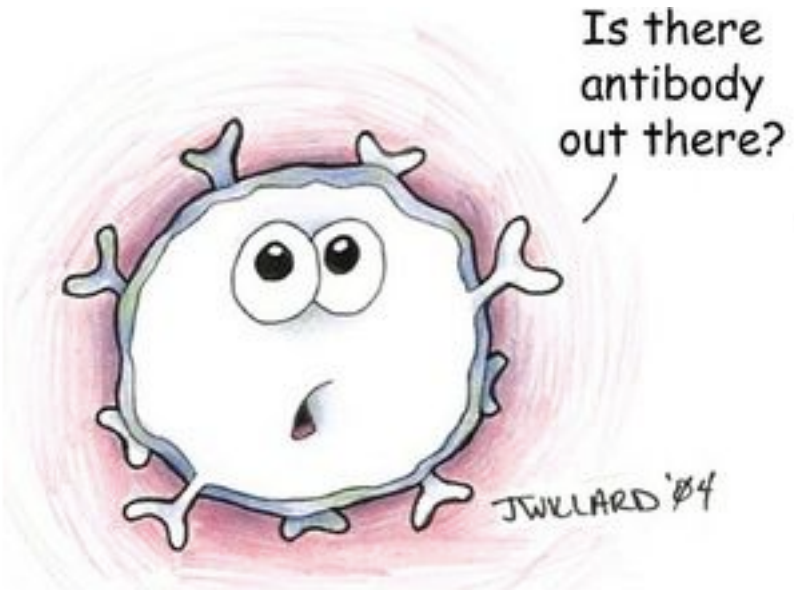


Monoclonal and engineered antibodies in cancer therapy

Dr. Claudia Duranti, PhD
Department of Experimental and Clinical Medicine
University of Florence, Italy

"Basic and translational oncology"
Italian-French Erasmus Intensive Course in Oncology organized in collaboration with
European Master of Genetics - University Paris7-Paris5

Talk outline:

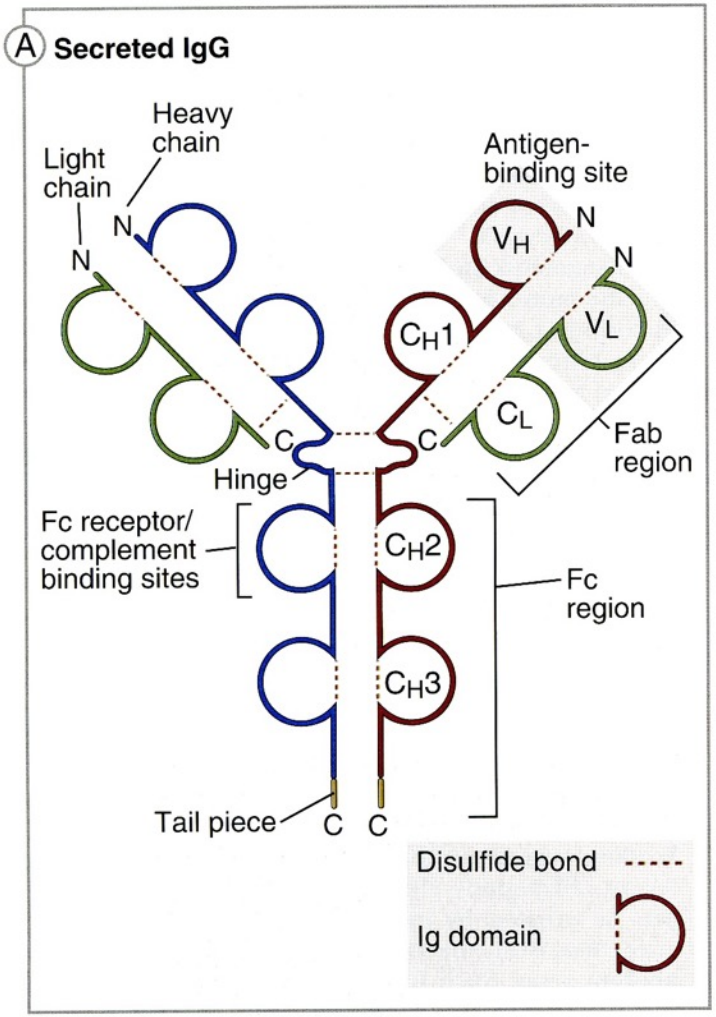
1. Experimental steps towards the production of monoclonal antibodies
2. Monoclonal antibodies in cancer therapy
3. What is next?…….Antibody engineering

Characteristics of Monoclonal Antibodies

Monoclonal antibodies are

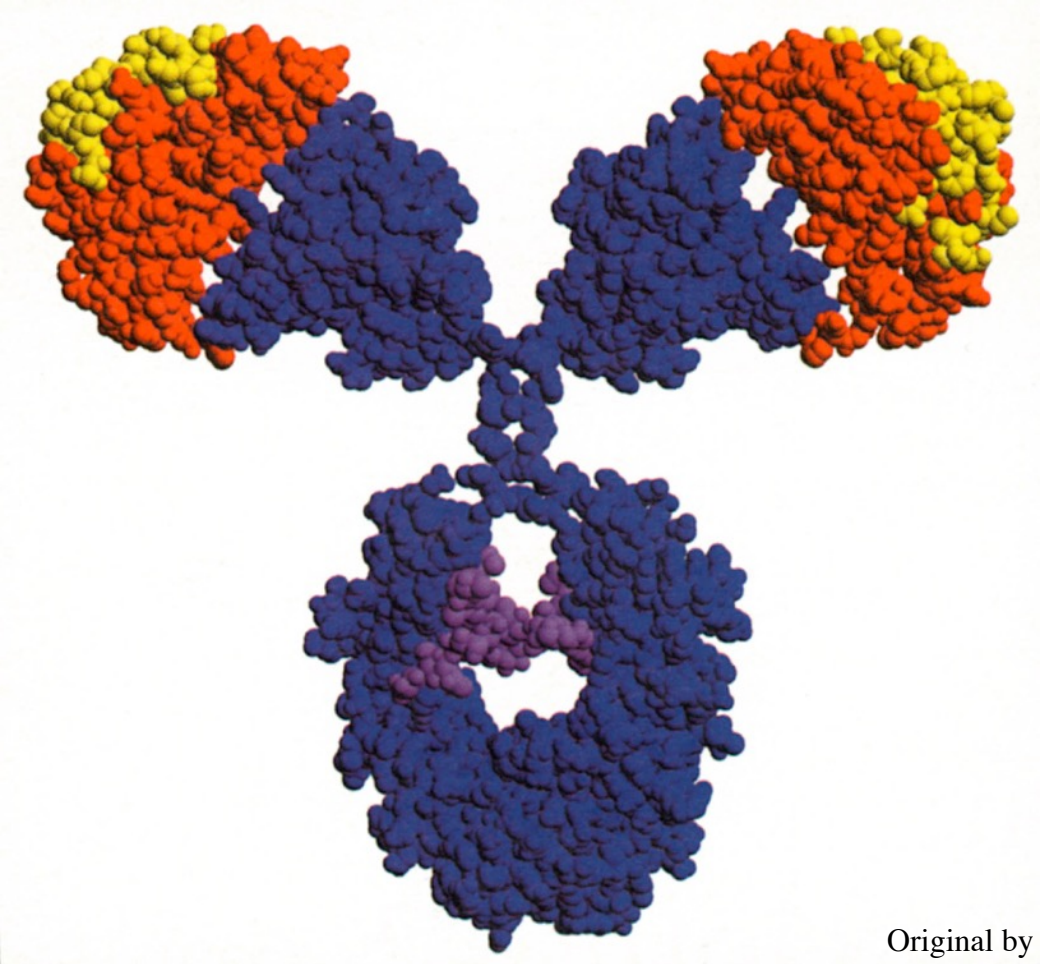
- monospecific
  - => recognize only one epitope (antigenic determinant)
- homogenous
  - => identical immunoglobulin molecules
  - => display identical binding strengths (affinity)
- produced in unlimited quantities
- usually derived from mouse

Model of Secreted IgG

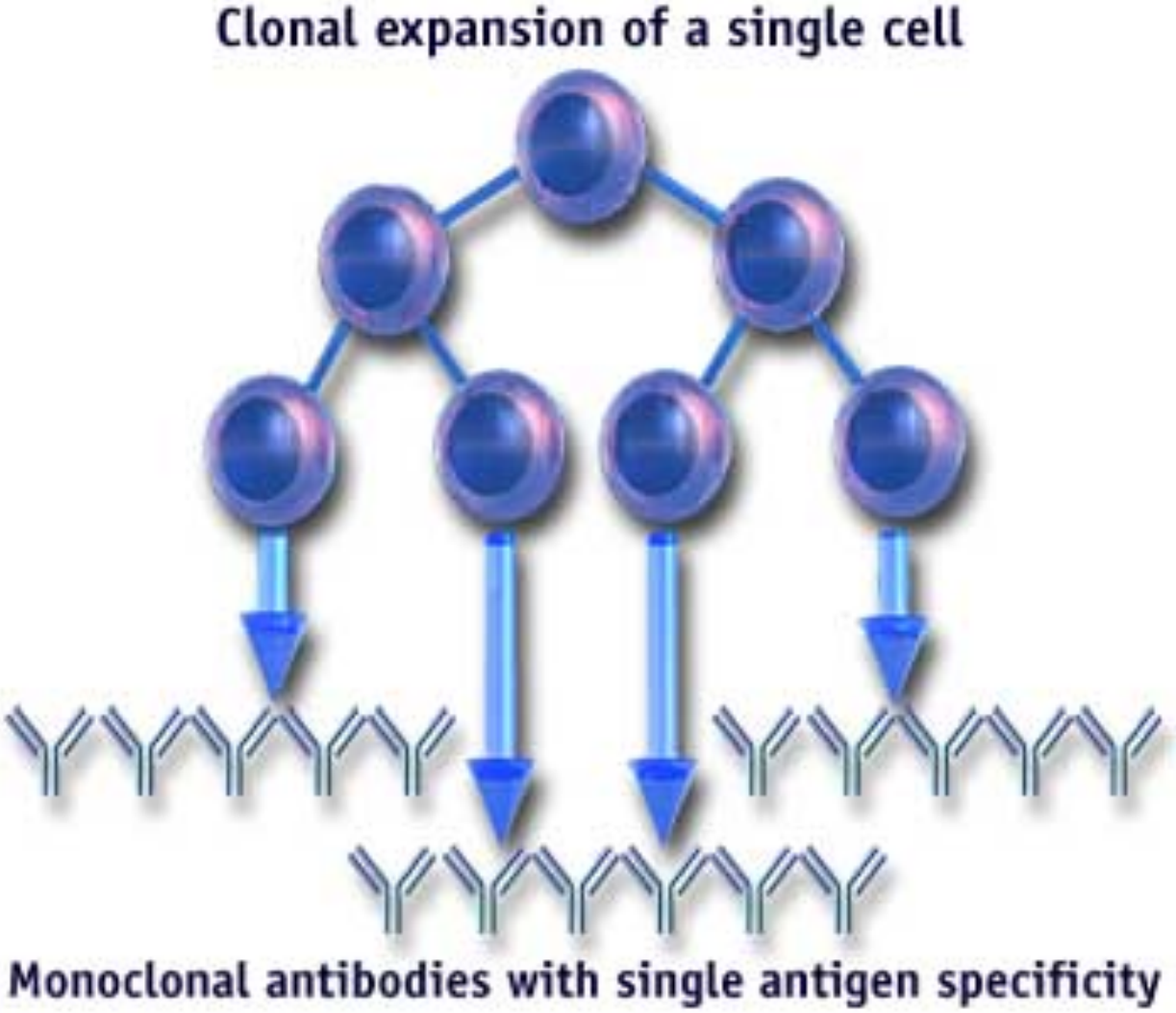


Abbas, A. K., Lichtman, A. H., & Pillai, S. Cellular and molecular immunology. Philadelphia: Saunders/Elsevier

Structure of an IgG Antibody



Original by Dr. Mike Clark

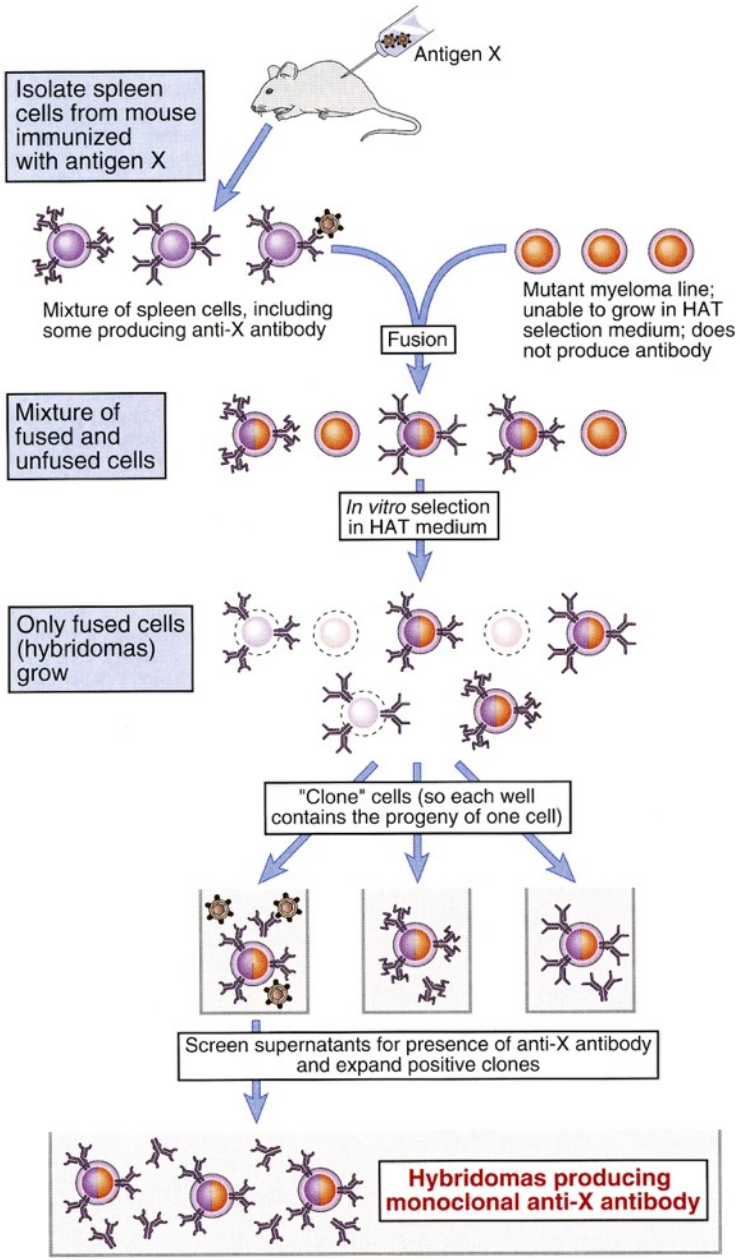

Clonal expansion of a single cell
Monoclonal antibodies with single antigen specificity

Monoclonal antibodies are proteins that respond to a single antigenic determinant and are highly specific.

The development of a technology for Ab production has had an extraordinary impact both in the research field and in the clinic.

For the first time, in 1975, Kohler and Milstein produced a cell line by melting a normal B lymphocyte, capable of responding to a single antigenic epitope with a tumor B cell capable of growing indefinitely in vitro.

Nobel prize **awarded in 1984 for the development of the HYBRIDOMA TECHNOLOGY**



Generation of Monoclonal Antibodies

G. Köhler and C. Milstein
1975

Protocol for monoclonal antibody (mAb) production

How to indefinitely amplify a B lymphocyte specific for a given antigen and select a single Ab producing clone?

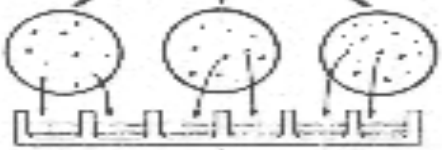
IMMUNIZED MICE

SPLEEN CELLS + **MYELOMA CELLS**

FUSION

HYBRID SELECTION

POSITIVE WELLS

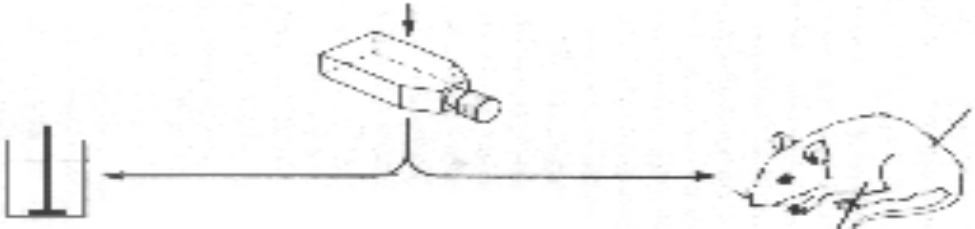


CELL CLONING BY PICKING

POSITIVE WELLS

(SOFT AGAR CLONING)

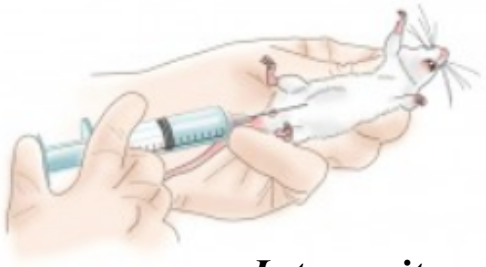
AMPLIFICATION



LARGE SCALE PRODUCTION

Immunization

- For production of mouse monoclonal antibodies, mice of a suitable strain are hyper-immunized with multiple doses of antigen either intraperitoneally or sub-cutaneously.

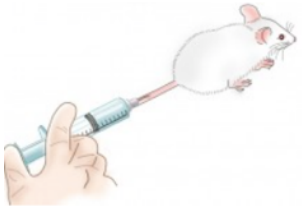


Intraperitoneal injection

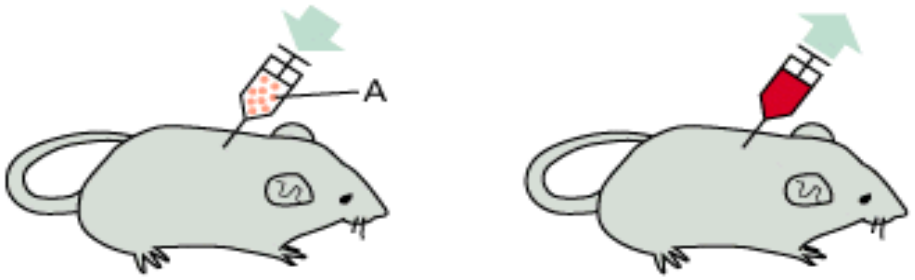
- Blood samples are taken before and a week after each immunization and the antibody titer is monitored.

Blood samples collection

- Three days before fusion mice are given a final intravenous boost in order to maximize the number of antigen-specific B cell blasts.



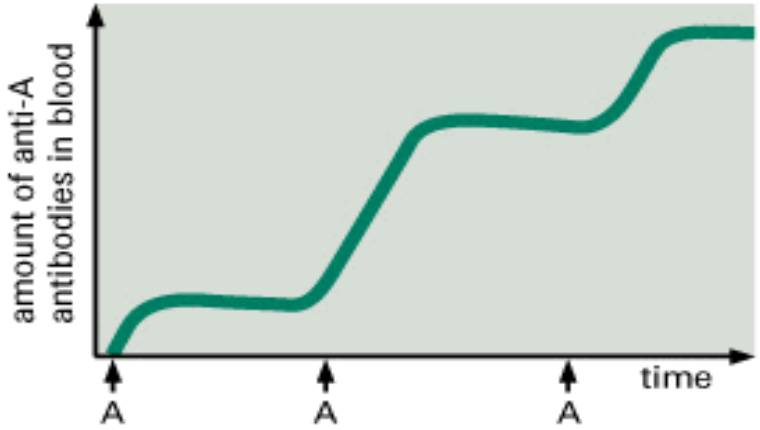
Intravenous injection



inject antigen A

take blood later

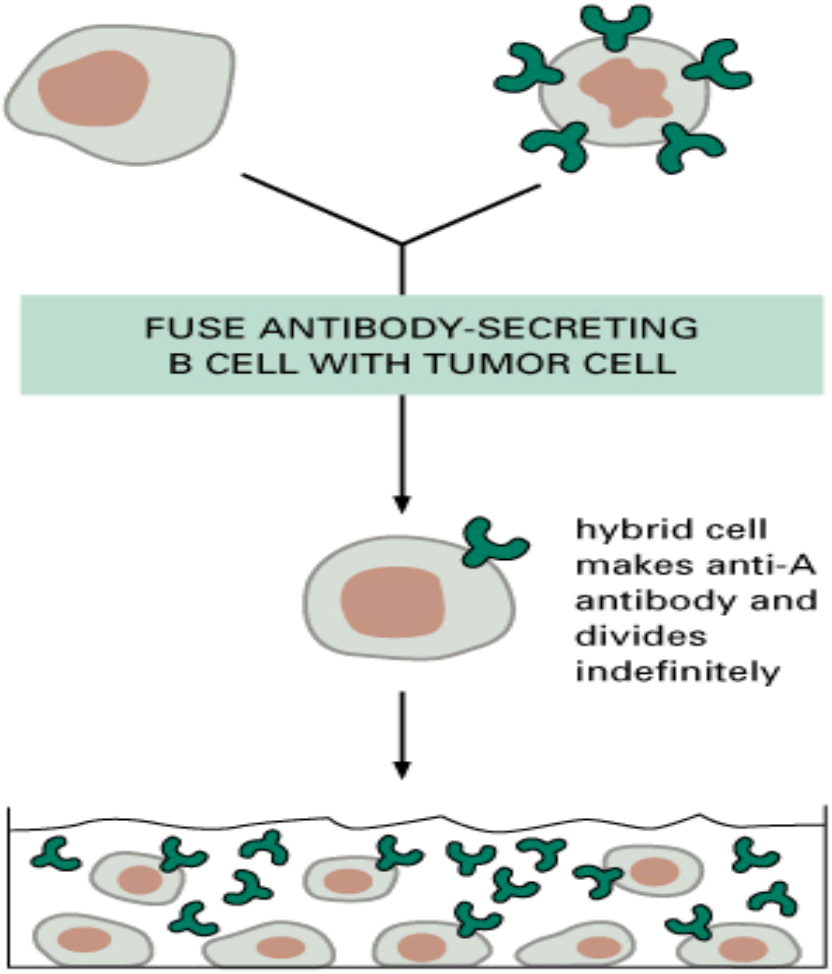
Repeated injections of the same antigen at intervals of several weeks stimulates specific B cells to secrete large amounts of anti-A antibodies into the bloodstream.



Because many different B cells are stimulated by antigen A, the blood will contain a variety of anti-A antibodies, each of which binds A in a slightly different way.

Cell fusion

Cell fusion
according to *Kohler and Milstein protocol (1975)*



CELL PROCESSING BEFORE FUSION

SPLEEN CELLS

On the day of fusion sacrifice the mouse by cervical dislocation. Open the abdominal cavity and harvest the spleen into a 25 ml plastic tube.

In a tissue culture hood transfer spleen and medium into a 90 mm Petri dish. Using sterile forceps and scissors, carefully remove any surrounding connective tissue.

With a 12 mm diameter Teflon homogeniser, gently squeeze the spleen fragments against the side of the tube in order to free the spleen cells.

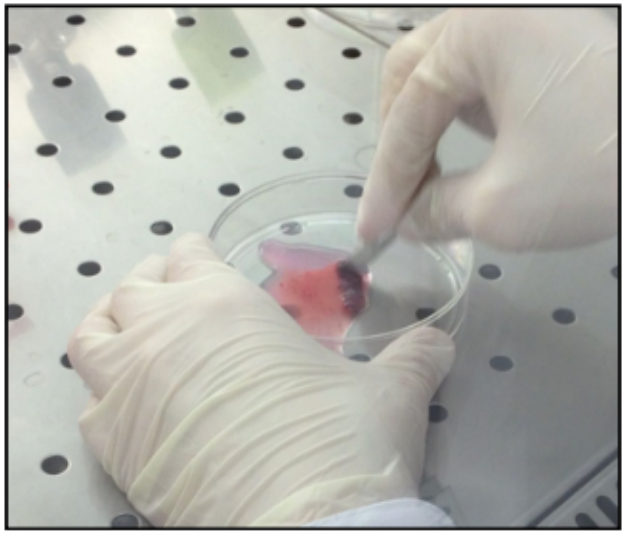
Add another 5 ml of 20% BSP in DMEM to the spleen fragments in the Teflon homogeniser and repeat the previous step.

Count the total number of spleen cells in a haemocytometre by making a 1/50 dilution in methyl violet stain. This lyses the red blood cells and stains the white cells blue-violet. Use 1 x 10^8 cells per fusion.

NS0 MYELOMA MURINE CELLS

- Maintain the mouse myeloma cells in log phase for at least 4-5 days before fusion in 10% BSP in DMEM (either in roller bottle or in 75 cm flasks at 37 C in a 5 % CO_2 incubator).
- Detach NS0 cells and count 100 *10^6

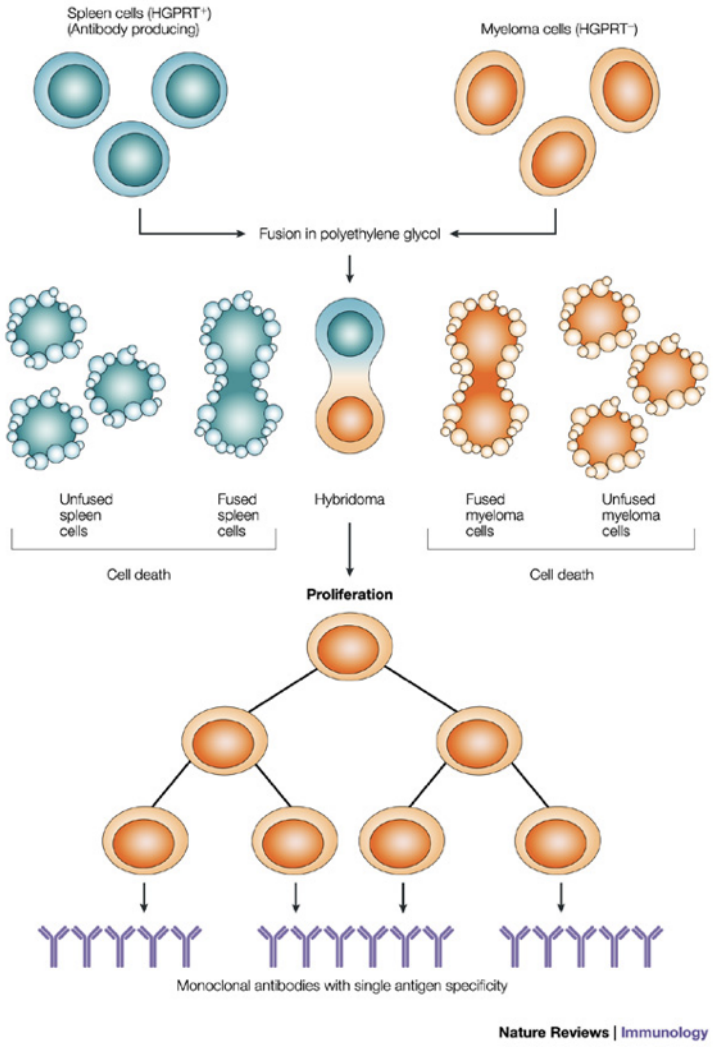
CELL FUSION



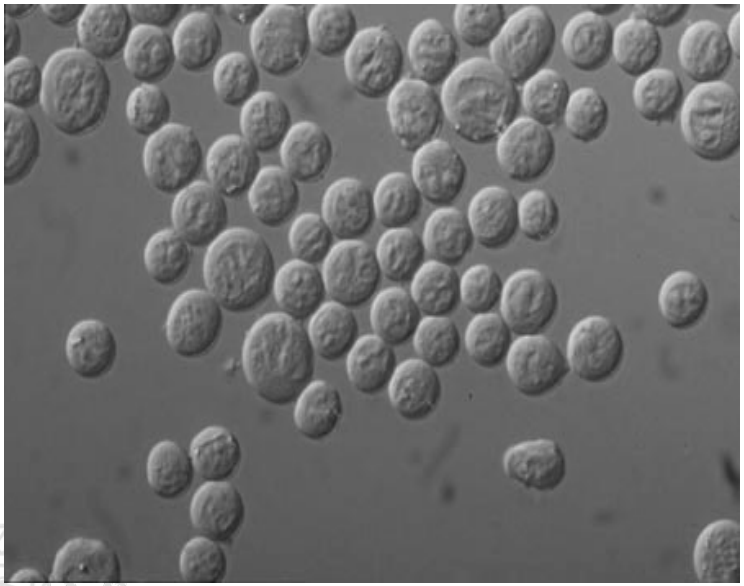
1. Add 1 ml of 37°C preheated PEG in 1 minutes, stirring;
2. Stir for 2 minutes;
3. Add 1 ml of 37°C preheated DMEM + 4 mM L-Gln in 1 minutes, stirring;
4. Repeat step 3;
5. Add 1 ml of 37°C preheated DMEM + 4 mM L-Gln in 30 sec, stirring;
6. Repeat step 5;
7. Add 6 ml of 37°C preheated DMEM + 4 mM L-Gln in 2 minutes, stirring;
8. Add 12 ml of 37°C preheated DMEM + 4 mM L-Gln drop to drop, stirring;
9. Centrifuge at 800 g for 5 minutes and discard supernatant.

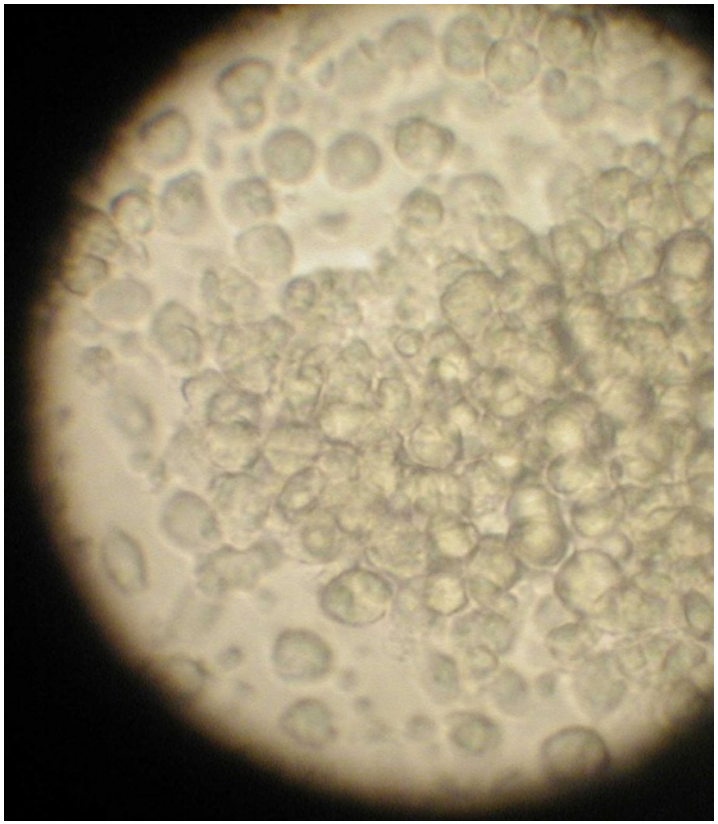
From cell fusion you will get:

- B lymphocytes not fused
- not fused myeloma NS0 cells
- Lymphocyte B-lymphocyte B
- myeloma NS0 cell-myeloma NS0 cell
- lymphocyte B-cell of myeloma NS0



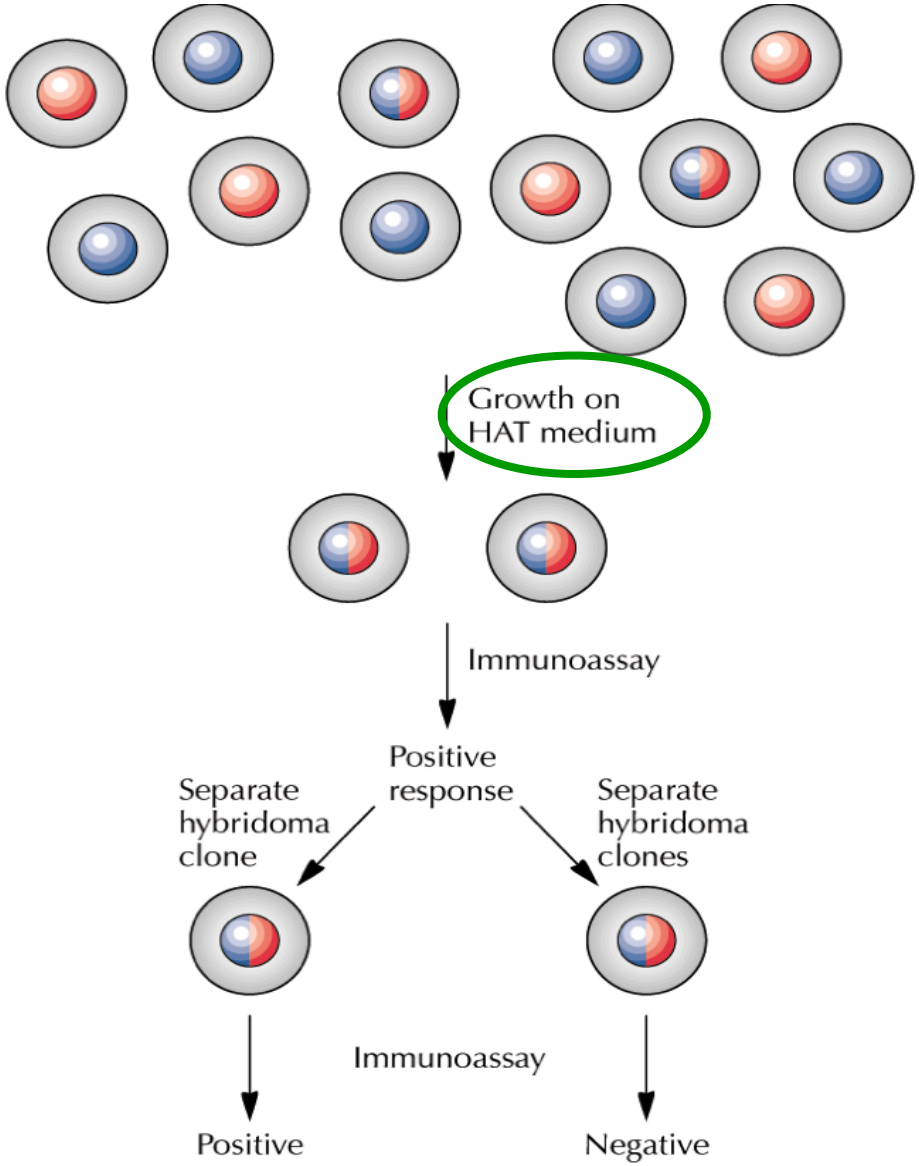
Hybridoma cells as they appear under microscopy examination





How to select hybridomas

(B lymphocyte-myeloma cells)?


Growth on
HAT medium
Immunoassay
Positive
response
Separate
hybridoma
clone
Separate
hybridoma
clones
Immunoassay
Positive
Negative

HAT (hypoxanthine-aminopterin-thymidine)

- B-cell/NS0 hybrid selection requires the use of a selective agent, added to the medium, which is HAT.
- HAT contributes to selection, exploiting the following mechanism: NS0 myeloma cells lack of the expression of ***Hypoxanthine-Guanine PhosphoRibosyl Transferase (HGPRT)*** enzyme.
- ***HGPRT*** is an enzyme that catalyses the conversion of hypoxanthine to inosine monophosphate and guanine to guanosine monophosphate, transferring the 5-phosphoribosyl group from 5-phosphoribosyl 1-pyrophosphate (PRPP) to the purine, thus playing a central role in the generation of purine nucleotides through the purine salvage pathway.
- Cells that do not express this enzyme can produce purine nucleotides only through the de novo pathway; but the latter is blocked by aminopterine.

- B lymphocytes not fused, hybrid Lymphocyte B-lymphocyte B have a short lifespan.

Only hybrids B lymphocytes- NS0 myeloma cells survive

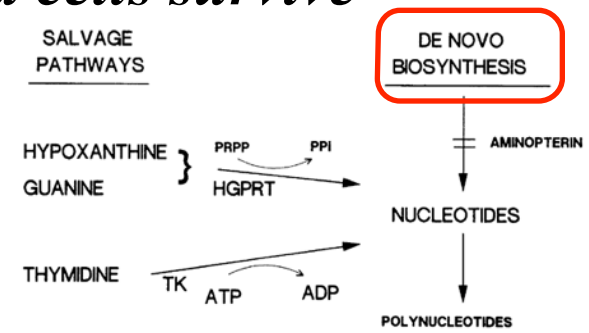


FIG. 21. Pathways of nucleotide biosynthesis showing enzymatic steps that are altered in mutant cells used as fusion partners. Mutant cells lacking HGPRT or TK cannot use the corresponding salvage pathway for nucleotide biosynthesis. Such mutants cannot survive in medium containing aminopterin to poison the de novo synthesis pathway. However, individual mutant cells that have fused with spleen cells and thus do contain the HGPRT or TK enzyme can survive in appropriate selective medium by using the salvage pathway. HGPRT, hypoxanthine guanine phosphoribosyl transferase; TK, thymidine kinase.

Media+HAT (Ipoxantina, Aminopterina, Timidina)

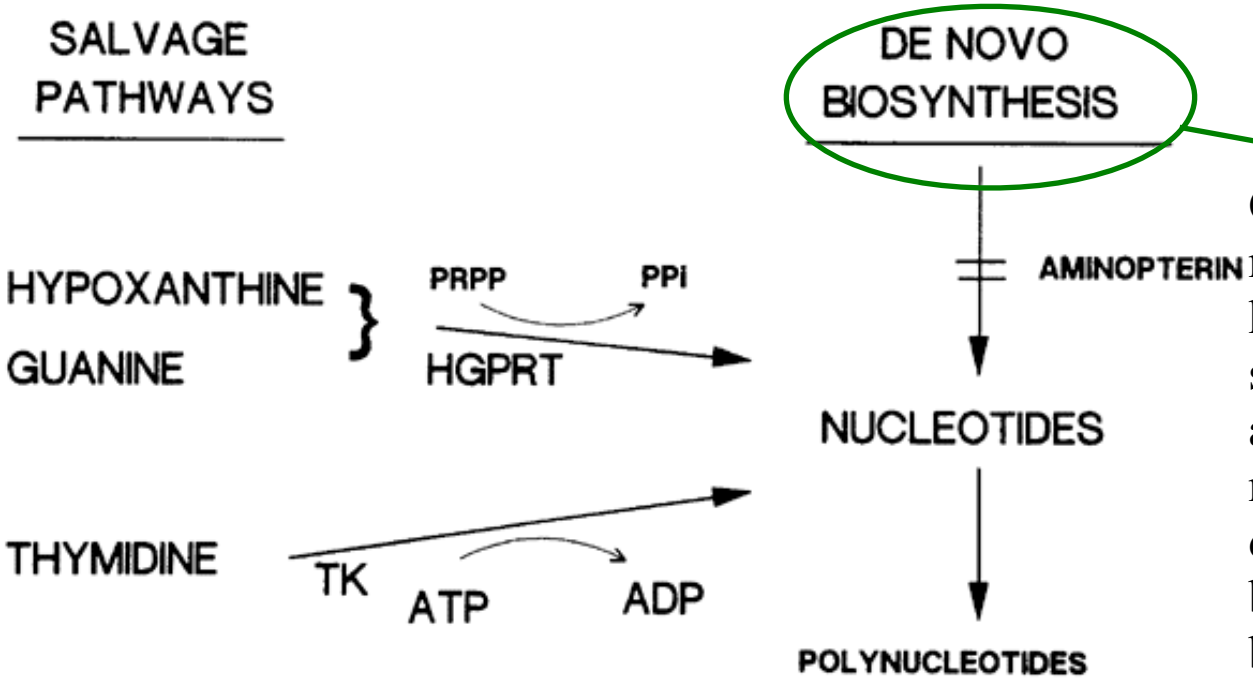
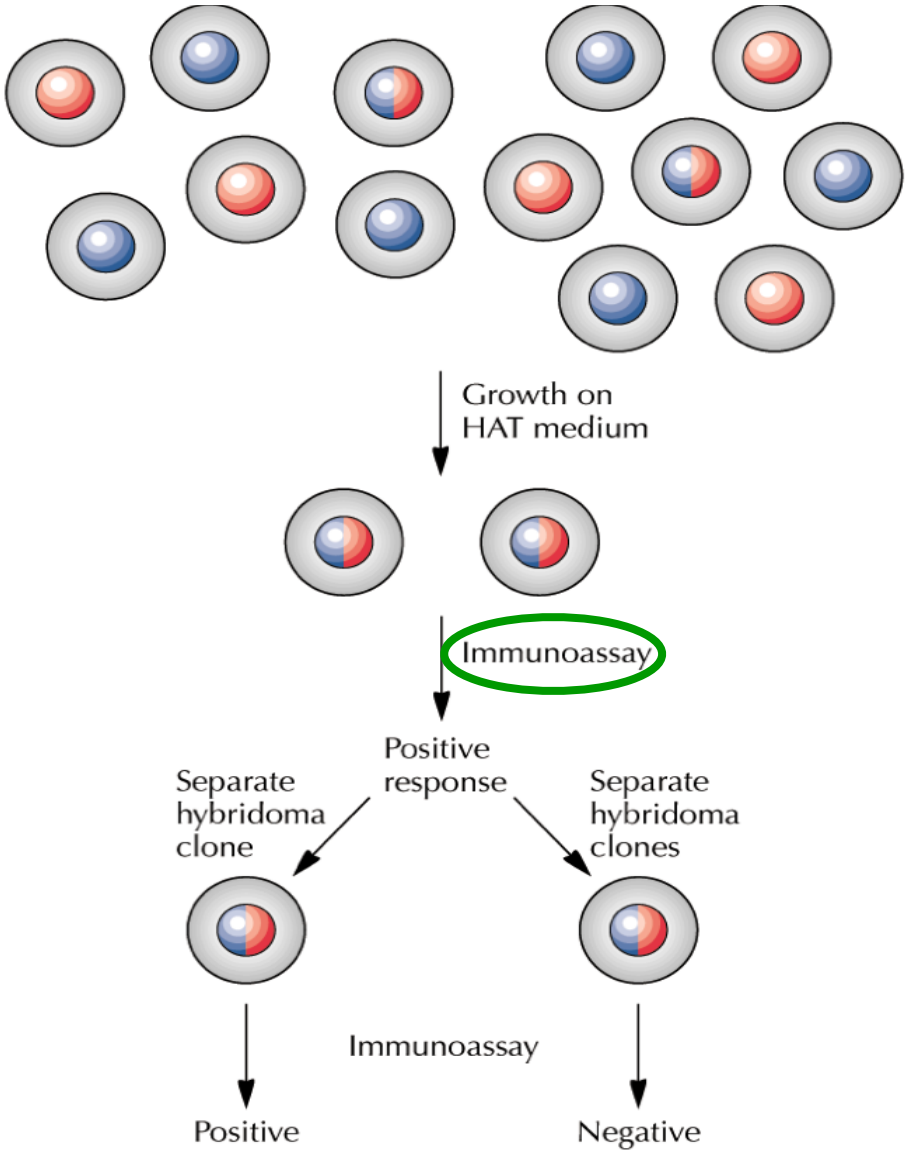
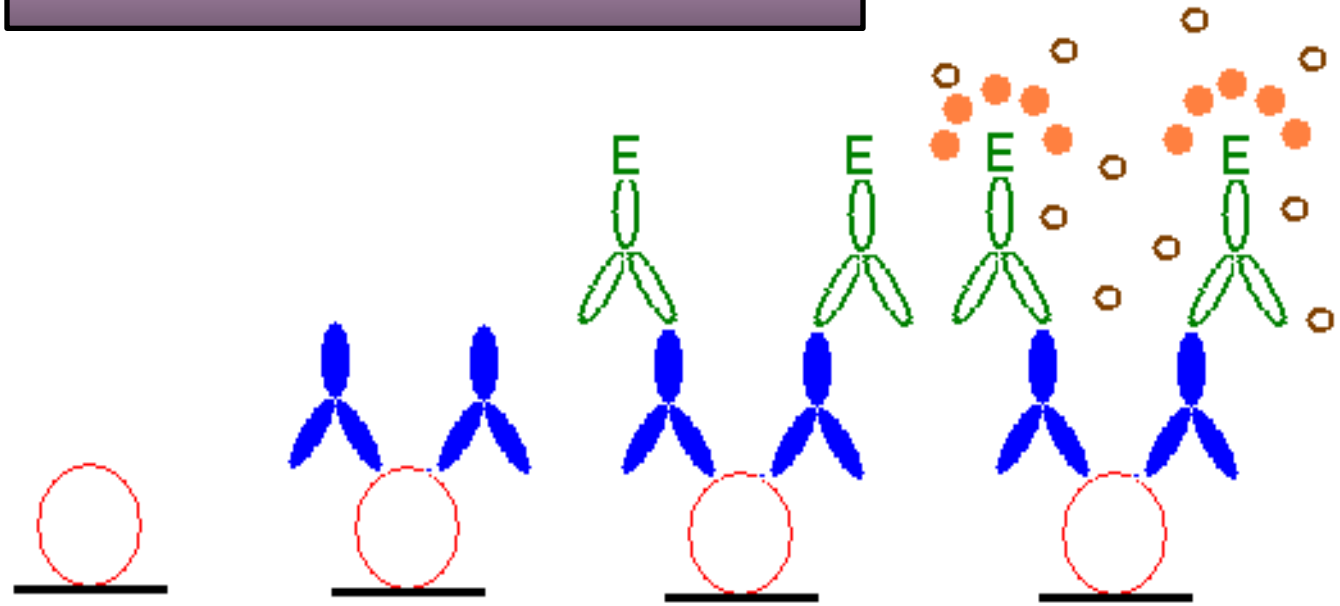


FIG. 21. Pathways of nucleotide biosynthesis showing enzymatic steps that are altered in mutant cells used as fusion partners. Mutant cells lacking HGPRT or TK cannot use the corresponding salvage pathway for nucleotide biosynthesis. Such mutants cannot survive in medium containing aminopterin to poison the de novo synthesis pathway. However, individual mutant cells that have fused with spleen cells and thus do contain the HGPRT or TK enzyme can survive in appropriate selective medium by using the salvage pathway. HGPRT, hypoxanthine guanine phosphoribosyl transferase; TK, thymidine kinase.

Cell Mieloma (HGPRT-) is not capable of using hypoxanthin for purine synthesis (Adenina Guanina) and uses the synthesis de novo based on the dihydrofolate reductase that is blocked by the aminopterin block this route)


Growth on
HAT medium
Immunoassay
Positive
response
Separate
hybridoma
clone
Separate
hybridoma
clones
Immunoassay
Positive
Negative

ELISA ASSAY

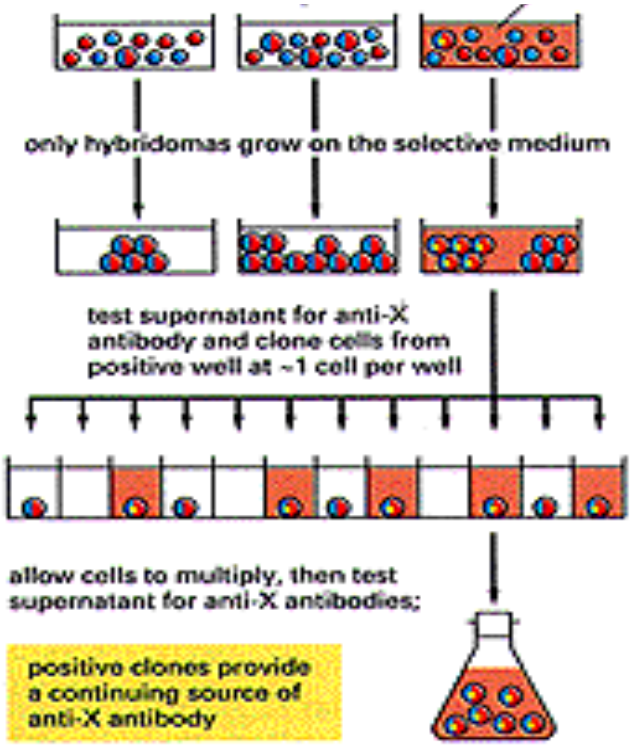


1. **Antigen**
2. **Antigen – antibody complex**
3. **Antigen – antibody complex + conjugate**
4. **Antigen – antibody complex + conjugate + substrate= coloured product**

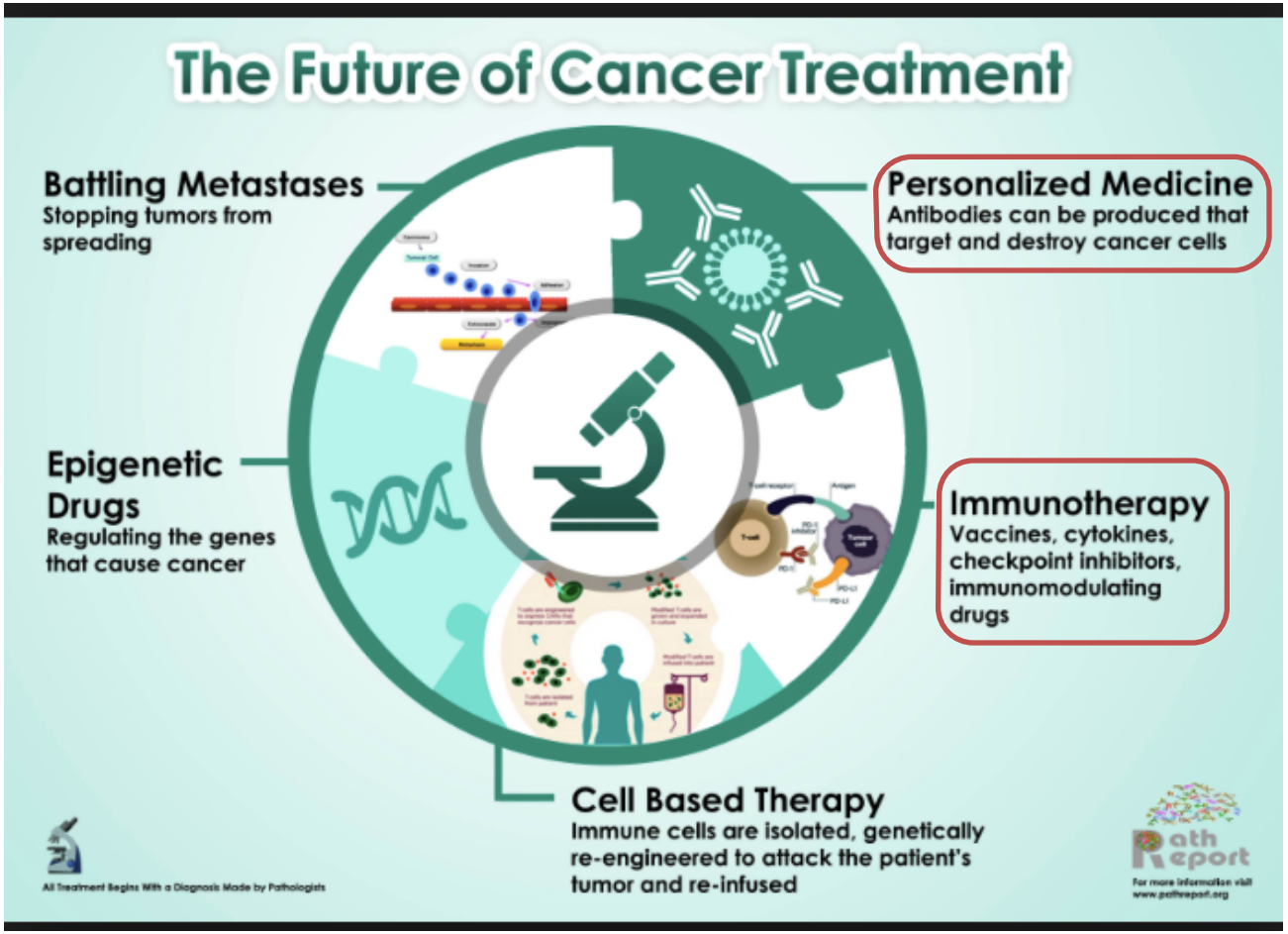
Antibody large scale production through…

Massive culture in specific media

…flasks



…fermentor


The Future of Cancer Treatment
Battling Metastases
Stopping tumors from spreading
Personalized Medicine
Antibodies can be produced that target and destroy cancer cells
Epigenetic Drugs
Regulating the genes that cause cancer
Immunotherapy
Vaccines, cytokines, checkpoint inhibitors, immunomodulating drugs
Cell Based Therapy
Immune cells are isolated, genetically re-engineered to attack the patient's tumor and re-infused
All Treatment Begins With a Diagnosis Made by Pathologists
Path Report
For more information visit www.pathreport.org

…..mAb therapeutic applications

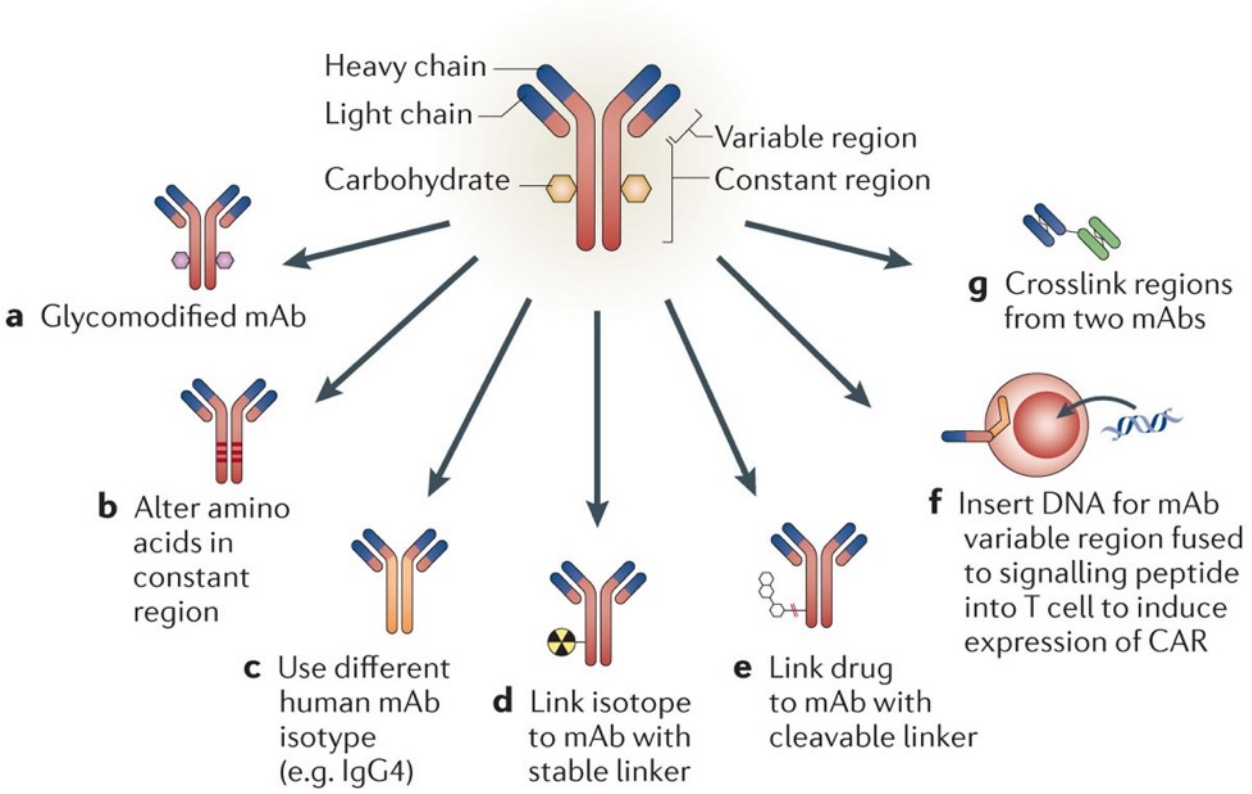


Table 2 | **Tumour-associated antigens targeted by therapeutic monoclonal antibodies in oncology**

Antigen category	Examples of antigens	Examples of therapeutic mAbs raised against these targets	Tumour types expressing antigen
Haematopoietic differentiation antigens	CD20	Rituximab	Non-Hodgkin's lymphoma
		Ibritumomab tiuxetan and tositumomab	Lymphoma
	CD30	Brentuximab vedotin	Hodgkin's lymphoma
	CD33	Gemtuzumab ozogamicin	Acute myelogenous leukaemia
	CD52	Alemtuzumab	Chronic lymphocytic leukaemia
Glycoproteins expressed by solid tumours	EpCAM	IGN101 and adecatumumab	Epithelial tumours (breast, colon and lung)
	CEA	Labetuzumab	Breast, colon and lung tumours
	gpA33	huA33	Colorectal carcinoma
	Mucins	Pemtumomab and oregovomab	Breast, colon, lung and ovarian tumours
	TAG-72	CC49 (minretumomab)	Breast, colon and lung tumours
	CAIX	cG250	Renal cell carcinoma
	PSMA	J591	Prostate carcinoma
	Folate-binding protein	MOv18 and MORAb-003 (farletuzumab)	Ovarian tumours
Glycolipids	Gangliosides (such as GD2, GD3 and GM2)	3F8, ch14.18 and KW-2871	Neuroectodermal tumours and some epithelial tumours
Carbohydrates	Le^y	hu3S193 and IgN311	Breast, colon, lung and prostate tumours
Targets of anti-angiogenic mAbs	VEGF	Bevacizumab	Tumour vasculature
	VEGFR	IM-2C6 and CDP791	Epithelium-derived solid tumours
	Integrin αVβ3	Etaracizumab	Tumour vasculature
	Integrin α5β1	Volociximab	Tumour vasculature
Growth and differentiation signalling	EGFR	Cetuximab, panitumumab, nimotuzumab and 806	Glioma, lung, breast, colon, and head and neck tumours
	ERBB2	Trastuzumab and pertuzumab	Breast, colon, lung, ovarian and prostate tumours
	ERBB3	MM-121	Breast, colon, lung, ovarian and prostate, tumours
	MET	AMG 102, METMAB and SCH 900105	Breast, ovary and lung tumours
	IGF1R	AVE1642, IMC-A12, MK-0646, R1507 and CP 751871	Glioma, lung, breast, head and neck, prostate and thyroid cancer
	EPHA3	KB004 and IIIA4	Lung, kidney and colon tumours, melanoma, glioma and haematological malignancies
	TRAILR1	Mapatumumab (HGS-ETR1)	Colon, lung and pancreas tumours and haematological malignancies
	TRAILR2	HGS-ETR2 and CS-1008	
	RANKL	Denosumab	Prostate cancer and bone metastases
Stromal and extracellular matrix antigens	FAP	Sibrotuzumab and F19	Colon, breast, lung, pancreas, and head and neck tumours
	Tenascin	81C6	Glioma, breast and prostate tumours

"Antibody therapy of cancer", Andrew M. Scott[1], Jedd D. Wolchok and Lloyd J. Old, Nature Reviews, 2012

Therapeutic Effects of Monoclonal Antibodies

Direct effects

- Blockade of growth factors / growth factor receptors
- Induction of apoptosis
- Inhibition of angiogenesis

Indirect effects

- Complement-dependent cytotoxicity (CDC)
- Antibody-dependent cellular cytotoxicity (ADCC)
- Vehicel for toxins, radionuclides and cytostatic drugs
- Anti-idiotype antibody formation
- Effector cell targeting using bispecific antibodies

Strategies for Therapeutic Application of Monoclonal Antibodies

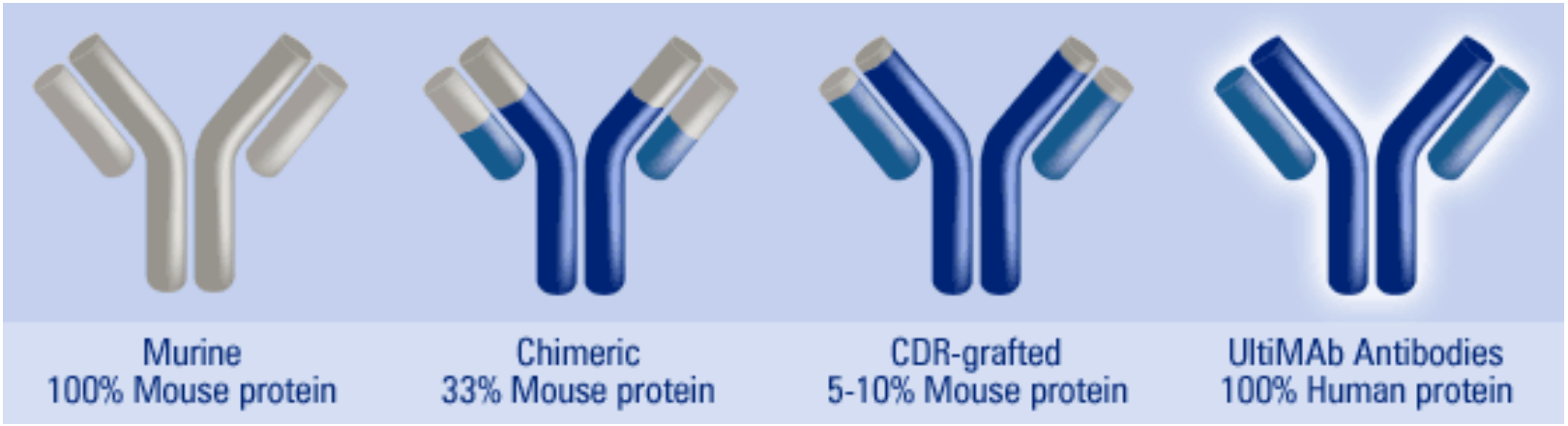
in vivo:

- mAb against differentiation antigens (ADCC, complement fixation)
- mAb with direct anti-proliferative effects (growth receptors, apoptosis)
- mAb interfering with angiogenesis
- mAb as carriers for radioisotopes (radioimmunotherapy)
- mAb as carriers for toxins (immunotoxins)
- Anti-idiotype mAb
- Bispecific mAb to focus effector cell activity (effector cell targeting)

ex vivo:

- Autologous BM/SC transplantation: purging of the autograft with mAb
- Allogeneic BM/SC transplantation: prevention of GVHD by T cell depletion

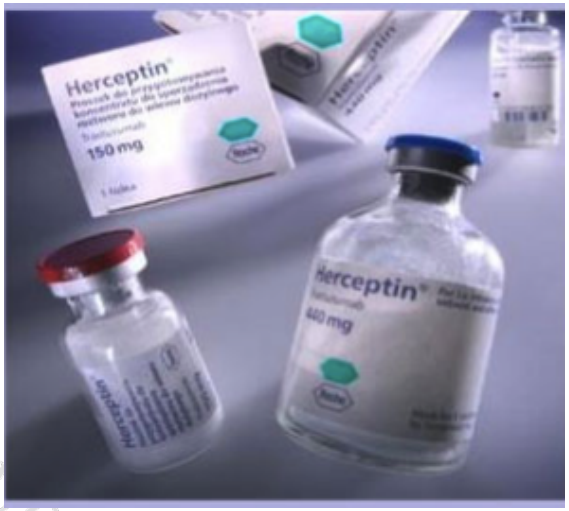
Monoclonal antibody evolution…



Milestone *mAbs* used in cancer therapy

Rituximab

Cetuximab

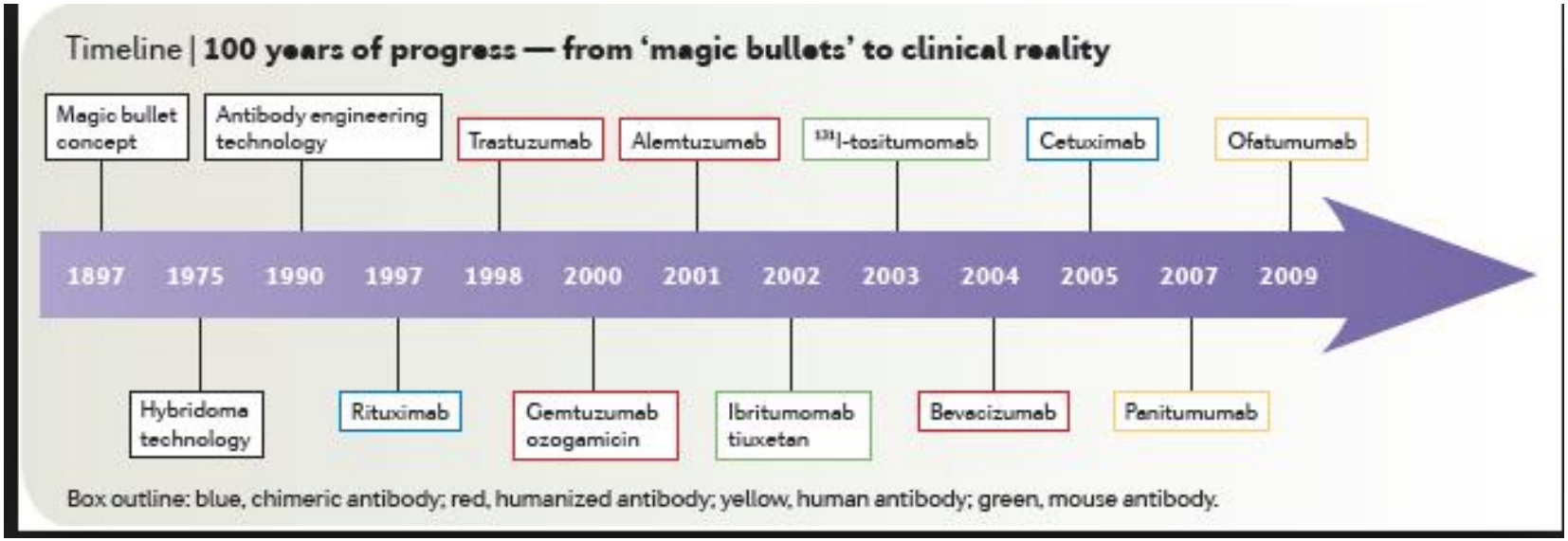


Trastuzumab

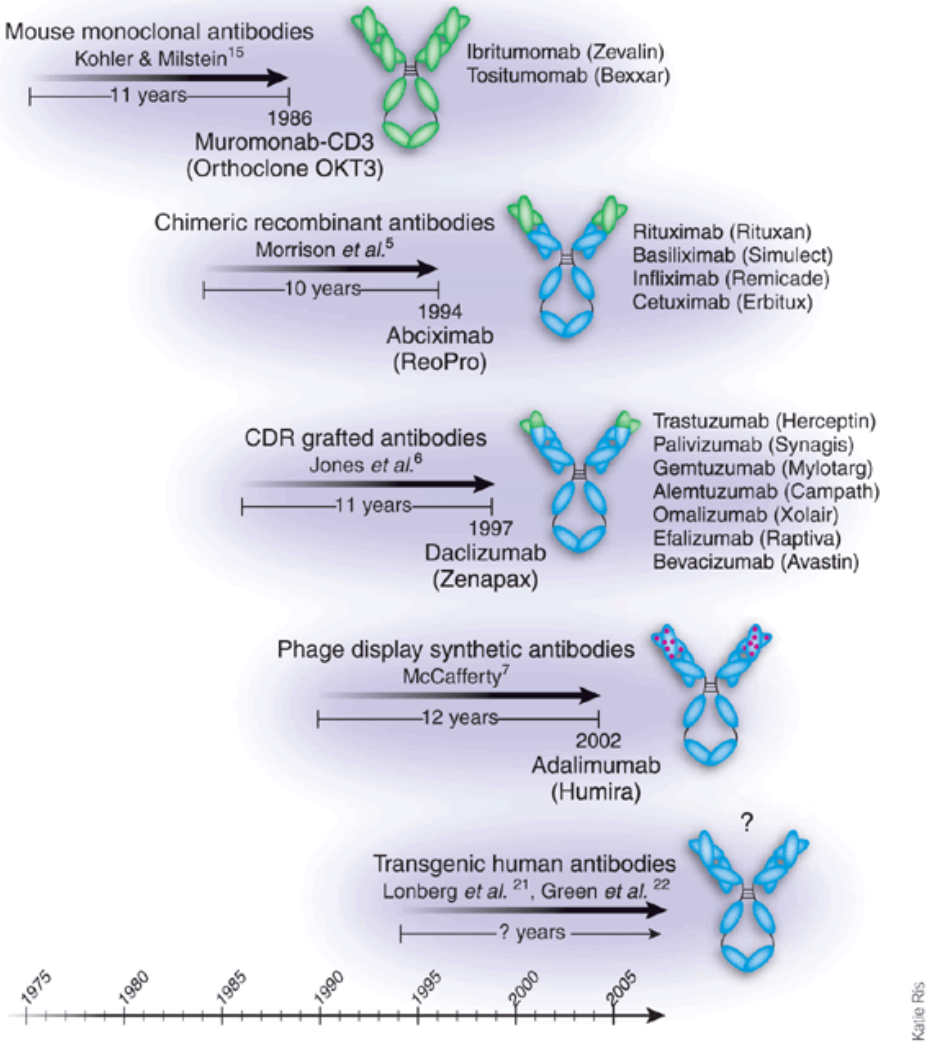
Bevacizumab

Monoclonal antibody development

…A FAST STORY



Monoclonal antibody development



MABS
2018, VOL. 10, NO. 2, 183–203
https://doi.org/10.1080/19420862.2018.1415671

Taylor & Francis
Taylor & Francis Group

PERSPECTIVE

Antibodies to watch in 2018

Hélène Kaplon[a] and Janice M. Reichert[b]

[a]Laboratory UMRS 1138 "Cancer, Immune Control and Escape", Cordeliers Research Centre, Paris, France; [b]The Antibody Society, Framingham, MA, USA

Table 1. Antibody therapeutics approved in the European Union or United States during 2017*.

International non-proprietary name	Brand name	Target; Format	Indication first approved	Date of first EU approval	Date of first US approval
Brodalumab	Siliq, Lumicef, Kyntheum	IL-17RA; Human IgG2	Plaque psoriasis	7/17/17	2/15/17
Avelumab	Bavencio	PD-L1; Human IgG1	Merkel cell carcinoma	9/18/17	3/23/17
Dupilumab	Dupixent	IL-4Rα; Human IgG4	Atopic dermatitis	9/27/17	3/28/17
Ocrelizumab	Ocrevus	CD20; Humanized IgG1	Multiple sclerosis	EC decision pending	3/29/17
Durvalumab	Imfinzi	PD-L1; Human IgG1	Bladder cancer	In review	5/1/17
Sarilumab	Kevzara	IL-6R; Human IgG1	Rheumatoid arthritis	6/23/17	5/22/17
Guselkumab	Tremfya	IL-23 p19; Human IgG1	Plaque psoriasis	11/23/17	7/13/17
Inotuzumab ozogamicin	Besponsa	CD22; Humanized IgG4; ADC	Acute lymphoblastic leukemia	6/29/17	8/17/17
Benralizumab	Fasenra	IL-5R α; Humanized IgG1	Asthma	EC decision pending	11/14/17
Emicizumab	Hemlibra	Factor IXa, X; Humanized IgG4, bispecific	Hemophilia A	In review	11/16/17

10 monoclonal antibodies (mAbs) have received

their first marketing approvals in 2017 by FDA and EMA

24 antibodies are undergoing evaluation in late-stage clinical studies of patients with cancer

Table 4. Antibody therapeutics in late-stage clinical studies for cancer indications

Primary sponsoring company	INN or code name	Molecular format	Target	Most advanced phase	Late-stage study indication(s)
Actinium Pharmaceuticals	I-131-BC8, Iomab-B	Murine IgG1, radiolabeled	CD45	Phase 3	Ablation of bone marrow prior to hematopoietic cell transplantation in AML patients
Sanofi	Isatuximab	Humanized® IgG1	CD38	Phase 3	Multiple myeloma
TG Therapeutics	Ublituximab	Chimeric IgG1	CD20	Phase 3	Chronic lymphocytic Leukemia, non-Hodgkin lymphoma, multiple sclerosis
AstraZeneca/ MedImmune LLC	Moxetumomab pasudotox	Murine IgG1 dsFv immunotoxin	CD22	Phase 3	Hairy cell leukemia
MorphoSys	XMAB-5574, MOR208	Humanized IgG1	CD19	Phase 2/3	Diffuse large B-cell lymphoma
Pfizer	Utomilumab	Human IgG2	4–1BB (CD137)	Phase 3	Diffuse large B-cell lymphoma
Hoffmann-La Roche	Polatuzumab vedotin	Humanized IgG1 ADC	CD79b	Phase 3	Diffuse large B-cell lymphoma
Viventia Bio	Oportuzumab monatox	Humanized scFv immunotoxin	EpCAM	Phase 3	Bladder cancer
Seattle Genetics	Enfortumab vedotin	Human IgG1 ADC	Nectin 4	Pivotal Phase 2	Urothelial cancer
Jiangsu HengRui Medicine Co., Ltd	Camrelizumab	Humanized IgG4	PD-1	Phase 2/3	Hepatocellular carcinoma, esophageal carcinoma
MacroGenics	Margetuximab	Chimeric IgG1	HER2	Phase 3	Breast cancer
Synthon Biopharmaceuticals BV	(vic-)trastuzumab duocarmazine	Humanized IgG1 ADC	HER2	Phase 3	Breast cancer
Immunomedics, Inc	Sacituzumab govitecan	IgG1 ADC	TROP-2 (epithelial glyco-protein-1)	Phase 3	Triple-neg. breast cancer
Celldex Therapeutics	Glembatumumab vedotin	Human IgG2 ADC	gpNMB	Pivotal Phase 2	gpNMB+ breast cancer, melanoma
Daiichi Sankyo	DS-8201	Humanized ADC	HER2	Pivotal Phase 2	HER2+ gastric or gastroesophageal junction adenocarcinoma
Gilead Sciences	Andecaliximab	Humanized® IgG4	MMP-9	Phase 3	Gastric cancer or gastroesophageal junction adenocarcinoma
AbbVie	Depatuxizumab mafodotin	IgG1 ADC	EGFR	Phase 2b/3	Glioblastoma
AstraZeneca/ MedImmune LLC	Tremelimumab	Human IgG2	CTLA-4	Phase 3	Non-small cell lung, head & neck, urothelial cancer, hepatocellular carcinoma
Recombio SL	Racotumomab	Murine IgG1	NGcGM3	Phase 3	Non-small cell lung cancer
Regeneron Pharmaceuticals	Cemiplimab	Human mAb	PD-1	Pivotal Phase 2; Phase 3	Cutaneous squamous cell carcinoma; non-small cell lung cancer, cervical cancer
Innovent Biologics (Suzhou) Co. Ltd.	IBI308	Human mAb	PD-1	Phase 3	Squamous cell non-small cell lung cancer
BeiGene	BGB-A317	Humanized mAb	PD-1	Phase 3	Non-small cell lung cancer
AbbVie	Rovalpituzumab tesirine	Humanized IgG1 ADC	DLL3	Phase 3	Small cell lung cancer
ImmunoGen	Mirvetuximab soravtansine	IgG1 ADC	Folate receptor 1	Phase 3	Epithelial ovarian cancer, peritoneal carcinoma, fallopian tube cancer
Biocad	BCD-100	Human mAb	PD-1	Phase 2/3	Melanoma
Novartis	PDR001	Humanized IgG4	PD-1	Phase 3	Melanoma
Philogen SpA	L19IL2/L19TNF	scFv immuno-conjugates	Fibronectin extra-domain B	Phase 3	Melanoma
Tracon	Carotuximab	Chimeric IgG1	Endoglin	Phase 3	Soft tissue sarcoma, angiosarcoma, renal cell carcinoma, wet age related macular degeneration

MABS
2019, VOL. 11, NO. 2, 219–238
https://doi.org/10.1080/19420862.2018.1556465

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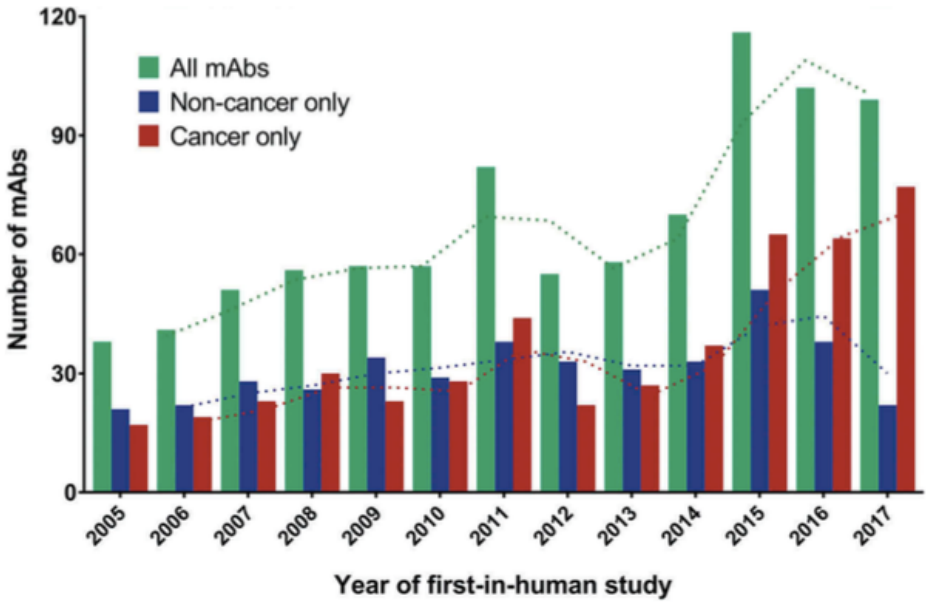
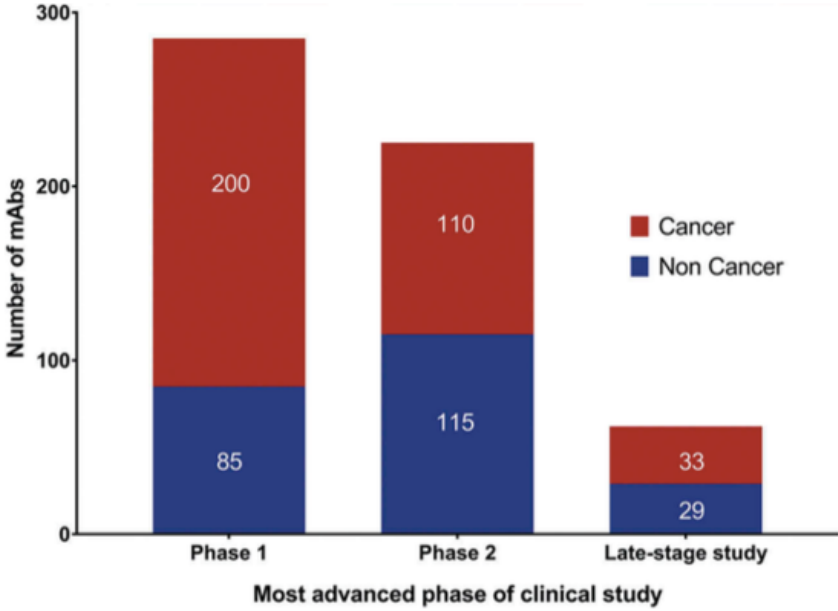
PERSPECTIVE

Antibodies to watch in 2019

Hélène Kaplon[a] and Janice M. Reichert[b]

[a]Institut de Recherches Servier, Croissy-sur-Seine, the Division of Biotechnology & Biomarker Research, France; [b]The Antibody Society, Framingham, MA, USA





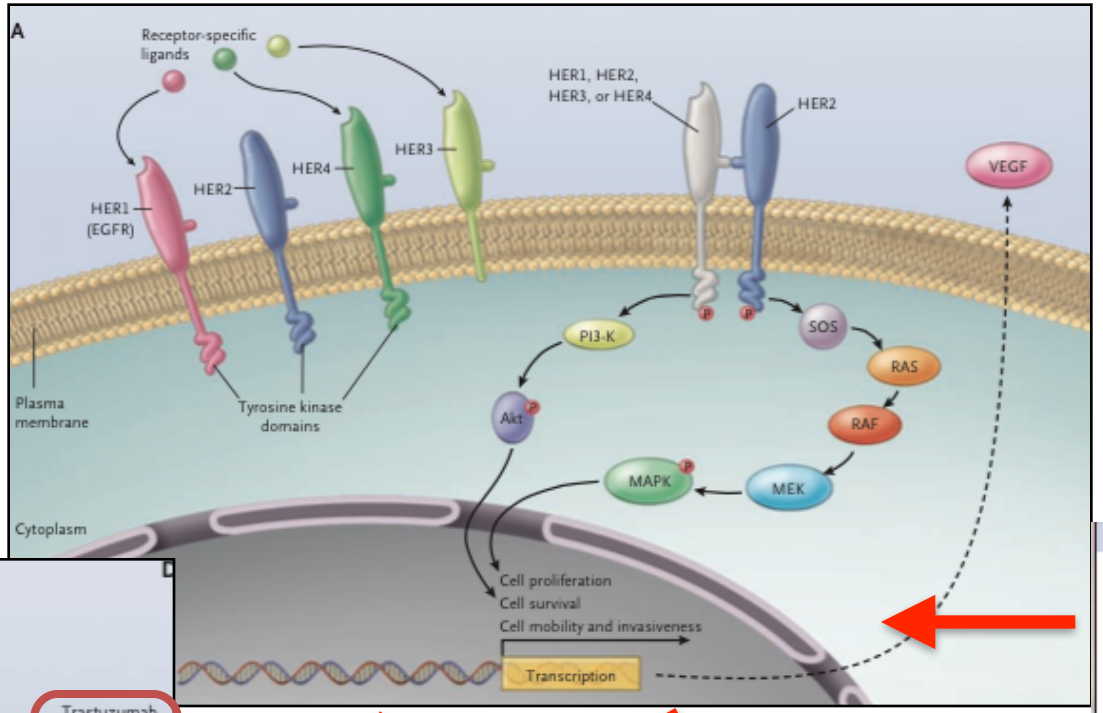
32 antibodies are undergoing evaluation in late-stage clinical studies of patients with cancer

Table 4. Investigational monoclonal antibodies in late-stage clinical studies for cancer indications.

Primary sponsoring company	INN or code name	Molecular format	Target(s)	Most advanced phase	Pivotal Phase 2, Phase 2/3 or 3 indications
TG Therapeutics	Ublituximab	Chimeric IgG1	CD20	Phase 3	Chronic lymphocytic leukemia
ADC Therapeutics Sarl	Loncastuximab tesirine	Humanized IgG1 ADC	CD19	Pivotal Phase 2	Diffuse large B-cell lymphoma
Hoffmann-La Roche	Polatuzumab vedotin	Humanized IgG1 ADC	CD79b	Phase 3	Diffuse large B-cell lymphoma
Pfizer	Utomilumab	Human IgG2	4-1BB (CD137)	Phase 3	Diffuse large B-cell lymphoma
MorphoSys	XMAB-5574, MOR208	Humanized IgG1	CD19	Phase 2/3	Diffuse large B-cell lymphoma
Sanofi	Isatuximab	Humanized IgG1	CD38	Phase 3	Multiple myeloma
Jiangsu HengRui Medicine Co., Ltd	Camrelizumab	Humanized IgG4	PD-1	Phase 3; regulatory review in China	Hodgkin's lymphoma, hepatocellular carcinoma
Actinium Pharmaceuticals	I-131-BC8, Iomab-B	Murine IgG1, radio-labeled	CD45	Phase 3	Ablation of bone marrow prior to hematopoietic cell transplantation in AML patients
Tracon	Carotuximab	Chimeric IgG1	Endoglin	Phase 3	Angiosarcoma
Alphamab Oncology	KN035	mAb, single domain	PD-L1	Phase 3	Bile tract carcinoma
Viventia Bio	Oportuzumab monatox	Humanized scFv immunotoxin	EpCAM	Phase 3	Bladder cancer
Bio-Thera Solutions	BAT8001	Humanized IgG1 ADC	HER2	Phase 3	Breast cancer
Synthon Biopharmaceuticals BV	(vic-)trastuzumab duocarmazine	Humanized IgG1 ADC	HER2	Phase 3	Breast cancer
MacroGenics	Margetuximab	Chimeric IgG1	HER2	Phase 3	Breast cancer
Daiichi Sankyo	Trastuzumab deruxtecan	Humanized ADC	HER2	Phase 3	Breast cancer, HER2+ gastric or gastroesophageal junction adenocarcinoma
Five Prime Therapeutics, Zai Lab Limited	Bemarituzumab	Humanized IgG1	FGFR2b	Phase 3	Gastric and gastro-esophageal junction adenocarcinoma
Astellas	Zolbetuximab, claudiximab	Chimeric IgG1	Claudin-18.2	Phase 3	Gastric and gastro-esophageal junction adenocarcinoma
Gilead Sciences	Andecaliximab	Humanized IgG4	MMP9	Phase 3	Gastric cancer or gastroesophageal junction adenocarcinoma
AbbVie	Depatuxizumab mafodotin	IgG1 ADC	EGFR	Phase 2b/3	Glioblastoma
Y-mabs Therapeutics	Naxitamab	Humanized mAb	GD2	Phase 3	High risk neuroblastoma and refractory osteomedullary disease
Bristol-Myers Squibb	Relatlimab (BMS-986016)	Human mAb	LAG-3	Phase 2/3	Melanoma
Biocad	BCD-100	Human mAb	PD-1	Phase 2/3	Melanoma
Novartis	Spartalizumab, PDR001	Humanized IgG4	PD-1	Phase 3	Melanoma
Philogen SpA	L19IL2 + L19TNF	scFv conjugates	Fibronectin extra-domain B	Phase 3	Melanoma
Y-mAbs Therapeutics	131I-omburtamab	Murine mAb, radiolabeled	B7-H3	Phase 2/3	Neuroblastoma central nervous system/ leptomeningeal metastases
BeiGene	Tislelizumab (BGB-A317)	Humanized mAb	PD-1	Phase 3; regulatory review in China	Non-small cell lung cancer, Hodgkin's lymphoma
Innovent Biologics (Suzhou) Co. Ltd.	IBI308	Human mAb	PD-1	Phase 3; regulatory review in China	Squamous cell non-small cell lung cancer
CStone Pharmaceuticals	CS1001	Human	PD-L1	Phase 3	Non-small cell lung cancer
AstraZeneca/ MedImmune LLC	Tremelimumab	Human IgG2	CTLA4	Phase 3	Non-small cell lung, head & neck, urothelial cancer
Tesaro, Inc.	TSR-042	Humanized mAb	PD-1	Phase 3	Ovarian cancer
ImmunoGen	Mirvetuximab soravtansine	IgG1 ADC	Folate receptor 1	Phase 3	Ovarian cancer
AbbVie	Rovalpituzumab tesirine	Humanized IgG1 ADC	DLL3	Phase 3	Small cell lung cancer
Seattle Genetics	Enfortumab vedotin	Human IgG1 ADC	Nectin 4	Phase 3	Urothelial cancer

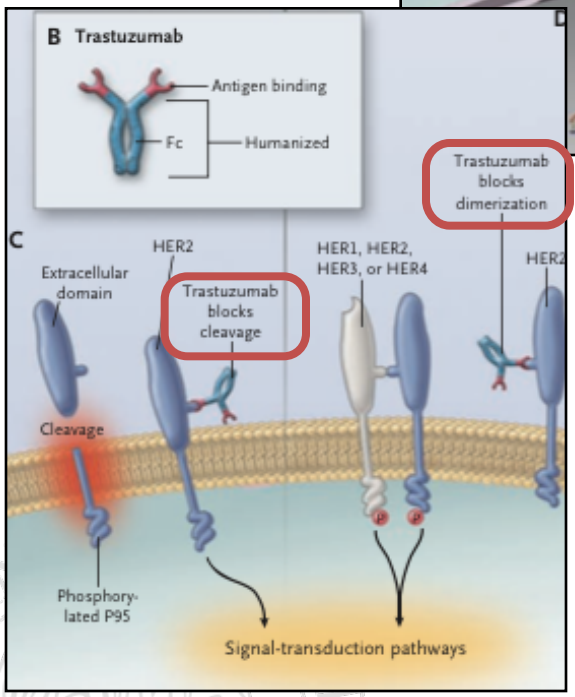
Data available as of November 30, 2018. Abbreviations: ADC, antibody drug conjugate; CTLA-4, cytotoxic T-lymphocyte–associated antigen 4; DLL3, delta-like protein 3; EGFR, epidermal growth factor receptor; EpCAM, epithelial cell adhesion molecule; FGFR2, fibroblast growth factor receptor 2; HER2, human epidermal growth factor receptor-2; MMP-9, matrix metallopeptidase 9; PD-1, programmed cell death 1; PD-L1, programmed death ligand-1.

Effects of Trastuzumab (Herceptin)



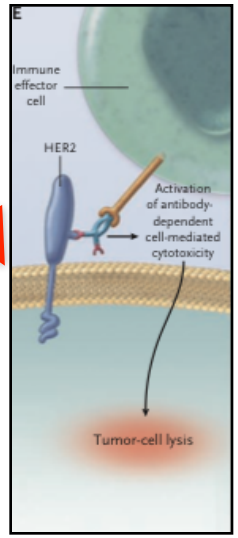
Intracellular effects of trastuzumab

- Induction of apoptosis
- Decreased cell proliferation
- HER2 down-regulation, dephosphorylation, or both
- Decreased VEGF production
- Potentiation of chemotherapy
- Modulation of downstream signal paths
- Altered cross-talk with other signal paths

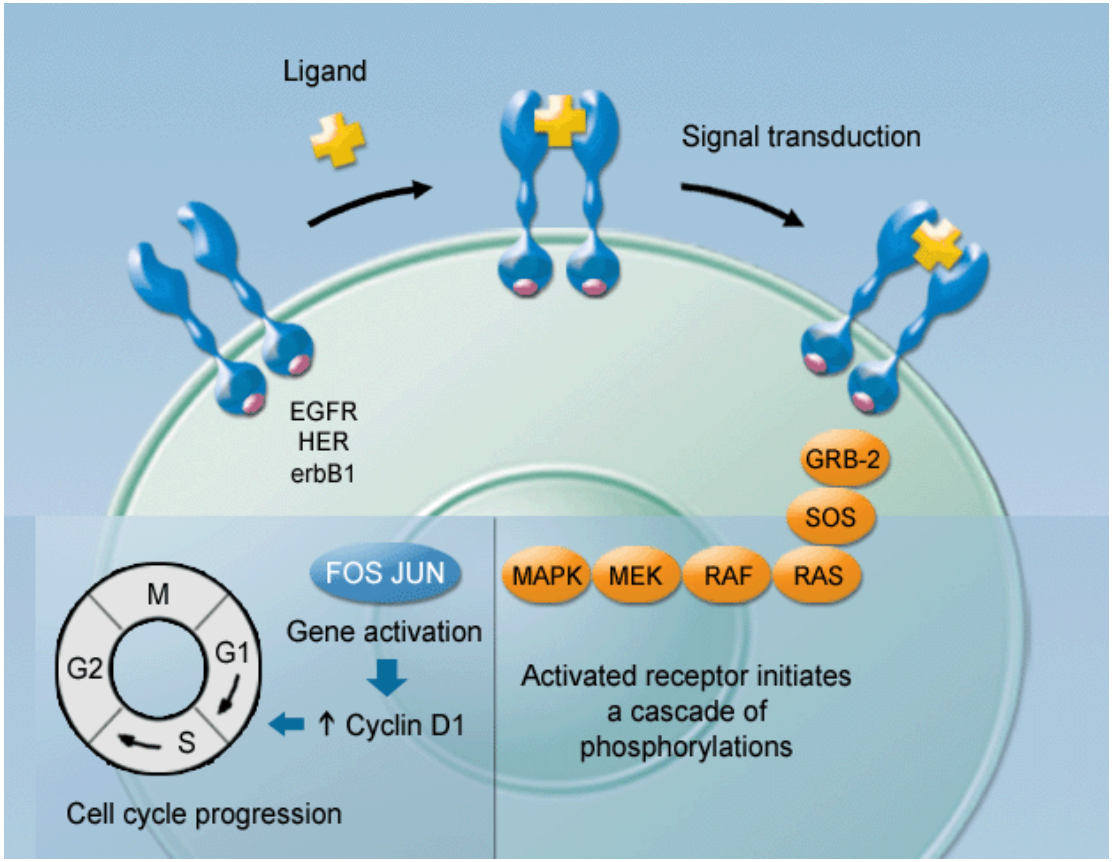


Extracellular effects of trastuzumab

- Inhibition of cleavage of HER2 extracellular domain
- Interference with homodimer and heterodimer formation between HER-family receptors
- Antibody-dependent immune mechanisms

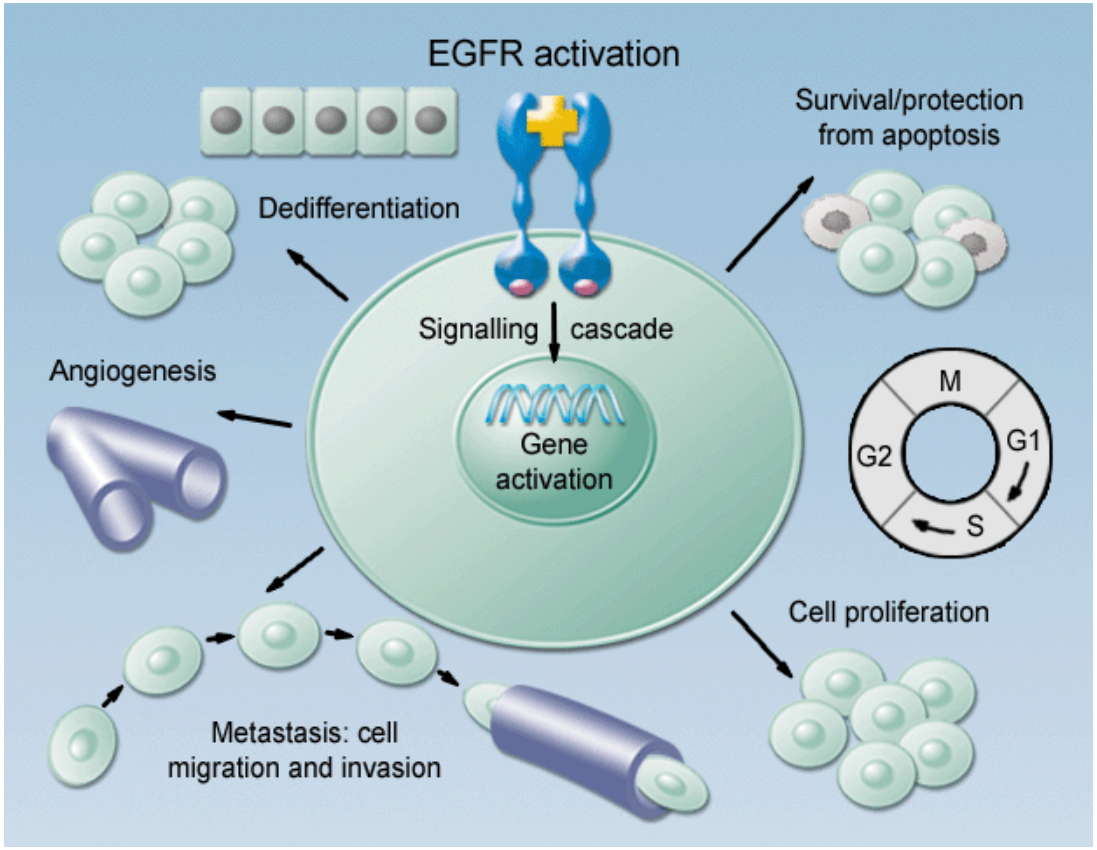


EGFR signaling



- The EGFR is activated by growth factors (e.g. epidermal growth factor (EGF) and transforming growth factor-α (TGF-α)).
- EGFR-activation leads to the building of either receptor homo- or hetero-dimers.
- Receptor dimerization initiates an intracellular signaling cascade, gene activation and the stimulation of cell cycle progression.

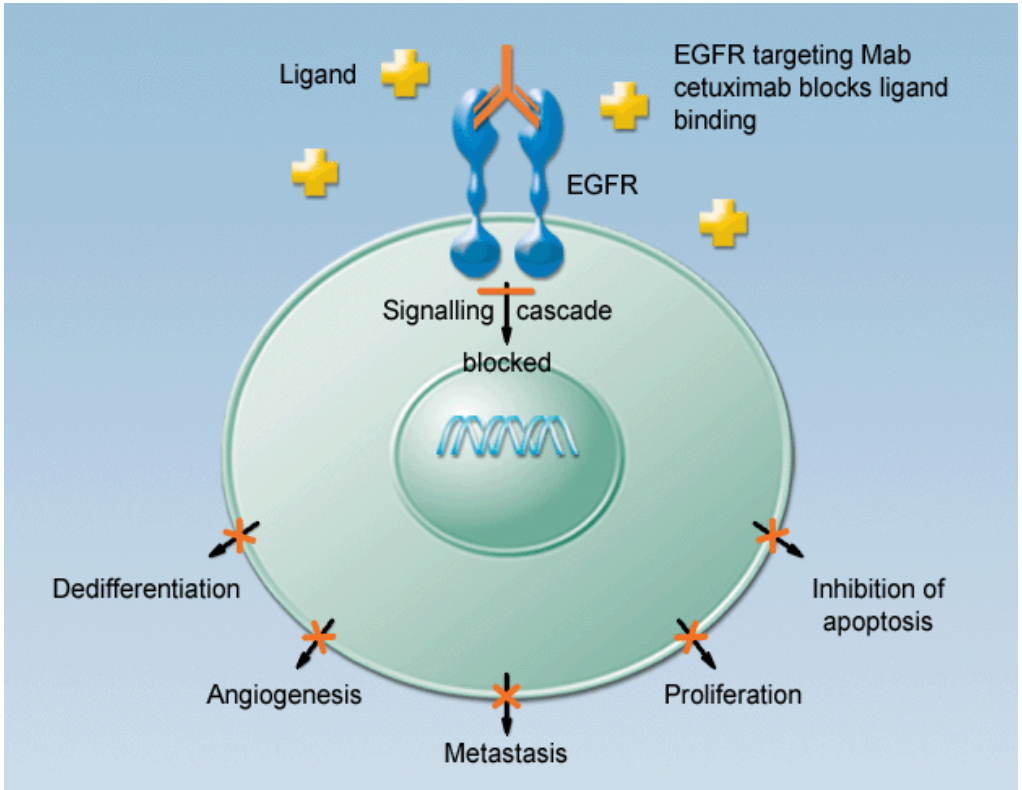
The importance of EGFR as a target



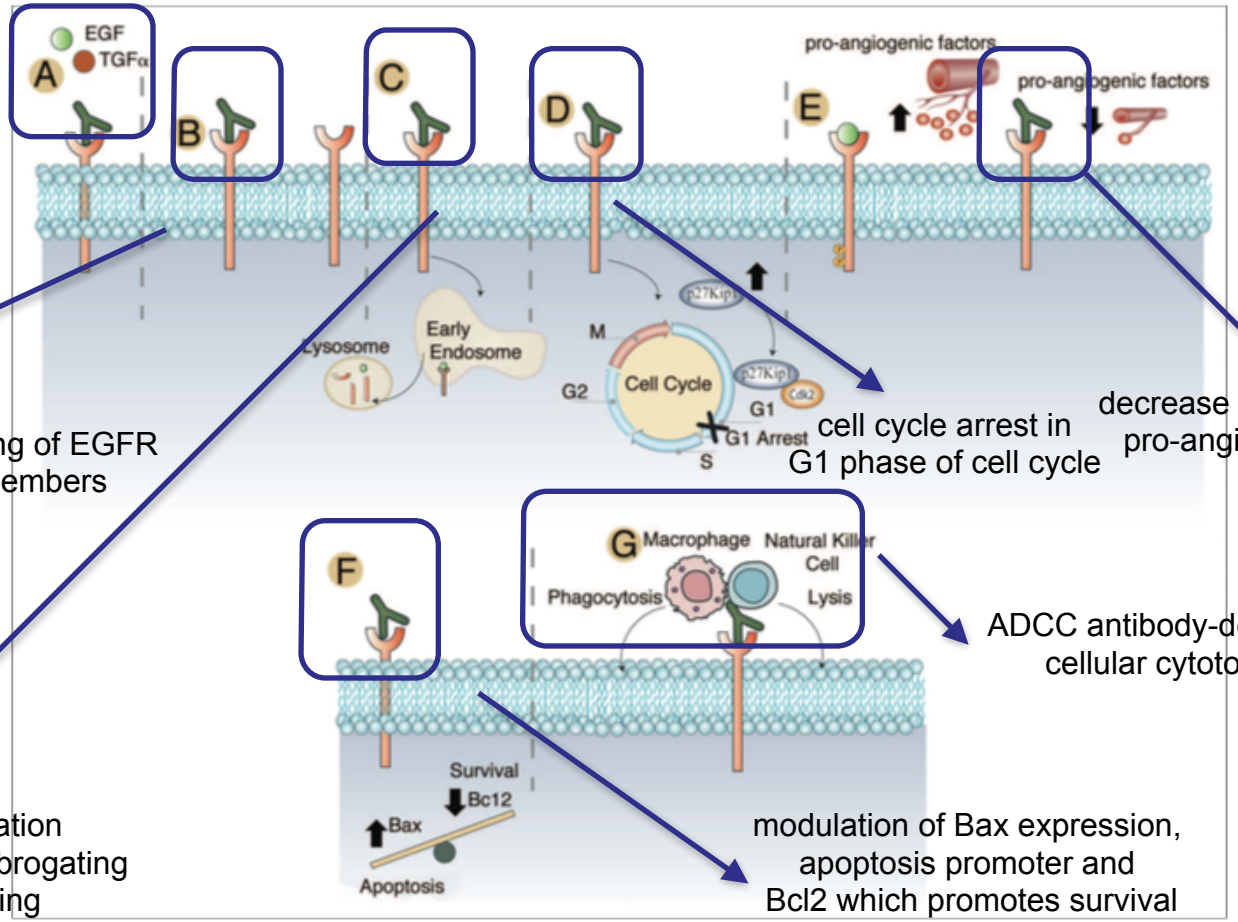
** Baselga 2002*

"Evidence for a role for the EGFR in the inhibition and pathogenesis of various cancers has led to the rational design and development of agents that selectively target this receptor."

Erbitux® (cetuximab)



- Erbitux® (cetuximab) is an IgG1 mAb targeting the EGFR
- Binding blocks EGFR signaling and inhibits proliferation, angiogenesis and metastasis, and stimulates apoptosis and differentiation
- The main toxicity is an acne-like rash that generally improves during treatment, and usually does not preclude continued treatment



higher affinity for EGFR, than TGF or EGF

sterically hinder the binding of EGFR to other HER family members

promotes the internalization and degradation of EGFR abrogating its downstream signaling

cell cycle arrest in G1 phase of cell cycle

decrease of expression of pro-angiogenic factors

ADCC antibody-dependent cellular cytotoxicity

modulation of Bax expression, apoptosis promoter and Bcl2 which promotes survival

Figure 3. Mechanisms of action of cetuximab. (A) Cetuximab has a higher affinity for the EGFR than either TGFα or EGF and effectively blocks ligand binding and ligand induced EGFR phosphorylation.[77-80] (B) Cetuximab has been noted to sterically hinder the binding of EGFR to other HER family members.[81] (C) Cetuximab promotes the internalization and degradation of the EGFR, abrogating its downstream signaling cascades.[83] (D) Cetuximab treatment of cancer cell lines and human tumor xenografts have shown a dramatic cell cycle arrest in the G_1 phase of the cell cycle. Further investigations indicated that this was due to an increased expression of the cell cycle inhibitor $p27^{Kip1}$. This increased expression led to the formation of $p27^{Kip1}$-Cdk2 complexes and the prevention of cells from exiting the G_1 phase of the cell cycle.[86-88] (E) It has been noted that EGFR expressing tumor lines display a significant increase in pro-angiogenic factors leading to increased angiogenesis to the tumor. Treatment with cetuximab has been shown to dramatically decrease the expression of pro-angiogenic factors. In addition to decreased angiogenesis, there is evidence that cetuximab therapy may lead to decreased invasion and metastatic spread of tumor cell.[86,94-97] (F) Cetuximab treatment has also been noted to influence the balance of apoptosis and cell survival through modulation of the expression of Bax, which promotes apoptosis and Bcl2, which promotes survival. Treatment with cetuximab increased expression of Bax and decreased Bcl2.[95-97] (G) Antibody-dependent cellular cytotoxicity mediated by cetuximab has also been noted in several studies.[98,99]

Strategies for Therapeutic Application of Monoclonal Antibodies

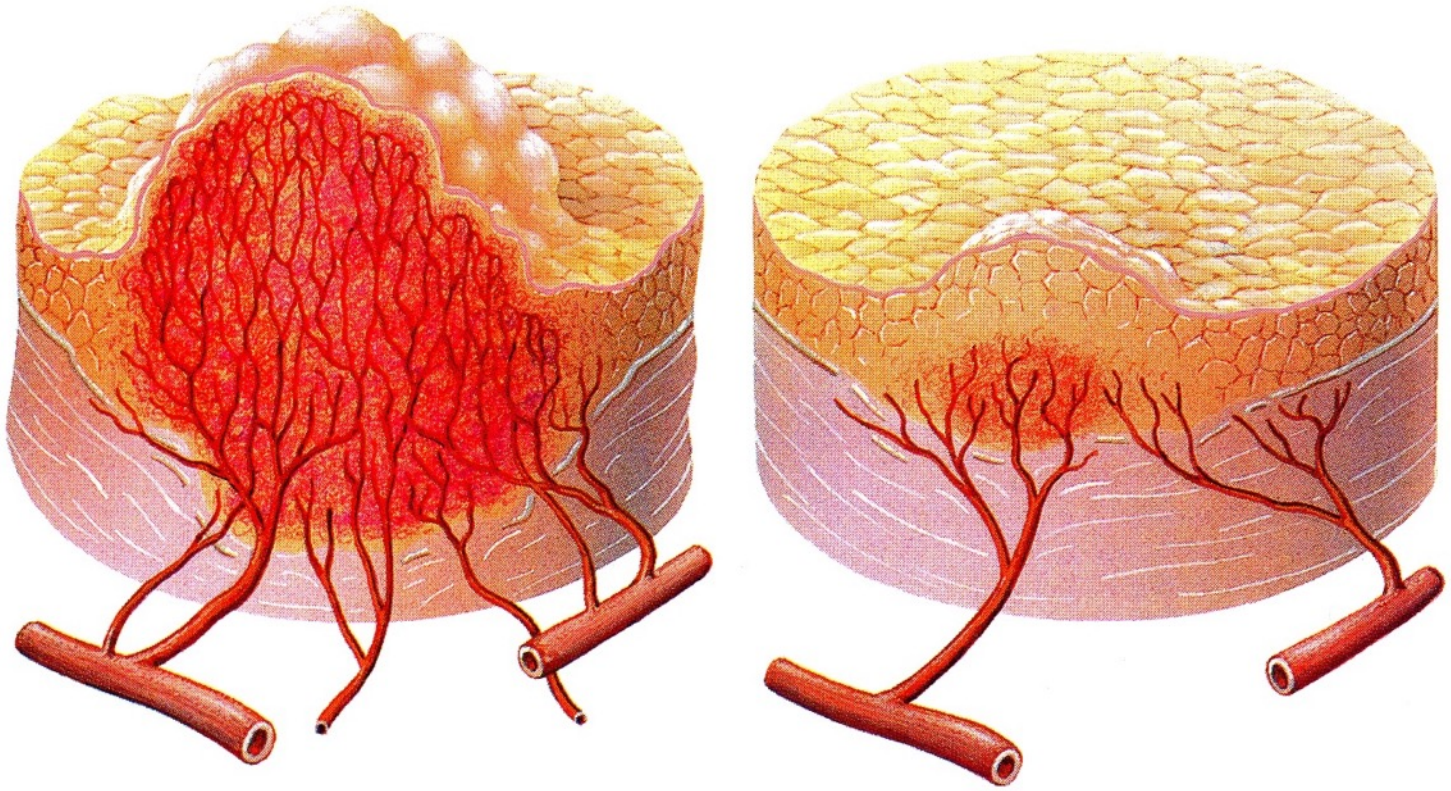
in vivo:

- mAb against differentiation antigens (ADCC, complement fixation)
- mAb with direct anti-proliferative effects (growth receptors, apoptosis)
- mAb interfering with angiogenesis
- mAb as carriers for radioisotopes (radioimmunotherapy)
- mAb as carriers for toxins (immunotoxins)
- Anti-idiotype mAb
- Bispecific mAb to focus effector cell activity (effector cell targeting)

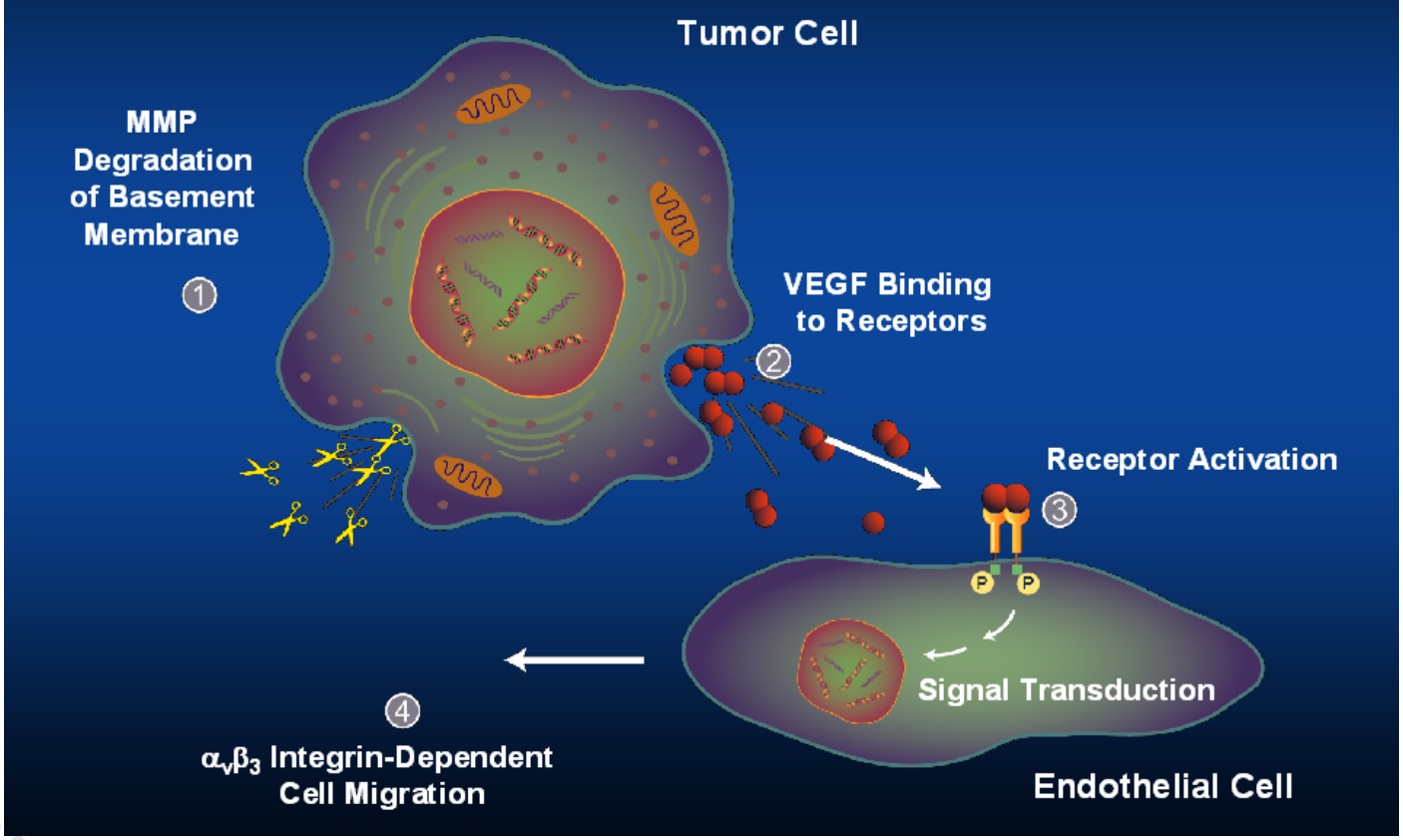
ex vivo:

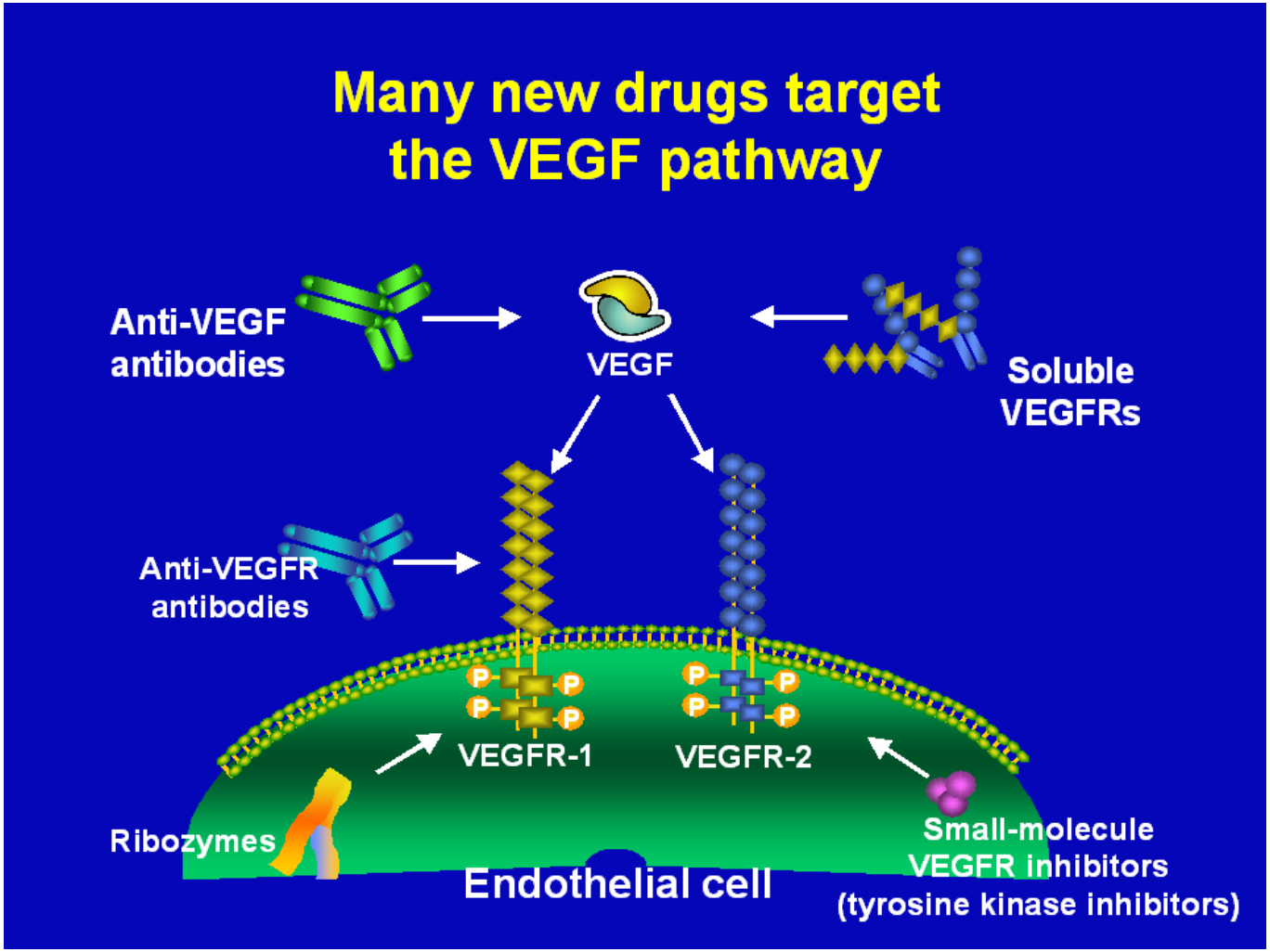
- Autologous BM/SC transplantation: purging of the autograft with mAb
- Allogeneic BM/SC transplantation: prevention of GVHD by T cell depletion

Inhibition of Angiogenesis

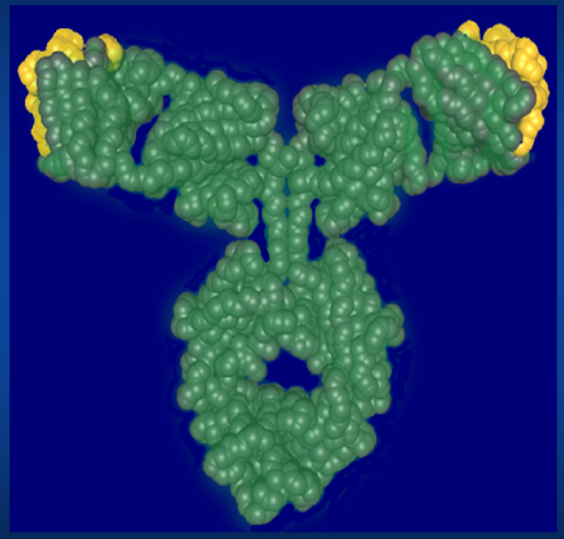


Targets for Angiogenesis Inhibition



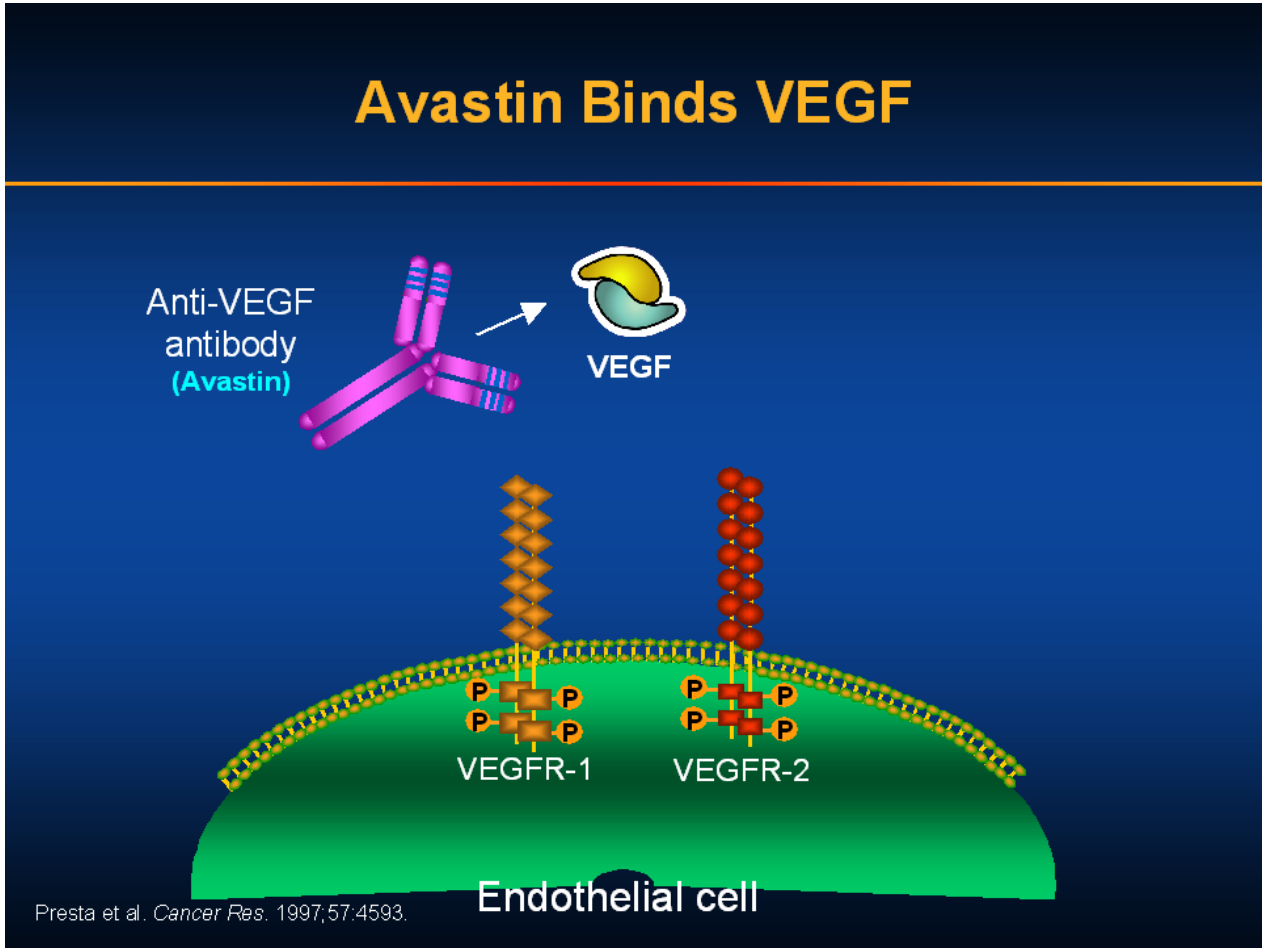

Many new drugs target the VEGF pathway
Anti-VEGF antibodies
VEGF
Soluble VEGFRs
Anti-VEGFR antibodies
P
VEGFR-1
VEGFR-2
Ribozymes
Endothelial cell
Small-molecule VEGFR inhibitors (tyrosine kinase inhibitors)

Avastin™ (bevacizumab)



- Recombinant humanized monoclonal IgG_1 antibody[1]
- Recognizes all isoforms of VEGF[2]
- Estimated half-life is approximately 20 days (range, 11-50 days)[1]

1. Avastin™ (bevacizumab) PI. February 2004.
2. Presta et al. *Cancer Res*. 1997;57:4593.


Avastin Binds VEGF
Anti-VEGF
antibody
(Avastin)
VEGF
P
VEGFR-1
VEGFR-2
Endothelial cell
Presta et al. Cancer Res. 1997;57:4593.

NANOBODIES APPLICATIONS IN ONCOLOGY

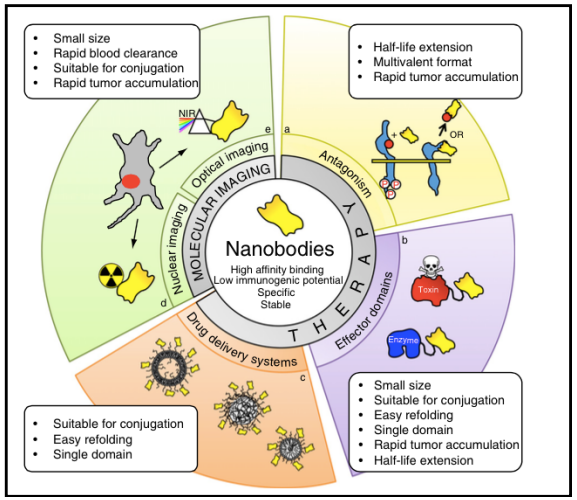


Table 1 | **Antibody constructs and potential uses in oncology**

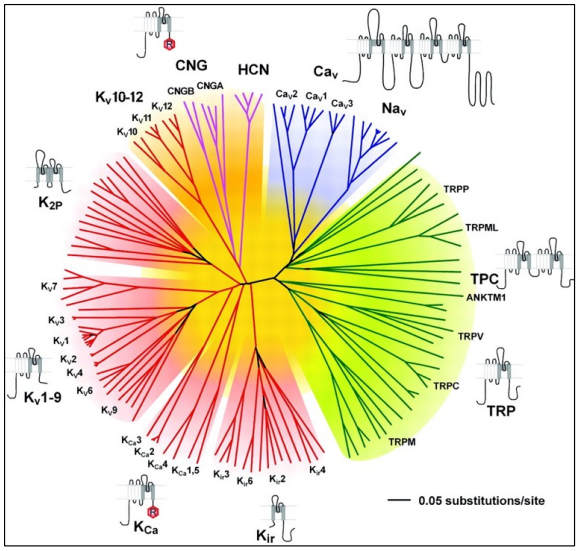
Antibody constructs	Examples of targets	Potential clinical use
scFv	CC49, ERBB2 and Le^y	Imaging and cell targeting
Diabody	Le^y and TAG-72	Imaging and drug delivery
Affibody	ERBB2	Imaging and drug delivery
Minibody	CEA and ERBB2	Imaging and drug delivery
Protein–Fc	Angiopoietin 1, angiopoietin 2, VEGFR1 and VEGFR2	Imaging and therapy
Intact IgG	CD20, CD33, EGFR, ERBB2 and VEGF	Imaging therapy and drug delivery
IgE and IgM	GM2	Therapy
Drug conjugates	CD30, CD33 and ERBB2	Therapy
Loaded nanoparticles	A33, EGFR and transferrin	Drug delivery
Bispecifics	CD19–CD3, EPCAM–CD3 and gp100–CD3	Therapy

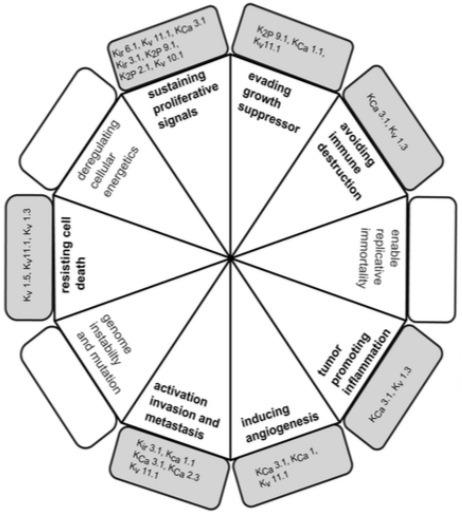
- Nanobodies have great applications in clinical and diagnostic setting, **with remarkable results especially for molecular imaging.**

Oliveira S. *et al., Journal of Controlled Release, 2013*

Scott et al. Nature Reviews, 2012

ION CHANNELS AND TRANSPORTERS… IN CANCER





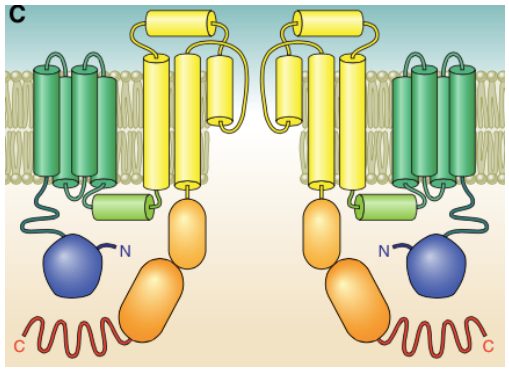
- Ion channels and transporters are an heterogeneous group of membrane proteins, which are also involved in many aspects of ***cancer establishment and progression.***

D'Amico et al., Recent Patents on Anti-Cancer Drug Discovery, 2013

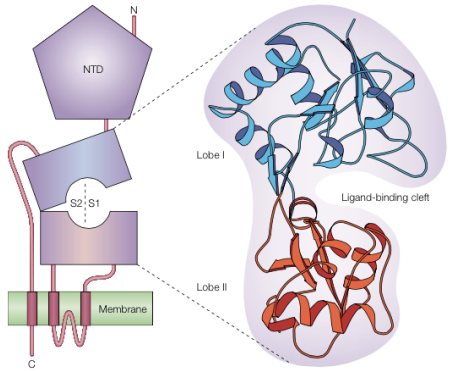
Vandenberg J.I., Physiologica Reviews, 2012

Madden D.R., Nature Review, 2002

they are now considered as TARGET

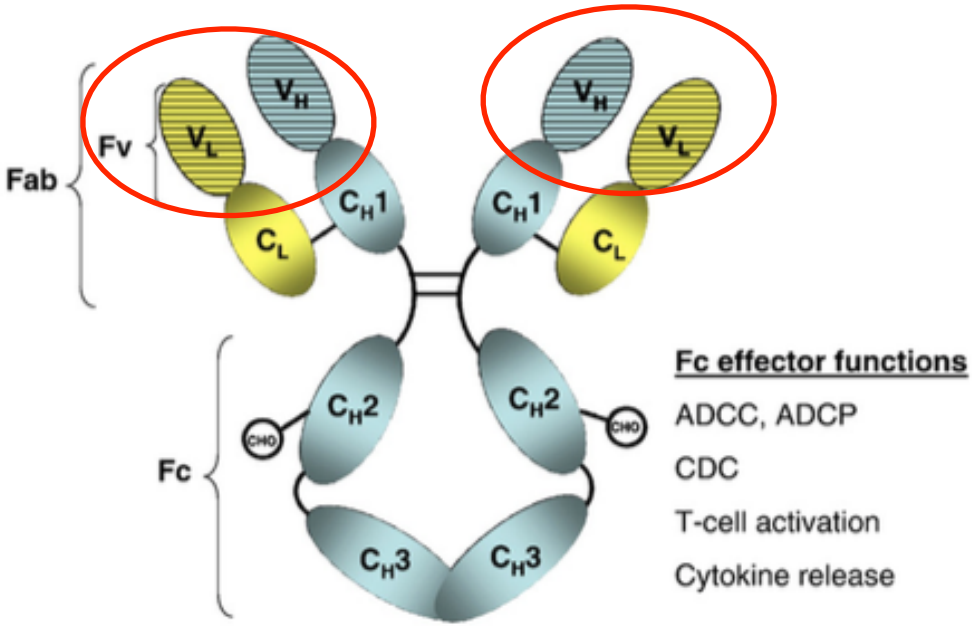


hERG1, Potassium channel



GluR4, Glutamate Receptor ion channel

IgG structure



Fc effector functions

ADCC, ADCP

CDC

T-cell activation

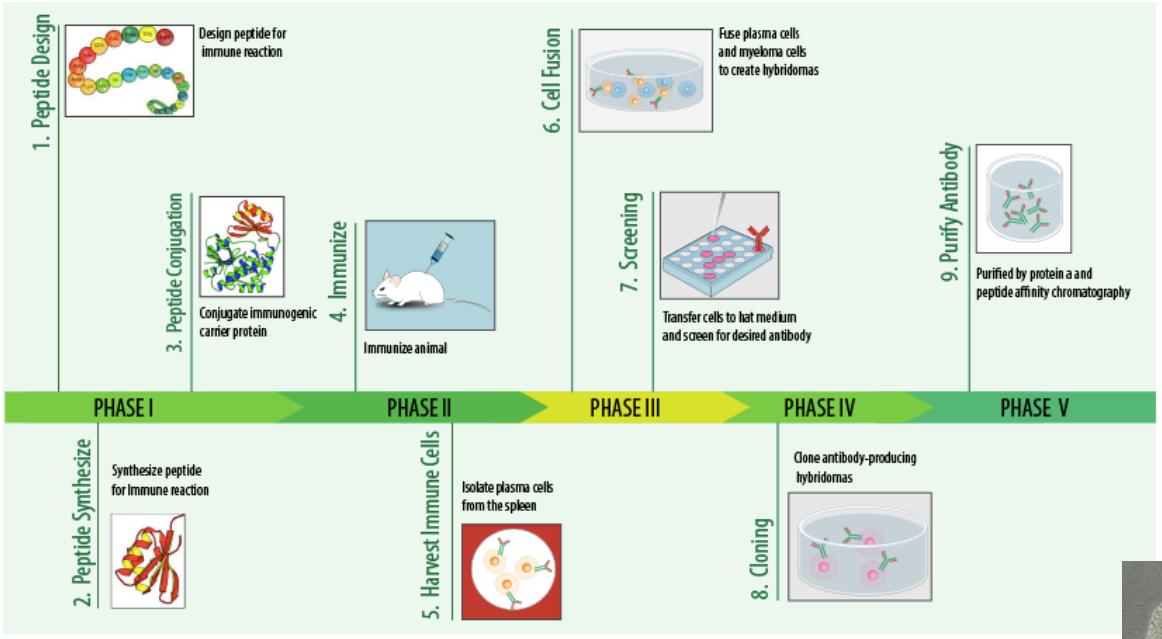
Cytokine release

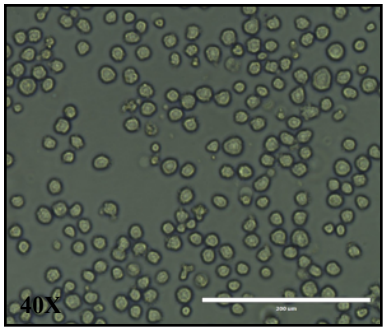
Recombinant Antibody Fragments

"Applications of single-chain variable fragment antibodies

in therapeutics and diagnostics "

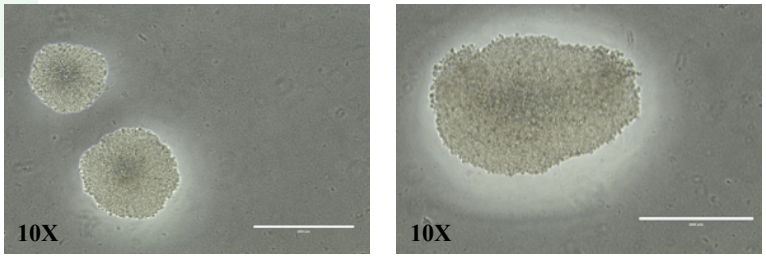
Nina E. Weisser , J. Christopher Hall, ***Biotechnology Adavances*****, 2009**





B5 Hybridoma obtained from cellular fusion

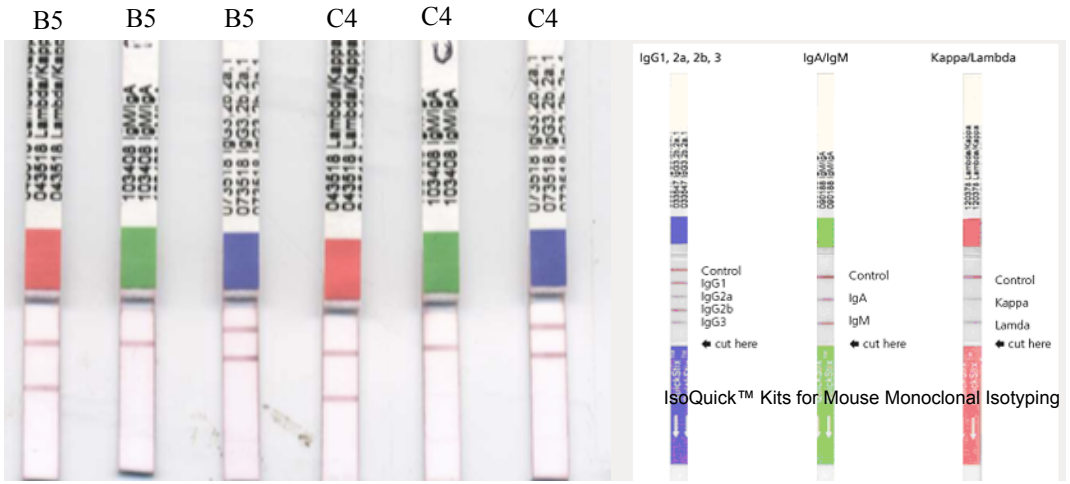
Soft agar cloning



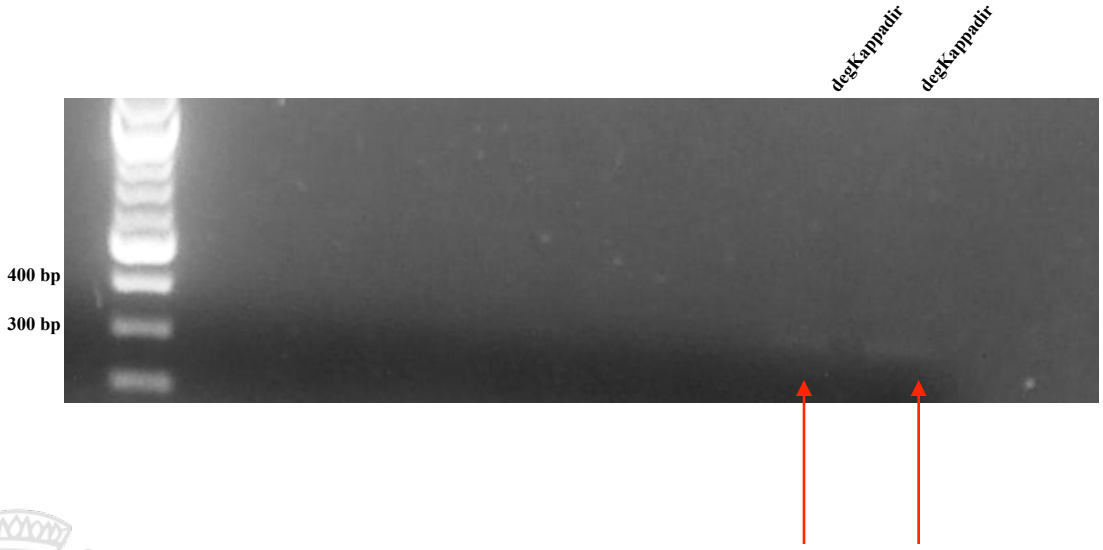
B5 Clones obtained from soft agar cloning

- Production of a **monoclonal antibody** against the *glutamate receptor, GluR4*.
- ***Antibody engineering***: production of scFv, to be used as a diagnostic and drug delivery agents.
- After ***soft agar cloning***, 102 grown clones were picked, grown in 24 well-plates and screened through ELISA assay: 28 gave out of scale absorbance (OD_{450})

Antibody engineering



IsoQuick™ Kits for Mouse Monoclonal Isotyping



B5 clone anti- GluR4 VL isolation

C4 clone anti- GluR4 VL isolation

Antibody Isotyping determination IgG1

↓

RNA extraction and RT-PCR

↓

PCR for VH and VL isolation using degenerated primers (Wang et al. 2000)

↓

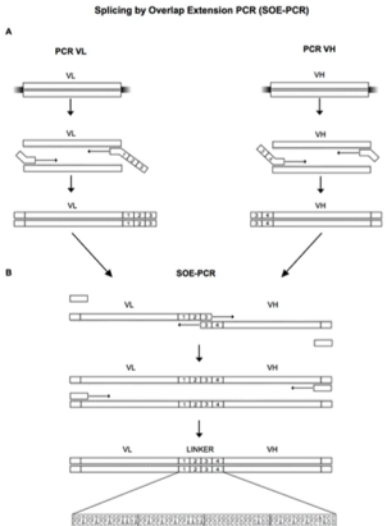
Cloning in a vector for DNA Sequencing, Zero-Blunt Cloning Kit (Invitrogen)

↓

DNA Sequencing and Sequence analysis and to identify the conserved aa determing regions CDR

↓

VH and VL amplification to assemble the scFv construct by Splicing by Overlap extension PCR (SOE-PCR)



- GluR4 antibody engineering to produce a scFv antibody
- ***DRUG DELIVERY SYSTEM*** , *scFv conjugated with nanoparticles*, in order to overcome the problem of the passage through the *blood-brain barrier, BBB* in the treatment of epileptic activity triggered by glia.

What is the purpose of developing mAb and/or scFv?

Table 1 | **Antibody constructs and potential uses in oncology**

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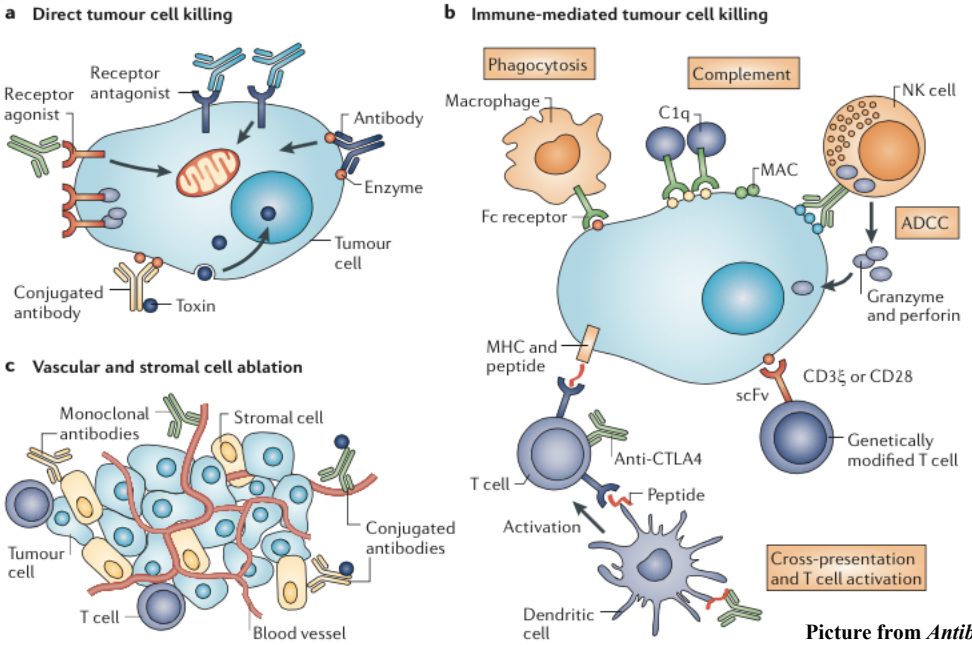
Intact IgG	CD20, CD33, EGFR, ERBB2 and VEGF	Imaging therapy and drug delivery
IgE and IgM	GM2	Therapy
Drug conjugates	CD30, CD33 and ERBB2	Therapy
Loaded nanoparticles	A33, EGFR and transferrin	Drug delivery
Bispecifics	CD19–CD3, EPCAM–CD3 and gp100–CD3	Therapy



Picture from *Antibody therapy of cancer* (Scott et al. 2012).

Antibody expression

www.oncotarget.com Oncotarget, 2018, Vol. 9, (No. 79), pp: 34972-34989

Research Paper

Generation and characterization of novel recombinant anti-hERG1 scFv antibodies for cancer molecular imaging

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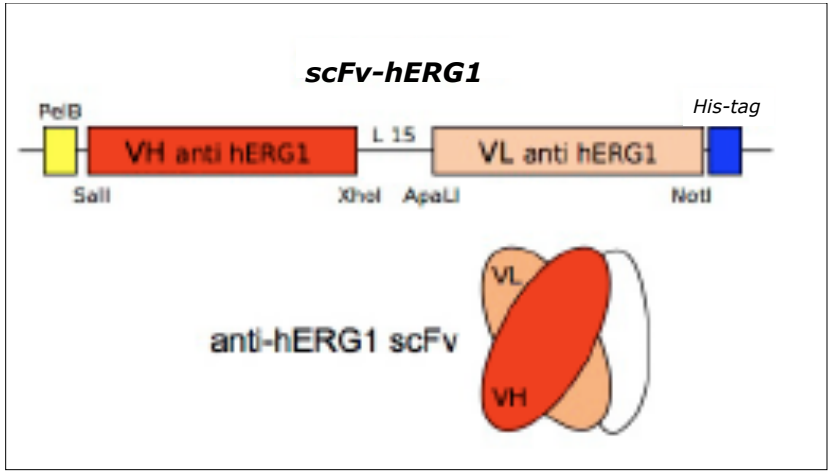
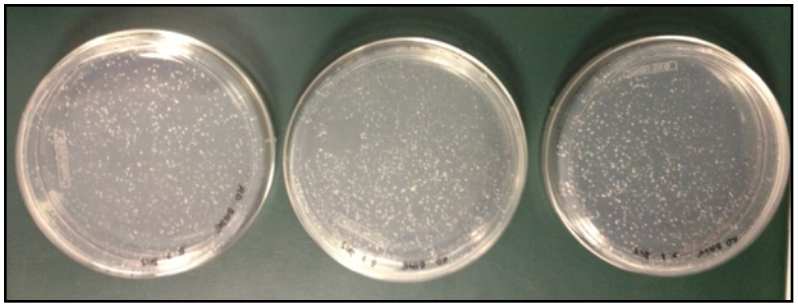
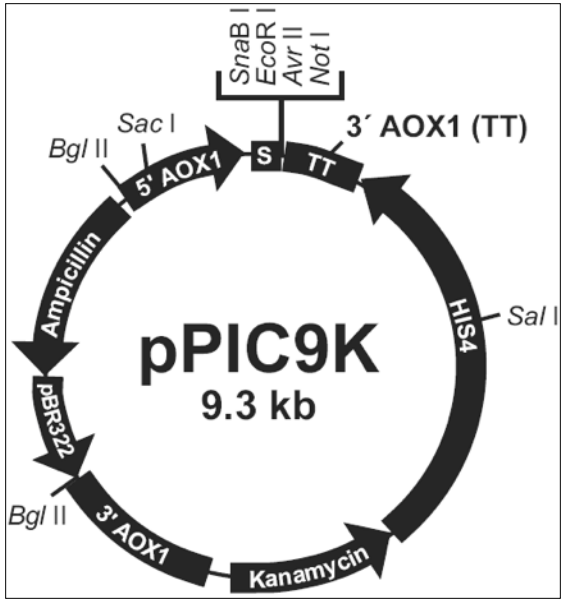
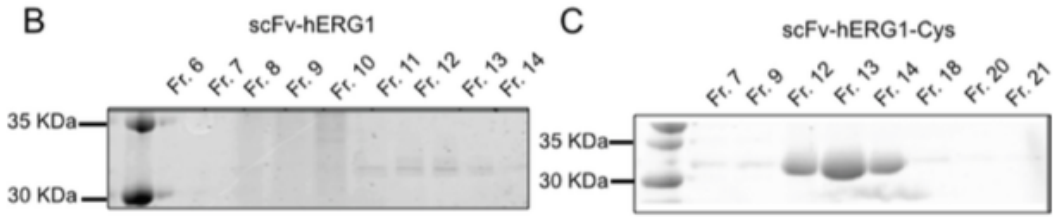
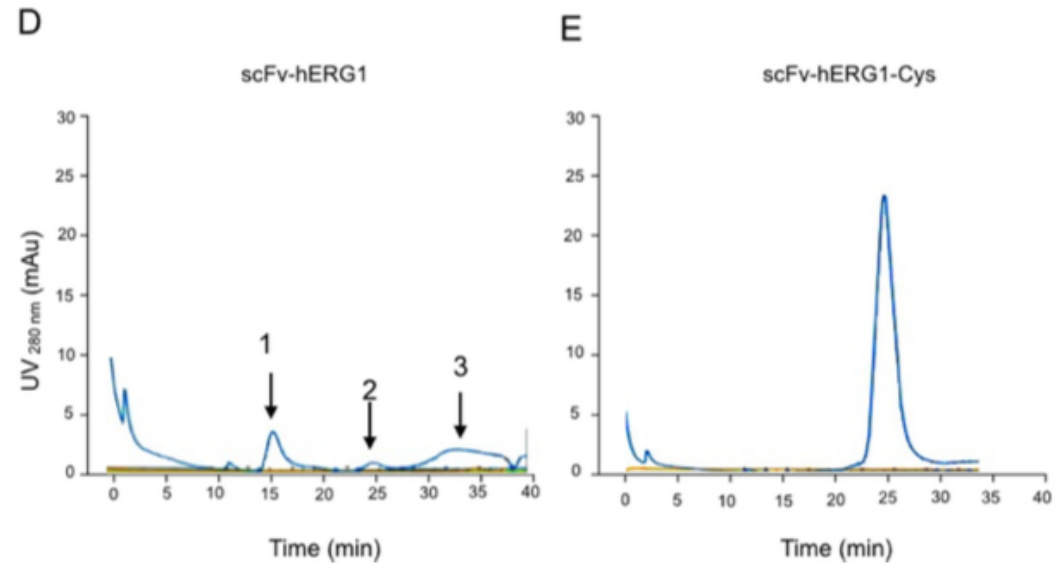




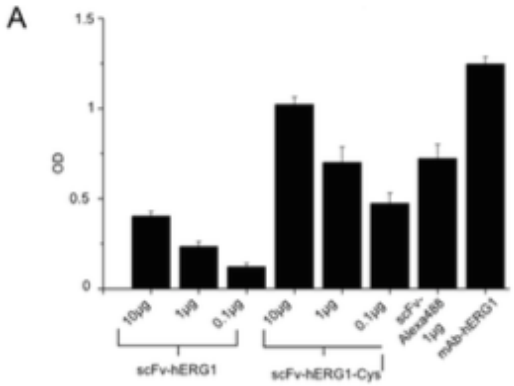


- hERG1 is an ion channel overexpressed in several types of tumors.
- scFv- hERG1 to be used as a diagnostic molecular tool.
- The cDNA of the construct expressing the scFv-hERG1 was cloned into pPIC9K vector to express the protein in *Pichia Pastoris*. The vector has been modified to carry the His tag useful for purification.

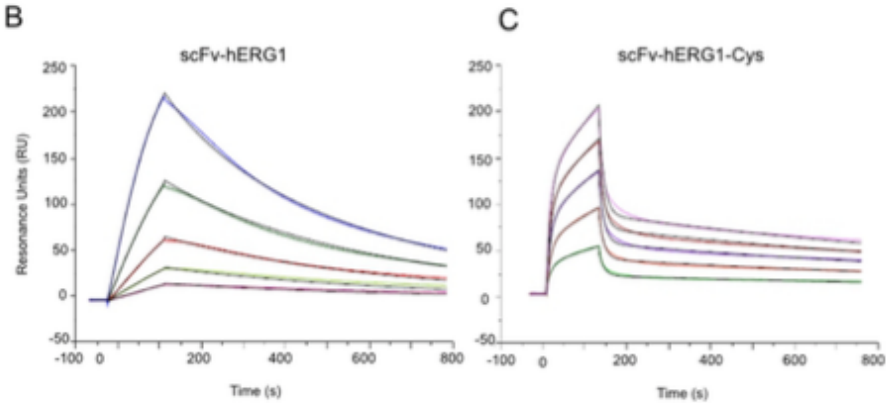


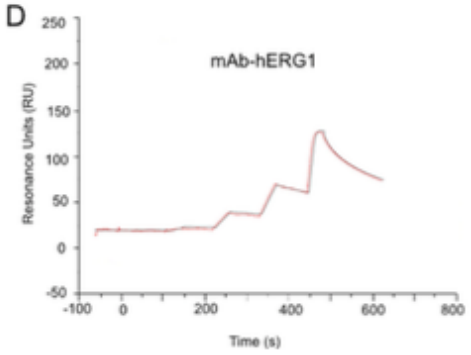


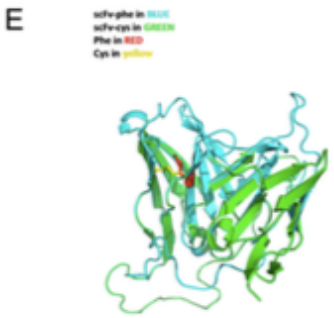
- **in silico studies indicated that there was a point mutation in a peculiar position**



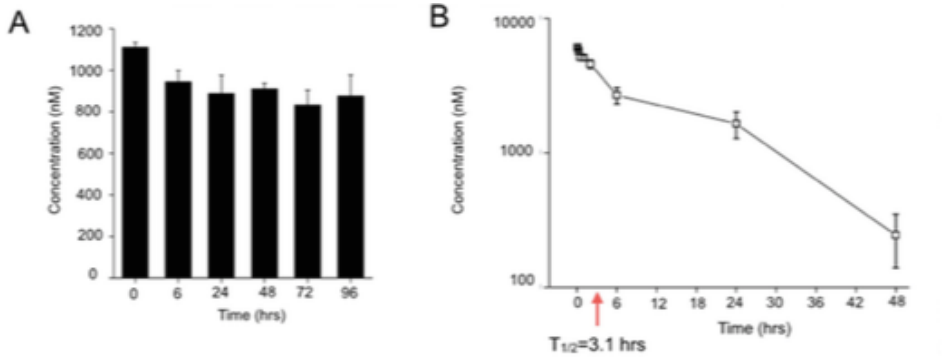
- **we evaluated the binding properties of the two different antibodies**

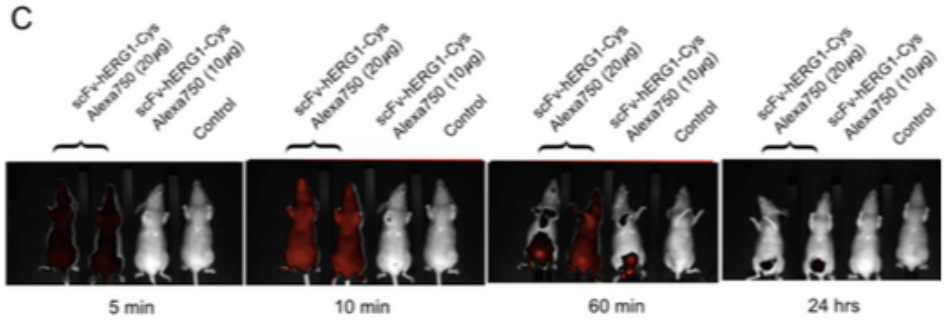


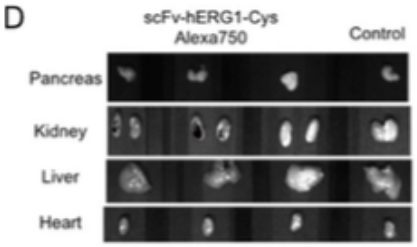


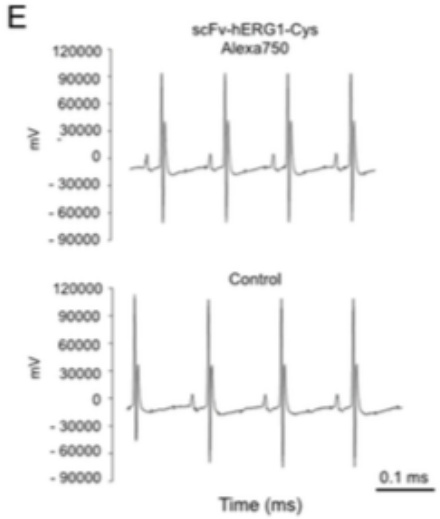


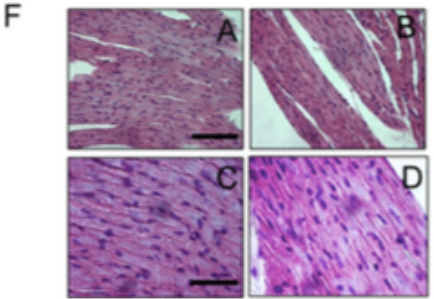
- **scFv is stable;**
- **scFv is cleared;**
- **No signs of toxicity both from ECG and ex vivo analysis**



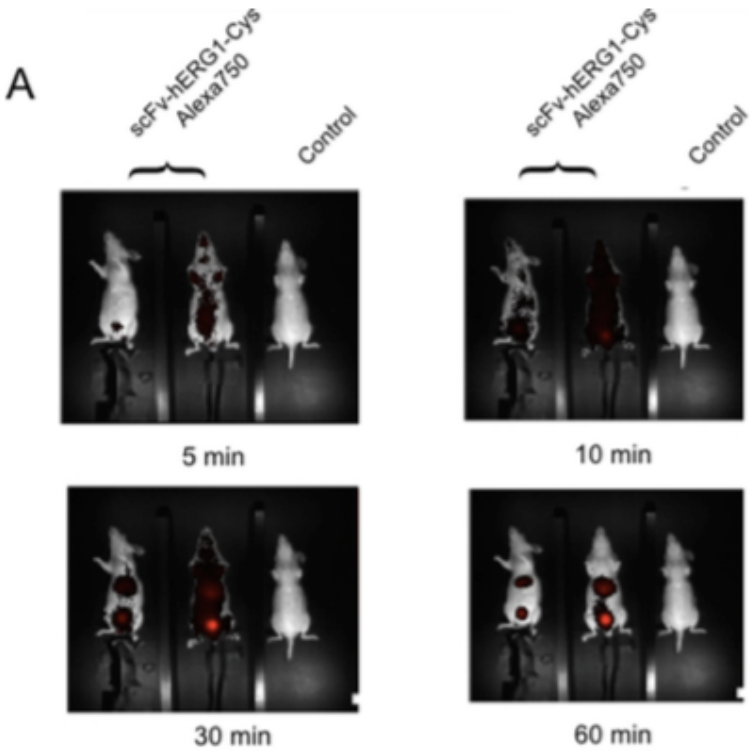


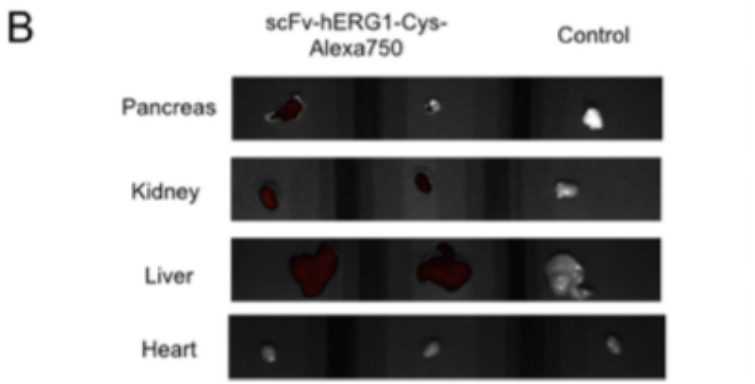




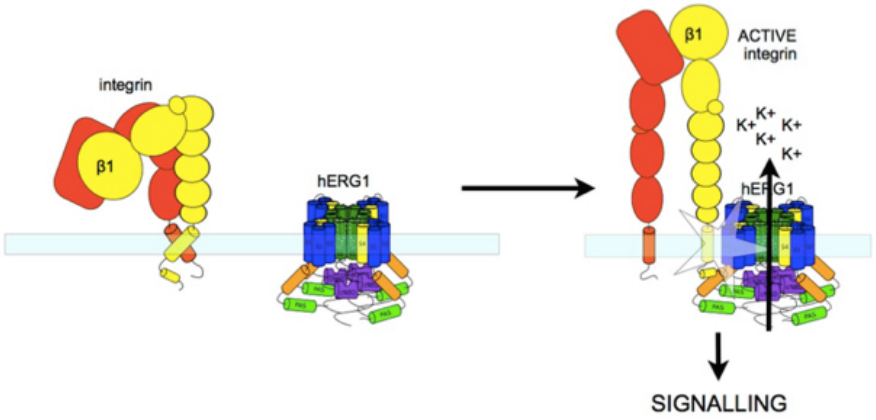


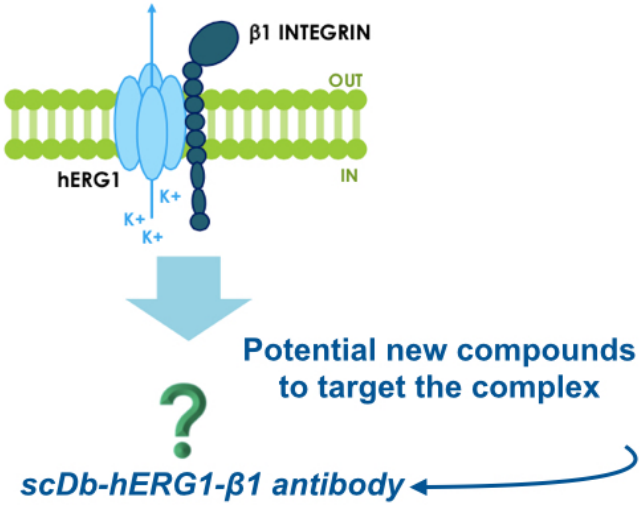
- **scFv as imaging tool tested in PDAC (pancreatic ductal adenocarcinoma) mouse model**

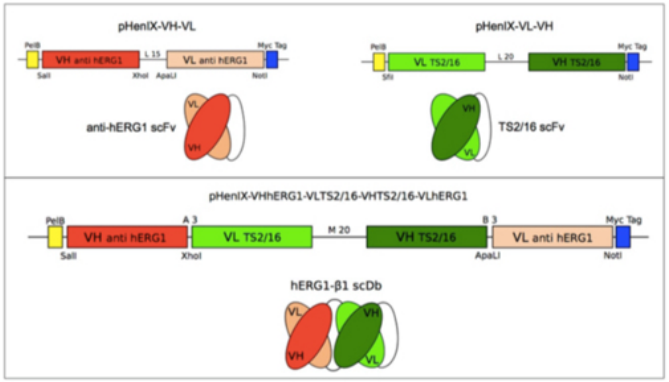




Bispecific antibodies







- Integrin activates hERG1 channel (Arcangeli et al. 1993), and hERG1 channel modulate integrin downstream signalling
- Targeting hERG1 channel in specific macromolecular complexes selectively expressed on cancer cells, such as hERG1/β1integrin complex could be a valid strategy for cancer therapy (Becchetti et al., 2017).

Patent. Inventors: Arcangeli A, Duranti C. et al.

Patent Ref: 102017000083637

- *mAbs*
- *Engeneered antibodies: scFv, bispecifics, T-CAR*

3rd generation of cancer therapeutics

Thank you for your attention!

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human epidermal growth factor receptor Phosphorylation of the tyrosine kinase domain by means of homodimerization or heterodimerization induces both cell proliferation and survival signaling HER2 is the preferred dimerization partner for the other HER family members. The phosphorylated (activated) tyrosine residues on the intra- cellular domain of HER2 activate the lipid kinase phos- phoinositide 3-kinase (PI3-K)

Binding of trastuzumab to a juxta- membrane domain of HER2 reduces shedding of the extracellular domain, thereby reducing p95 (Panel C). Trastuzumab may reduce HER2 signaling by physically inhibiting either homodimerization, as shown, or het- erodimerization

Bevacizumab acts by selectively binding circulating VEGF, thereby inhibiting the binding of VEGF to its cell surface receptors. This inhibition leads to a reduction in mi- crovascular growth of tumor blood vessels and thus limits the blood supply to tumor tissues. These effects also lower tissue interstitial pressure, increase vascular permeability, may increase delivery of chemotherapeutic agents, and fa- vor apoptosis of tumor endothelial cells