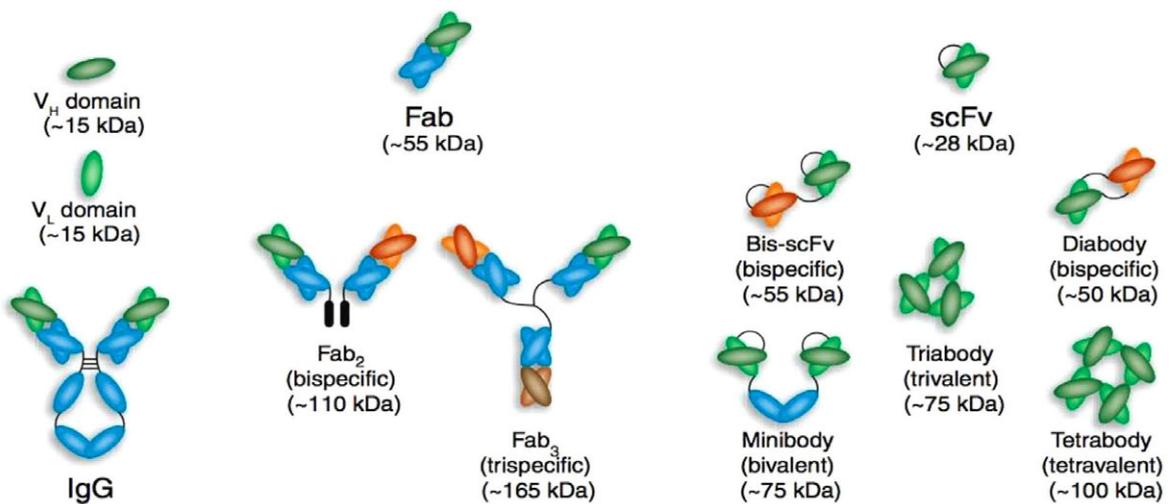
Manipulating antibody activities



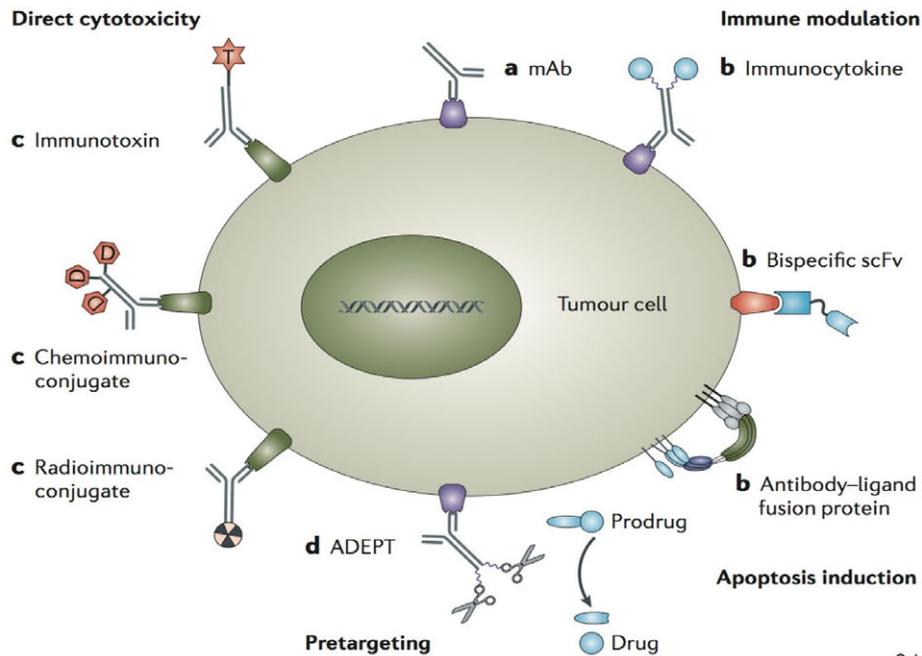
Manipulating antibody activities



Recombinant antibody fragments

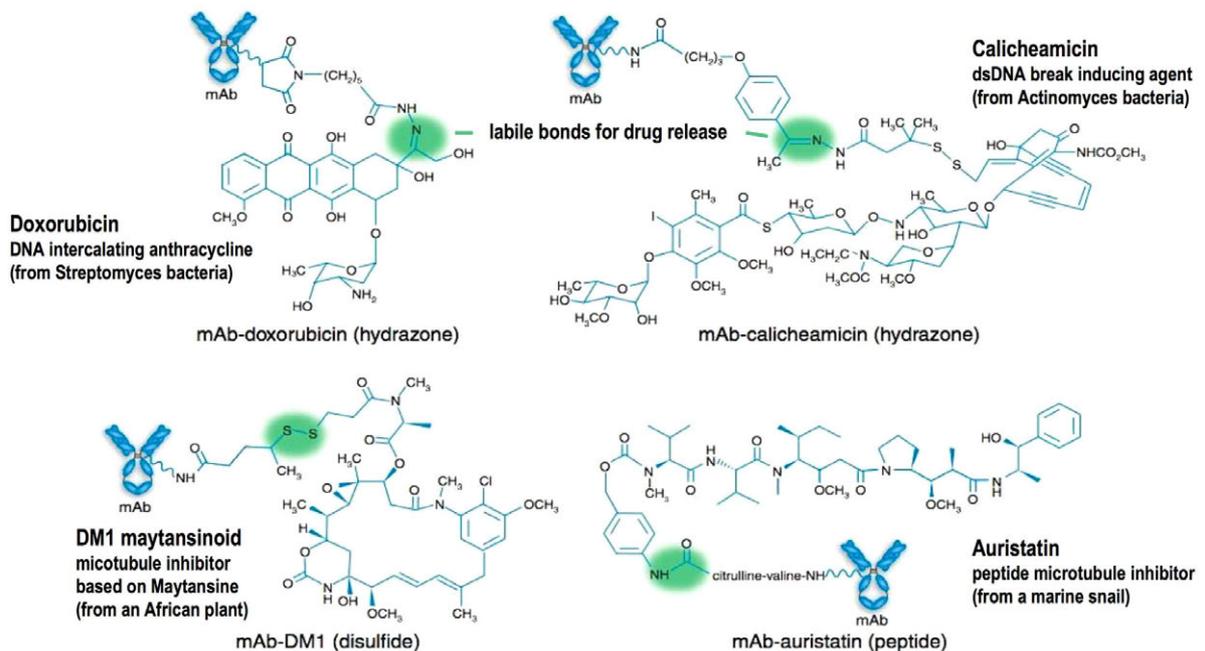


Arming antibodies for enhanced antitumoral activity



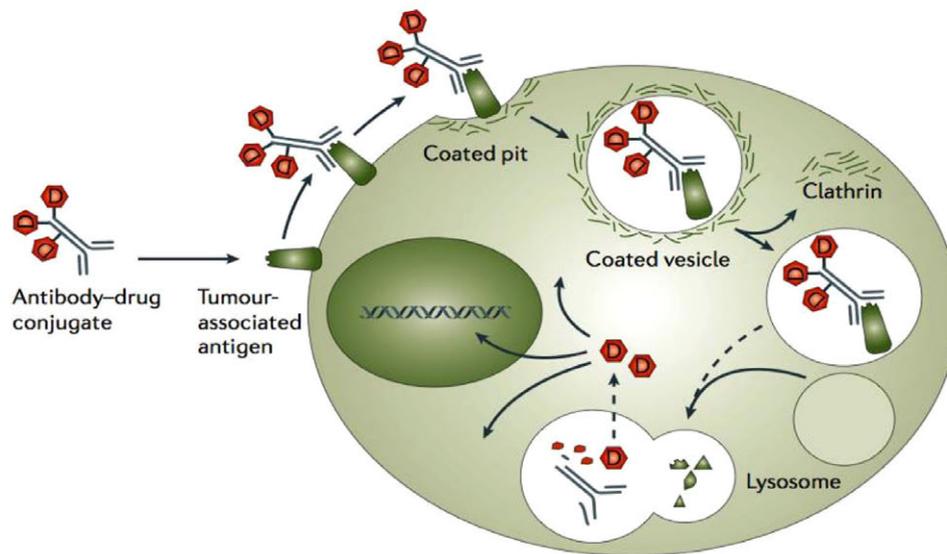
Schrama et al., 2006

Chemical structures of antibody-drug conjugates



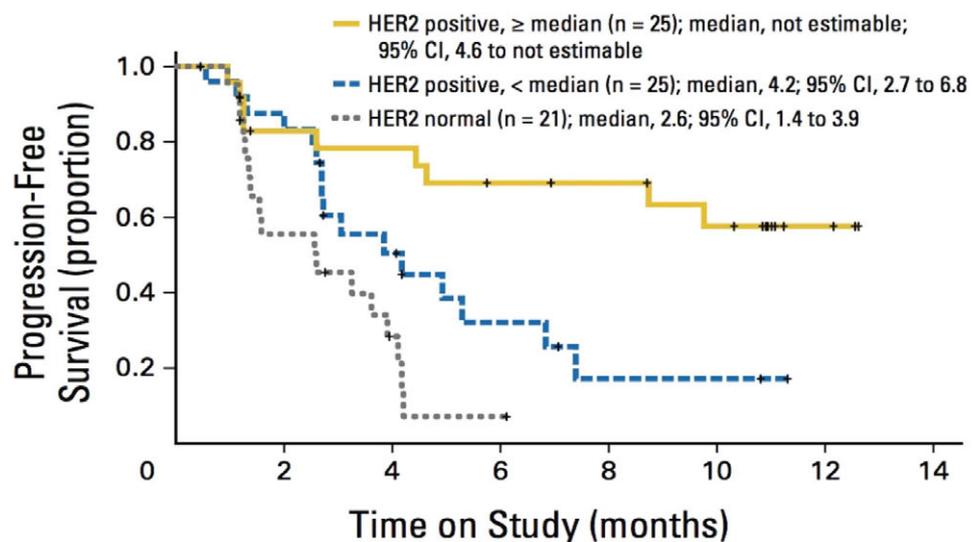
Wu & Senter, 2005

Internalization of antibody-drug conjugates



Schrama et al., 2006

Phase II study of the antibody drug conjugate trastuzumab-DM1 for the treatment of HER2/ErbB2-positive breast cancer



Burris et al., 2011

Approved Therapeutic Antibodies (March 2011)

International non-proprietary name	Trade name	Type	Indication first approved	First EU (US) approval year
Muromonab-CD3	Orthoclone Okt3	Anti-CD3; Murine IgG2a	Reversal of kidney transplant rejection	1986* (1986#)
Abciximab	Reopro	Anti-GPIIb/IIIa; Chimeric IgG1 Fab	Prevention of blood clots in angioplasty	1995* (1994)
Rituximab	MabThera, Rituxan	Anti-CD20; Chimeric IgG1	Non-Hodgkin's lymphoma	1998 (1997)
Basiliximab	Simulect	Anti-IL2R; Chimeric IgG1	Prevention of kidney transplant rejection	1998 (1998)
Daclizumab	Zenapax	Anti-IL2R; Humanized IgG1	Prevention of kidney transplant rejection	1999 (1997); #
Palivizumab	Synagis	Anti-RSV; Humanized IgG1	Prevention of respiratory syncytial virus infection	1999 (1998)
Infliximab	Remicade	Anti-TNF α ; Chimeric IgG1	Crohn disease	1999 (1998)
Trastuzumab	Herceptin	Anti-HER2; Humanized IgG1	Breast cancer	2000 (1998)
Gemtuzumab ozogamicin	Mylotarg	Anti-CD33; Humanized IgG4	Acute myeloid leukemia	NA (2000#)
Alemtuzumab	MabCampath, Campath-1H	Anti-CD52; Humanized IgG1	Chronic myeloid leukemia	NA (2003)
Adalimumab	Humira	Anti-TNF; Human IgG1	Rheumatoid arthritis	2003 (2002)
Tositumomab-I131	Bexxar	Anti-CD20; Murine IgG2a	Non-Hodgkin lymphoma	NA (2003)
Efalizumab	Raptiva	Anti-CD11a; Humanized IgG1	Psoriasis	2004 (2003); #
Cetuximab	Erbix	Anti-EGFR; Chimeric IgG1	Colorectal cancer	2004 (2004)
Ibritumomab tiuxetan	Zevalin	Anti-CD20; Murine IgG1	Non-Hodgkin's lymphoma	2004 (2002)
Omalizumab	Xolair	Anti-IgE; Humanized IgG1	Asthma	2005 (2003)
Bevacizumab	Avastin	Anti-VEGF; Humanized IgG1	Colorectal cancer	2005 (2004)
Natalizumab	Tysabri	Anti- α 4 integrin; Humanized IgG4	Multiple sclerosis	2006 (2004)
Ranibizumab	Lucentis	Anti-VEGF; Humanized IgG1 Fab	Macular degeneration	2007 (2006)
Panitumumab	Vectibix	Anti-EGFR; Human IgG2	Colorectal cancer	2007 (2006)
Eculizumab	Soliris	Anti-C5; Humanized IgG2/4	Paroxysmal nocturnal hemoglobinuria	2007 (2007)
Certolizumab pegol	Cimzia	Anti-TNF; Humanized Fab, pegylated	Crohn disease	2009 (2008)
Golimumab	Simponi	Anti-TNF; Human IgG1	Rheumatoid and psoriatic arthritis, ankylosing spondylitis	2009 (2009)
Canakinumab	Ilaris	Anti-IL1b; Human IgG1	Muckle-Wells syndrome	2009 (2009)
Catumaxomab	Removab	Anti-EPCAM/CD3; Rat/mouse bispecific mAb	Malignant ascites	2009 (NA)
Ustekinumab	Stelara	Anti-IL12/23; Human IgG1	Psoriasis	2009 (2009)
Tocilizumab	RoActemra, Actemra	Anti-IL6R; Humanized IgG1	Rheumatoid arthritis	2009 (2010)
Ofatumumab	Arzerra	Anti-CD20; Human IgG1	Chronic lymphocytic leukemia	2010 (2009)
Denosumab	Prolia	Anti-RANK-L; Human IgG2	Bone Loss	2010 (2010)