Development of (bio)pharmaceuticals: structure and formulation considerations

EPSA Autumn Symposium 2014
29 Oct 2014, Hradec Kralove (CZ)

Dr. Amon R. Wafelman
EIPG SIG Production, co-chair /
QP Parenterals, TEVA Haarlem (NL)

Pharma Industry from bench to bedside

Computational chemistry, gene isolation + transfection
Biopharmaceuticals more complex

Drug discovery

Target finding, genomics

Initial characterization

Engineering batch(es)

Pre-clinical trials

PD, toxicology, translation to F-i-H, MABEL

Regulatory approval sought to commence trials in humans

Upgrade components, scale up, cleaning validation, media fill, qualification batch(es), analytical validation (partial)

PBPK/PD modeling, genotyping

Clinical trials (Phases I, II & III)

Process validation/registration batches, analytical validation (full)

Clinical trial feedback

Submission of marketing/manufacturing authorization applications to regulatory authorities

Regulatory authorities review information and grant (or refuse) marketing/manufacturing licences

Commercial batches, gowning, media fill, PQR/APR

Product goes on sale

Post-marketing surveillance

Complaints, adverse reactions

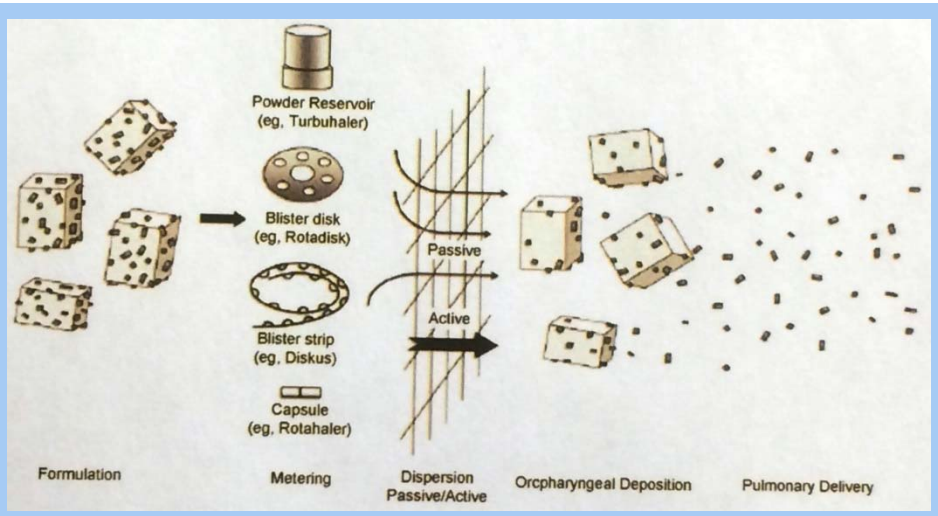
Adapted from: Walsh G. Biopharmaceuticals: biochemistry and biotechnology. J.Wiley&Sons, Chichester (UK)1998, p. 38

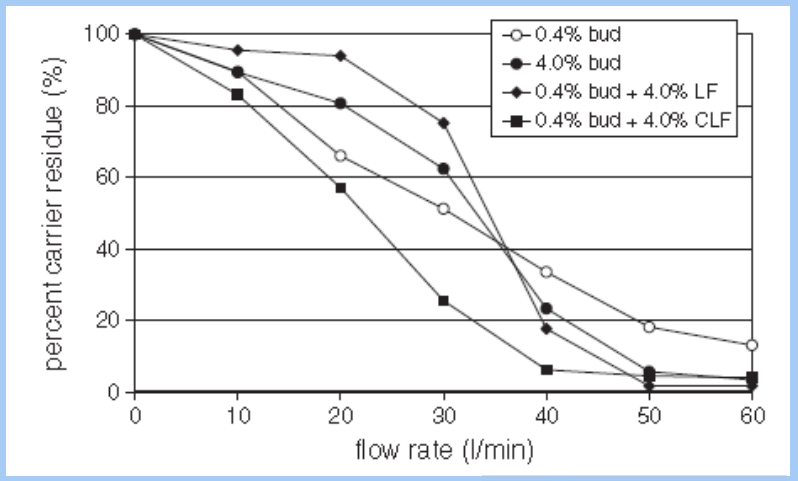
Multi-dose dry powder inhalers

EMA Guideline (2006) on pharmaceutical quality of inhalation products:

Necessary development studies a.o.
- Delivered dose uniformity + fine particle mass → through container life
- Delivery device development → over patient flow rate range

Material	Quality	x_{10}(±SD)	x_{50}(±SD)	x_{90}(±SD)	
Respitose® SV003	Sieved	14.13 µm (±4.91 µm)	56.76 µm (±0.74 µm)	93.04 µm (±1.44 µm)	Ternary
Lactohale® LH 300	Micronized	0.90 µm (±0.00 µm)	3.25 µm (±0.09 µm)	7.46 µm (±0.18 µm)	Ternary
Budesonide	Micronized	0.41 µm (±0.02 µm)	1.35 µm (±0.01 µm)	3.70 µm (±0.04 µm)	Binary





Cordts E et al. Eur J Pharm Biopharm (2012).
Pilcer G et al. Int J Pharmaceutics (2010).
Boer de A et al. Adv Drug Del Rev (2012)

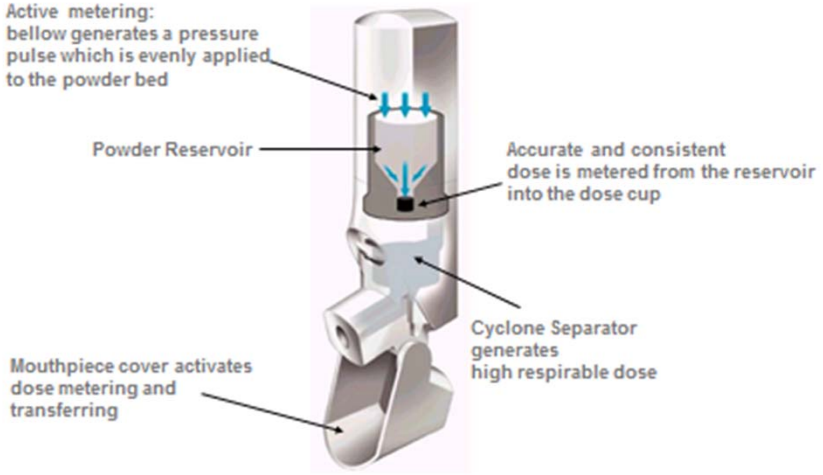
GPIE EIPG

3 Introduction to DuoResp® Spiromax®

- DuoResp® Spiromax® contains the same drug substances as AstraZeneca's Symbicort® Turbuhaler®
  - A blend of budesonide and formoterol with lactose as excipient

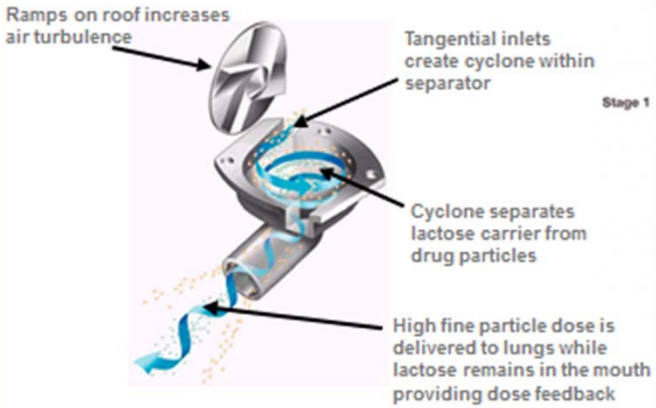
4 Spiromax® Innovative Operating Mechanism

- Active metering and cyclone separator technology differentiates Spiromax® from other inhalers



5 Spiromax® Innovative Operating Mech

- Cyclone Separator Creates Lung Dose



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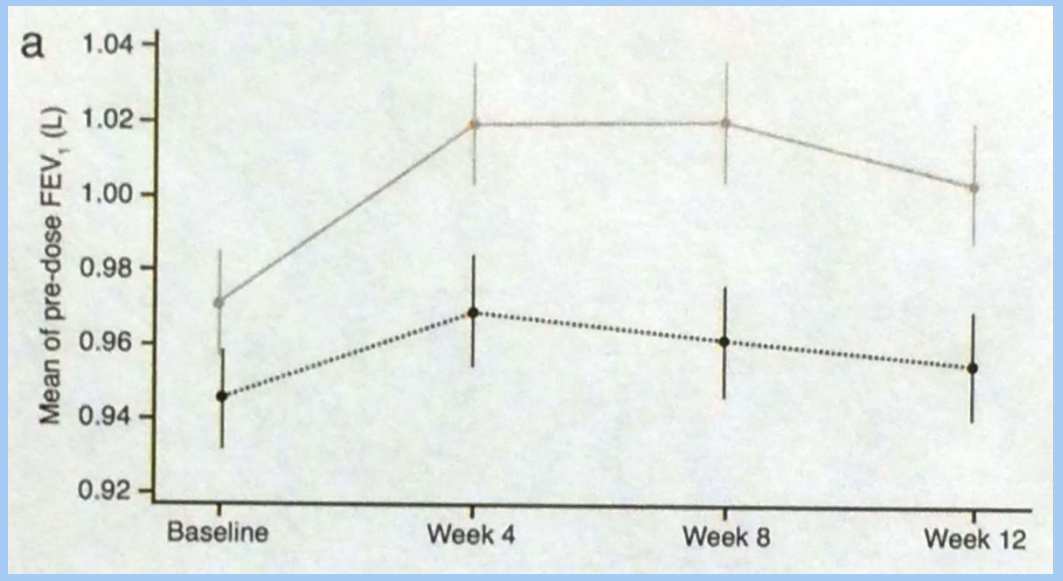
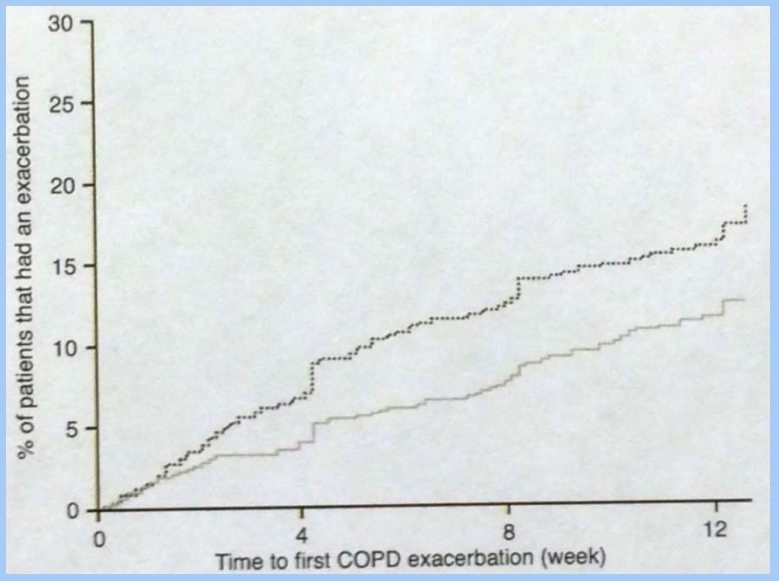
Phase III clinical trial Symbicort vs. formoterol





Grey, solid = budesonide 160 µg/formoterol 4.5 µg bid (via Turbuhaler, Symbicort)
Black, dotted = formoterol 4.5 µg bid (via Turbuhaler)

Fukuchi Y et al. Respirology (accepted in 2013).

In 2014 EMA approved TEVA's DuoResp Spiromax.

Comparable quality and bioequivalent to comparator Symbicort Turbuhaler.

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Injectable (bio)pharmaceuticals

Small molecules

- Levothyroxine: 777 Da (Mw)
  - Raw materials (tablets): 6
  - Analyses per lot: 67
- Chemically synthesized
- No immunogenicity
- Test bacteria, yeast/mld, BET
- In general stable
- 20th century: chemistry

Biopharmaceuticals

- rt-PA (Actilyse®): 64 kDa
  - Raw materials (inj.): 76
  - Analyses per lot: >700
- Produced by (micro)organisms
- Immunogenicity is a challenge
- See sm + virus, mycopl, TSE
- Beware of temp. + aggregates
- 21st century: biology

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Biosafety

Biologicals

Biopharmaceuticals

Efficacy/dose frequency

1st generation Biopharmaceuticals

Later generation Biopharmaceuticals

Adverse effects

Doxorubicin

Liposomal Doxorubicin

Targeting needs

WBC upregulation: NO

Cytostatics to tumour: YES

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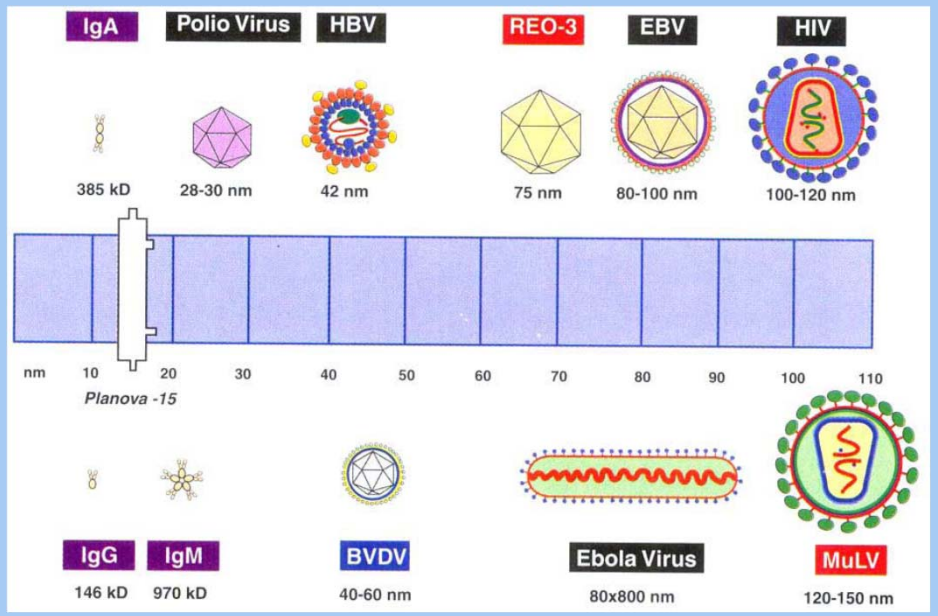
Biosafety

Table 1. Examples of the transmission of infectious agents by biological medicinal products

Material	Date	Incident	Cause	Solution	Ref.
Yellow fever vaccine	1947	Transmission of hepatitis B virus	Contaminated albumin stabilizer	Screen donors, pasteurize albumin	4
Polio vaccine	1954–61	Possible transmission of SV40	Infected cell substrates from animals caught in the wild	Screen animals, cells and final product for SV40	5
Polio vaccine	1955	Transmission of polio virus (Cutter incident)	Imperfectly inactivated virus	Change process to remove aggregates of virus; check final product for live virus	6
Clotting factors	1980–85	Transmission of human immunodeficiency virus (HIV)	Infected donors	Screen donors; validate process for virus removal	7,8
Growth hormone	1985	Transmission of Creutzfeldt-Jakob disease	Contaminated human pituitary glands	Produce hormone in bacteria by recombinant DNA methodology	9,10



Minor PD. TIBTECH 1994;12:257-61.

Maerz H et al. Nat Biotech 1996;14:651-2.

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Biosafety

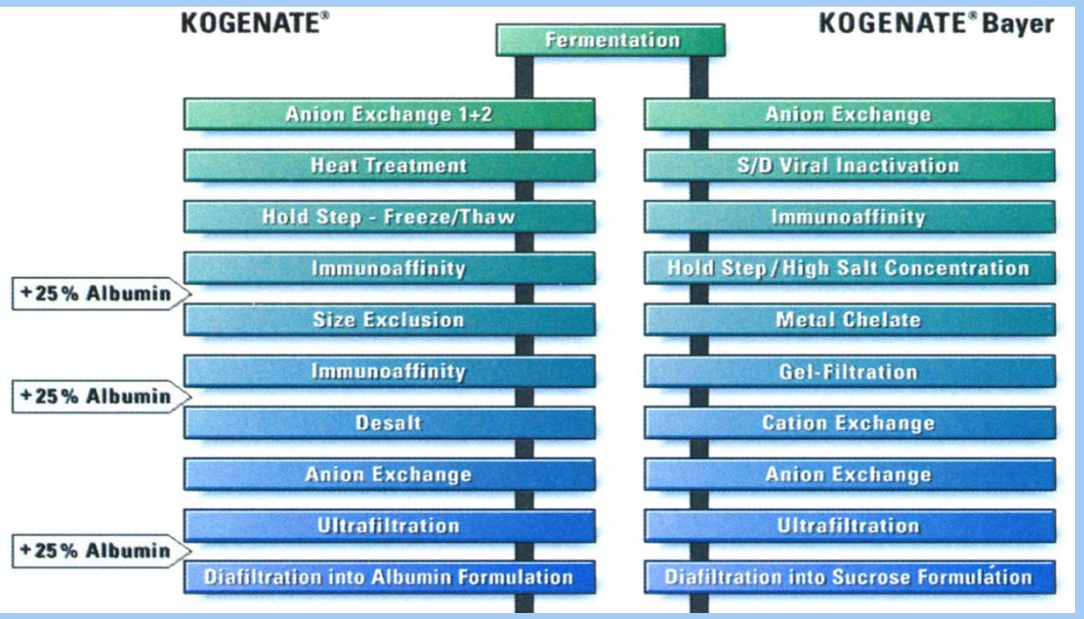
- Nanofiltration
- Animal derived component free production

Table 1. Currently available FIX concentrates.

Product (manufacturer)	FIX product type	Methods of purification	Methods of viral inactivation
Aimafix (Kedrion)	Plasma-derived	Anion exchange, DEAE sephadex/sepharose, heparin affinity chromatography	Solvent/detergent, dry heat (100°C for 30 min)
AlphaNine SD (Grifols)	Plasma-derived	Ion exchange and dual polysaccharide ligand chromatography	Solvent/detergent, nanofiltration
Berinin P (CSL Behring)	Plasma-derived	Multiple precipitation and adsorption steps, DEAE-sephadex, heparin affinity chromatography	Pasteurization (60°C for 10 h)
BETAFACT (LFB)	Plasma-derived	Ion exchange and affinity chromatography	Solvent/detergent, nanofiltration
Factor IX (Grifols)	Plasma-derived	Precipitation and multiple chromatography	Solvent/detergent, nanofiltration
Haemonine (Biotest)	Plasma-derived	Anion exchange, immunoaffinity and hydrophobic interaction chromatography	Solvent/detergent, nanofiltration
Hemo-B-RAAS (Shanghai RAAS)	Plasma-derived	Ion exchange and affinity chromatography	Solvent/detergent, dry heat, nanofiltration
Immunine (Baxter BioScience)	Plasma-derived	Ion exchange and hydrophobic interaction chromatography	Detergent, vapor heat (60°C for10 h, then 80°C for 1 h)
Mononine (CSL Behring)	Plasma-derived	Immunoaffinity chromatography	Sodium thiocyanate, ultrafiltration
Nanotiv (Octapharma)	Plasma-derived	Ion exchange and affinity chromatography	Solvent/detergent, nanofiltration
Nonafact (Sanquin)	Plasma-derived	Immunoaffinity and hydrophobic interaction chromatography	Solvent/detergent, nanofiltration
Octanine F (Octapharma)	Plasma-derived	Ion exchange and affinity chromatography	Solvent/detergent, nanofiltration
Replenine-VF (BioProducts Laboratory)	Plasma-derived	Metal chelate chromatography	Solvent/detergent, nanofiltration
TBSF FIX (CSL Biotherapies)	Plasma-derived	Ion exchange and heparin affinity chromatography	Solvent/detergent, nanofiltration
ALPROLIX (Biogen Idec)*	Recombinant	Affinity chromatography	Nanofiltration
BeneFIX (Pfizer)	Recombinant	Anionic chromatography	Nanofiltration
Rixubis (Baxter)	Recombinant	Chromatography	Solvent/detergent, nanofiltration

*Fc fusion protein with prolonged half-life.
FIX: Factor IX.

Mannucci PM et al. Exp.Opin.Emerg.Drugs 2014;19:407-14.



Pharmaceutical Visions 2001:35.

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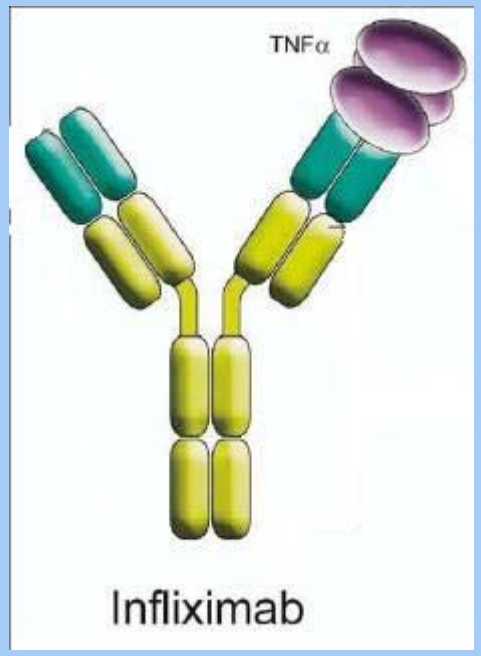
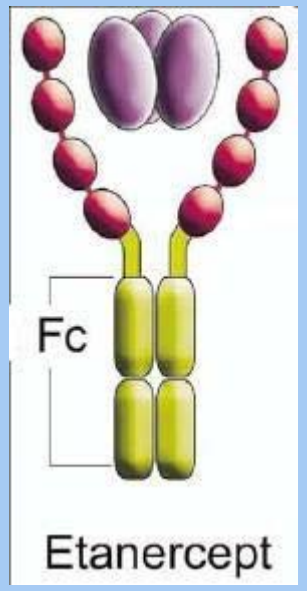


Sources: Anderson PJ. Semin Arthritis Rheum 2005;34(Suppl1):19-22

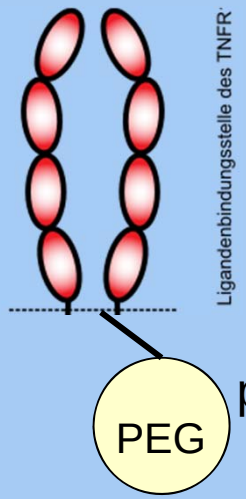
Melmed GY et al. Nat Rev Drug Discov 2008;7:641-2

Dingermann Th, Zündorf I. Biotechnol J 2006;1:47-57

MAb or Fab



Fusion protein or PEG-soluble cytokine receptor



pegsunercept

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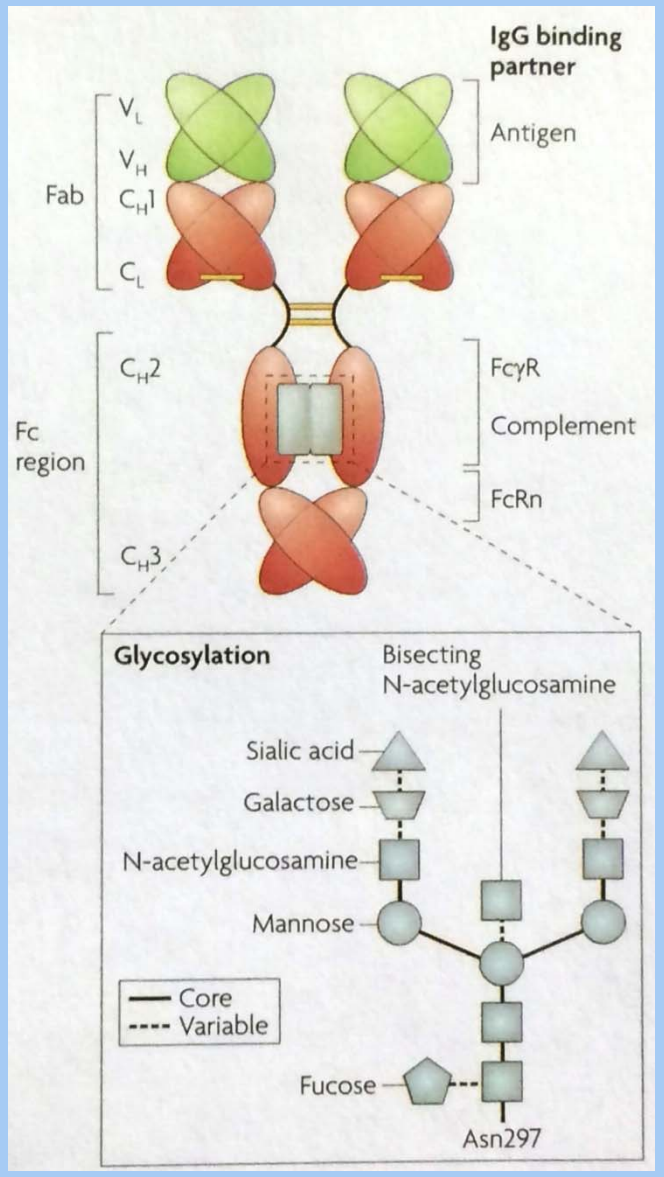
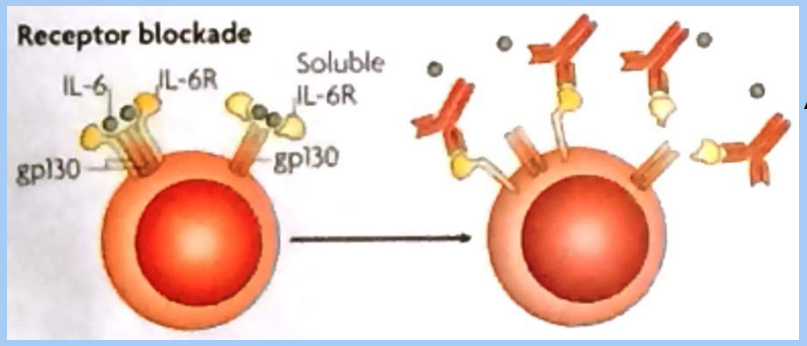
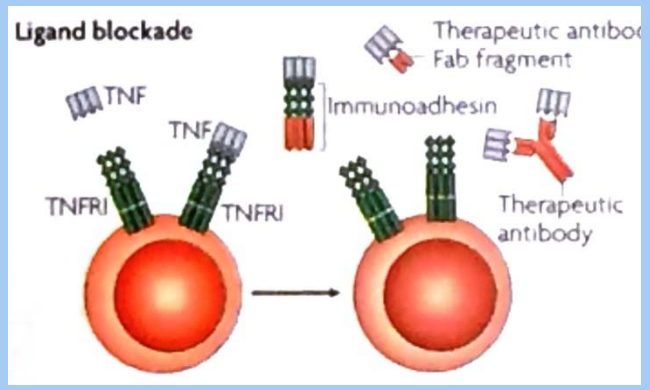
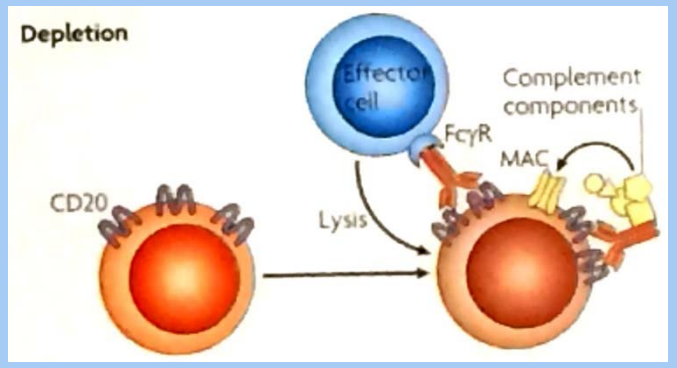




Abciximab



Infliximab
Etanercept
Certolizumab pegol
Rituximab



Rituximab

Chan AC, Carter PJ. Nat. Rev. Immunol 2010;10:301-16.

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Table 1: Pharmacologic data for several mAbs, a Fab, a PEGylated Fab, fusion proteins* and a PEGylated soluble cytokine receptor

mAb/Fab/FusProt/sCR	$T_{½}$ (plasma)	Immunogenicity	Target; indication	References
muro**mo**nab-CD3 Orthoclone-OKT3 **M**	18 h	80%	CD3; rejection transplant	[30, 55]
abci**xi**mab Reopro **Fab**	20–30 m	6%	GP2b/3a; profylaxis cardiac ischemia	[30, 32, 35]
ritu**xi**mab Mabthera **M**	3–17 d	1%	CD20; B-cell lymphoma	[30, 55]
infli**xi**mab Remicade **M**	8–10 d	8†-43% RA pat. 61% Crohn pat.	TNFα; RA, M. Crohn	[55-58]
trastu**zu**mab Herceptin **M**	6–28 d	0%	HER2/neu; breast cancer	[30, 59]
Alemtu**zu**mab Campath **M**	12 d	2% CLL pat. 63% RA pat.	CD52; CLL	[30]
Certoli**zu**mab pegol Cimzia **PEG-Fab**	14 d	8%	TNFα; M. Crohn	[60, 61]
abatacept Orencia **FuPr**	13 d	3%	CD80 and -86; RA	[62, 63]
etanercept Enbrel **FuPr**	3–5 d	6%	TNFα; RA, psoriasis	[56, 64-65]
pegsunercept **PEG-sCR**	3 d	5%	TNFα; RA	[34]
adali**mu**mab Humira **M**	14 d	1†–17%	TNFα; RA	[30, 66]
panitu**mu**mab Vectibix **M**	8–16 d	0%	EGFR; solid tumours	[31, 67,68]

*Fusion protein = a soluble (= extracellular domain of a) cytokine receptor connected to the Fc of a mAb. As for abatacept, the cytokine receptor is the human cytotoxic T-lymfocyte-associated antigen-4 (CTLA-4) receptor; as for etanercept, the cytokine receptor is a tumour necrose factor α-receptor. †These patients were also administered methotrexate. h: hours; m: minutes; d: days; TNFα: tumour necrose factor α; EGFR: epidermal growth factor receptor.

Wafelman AR. EurJHospPharm Practice 2011;17:30-35.

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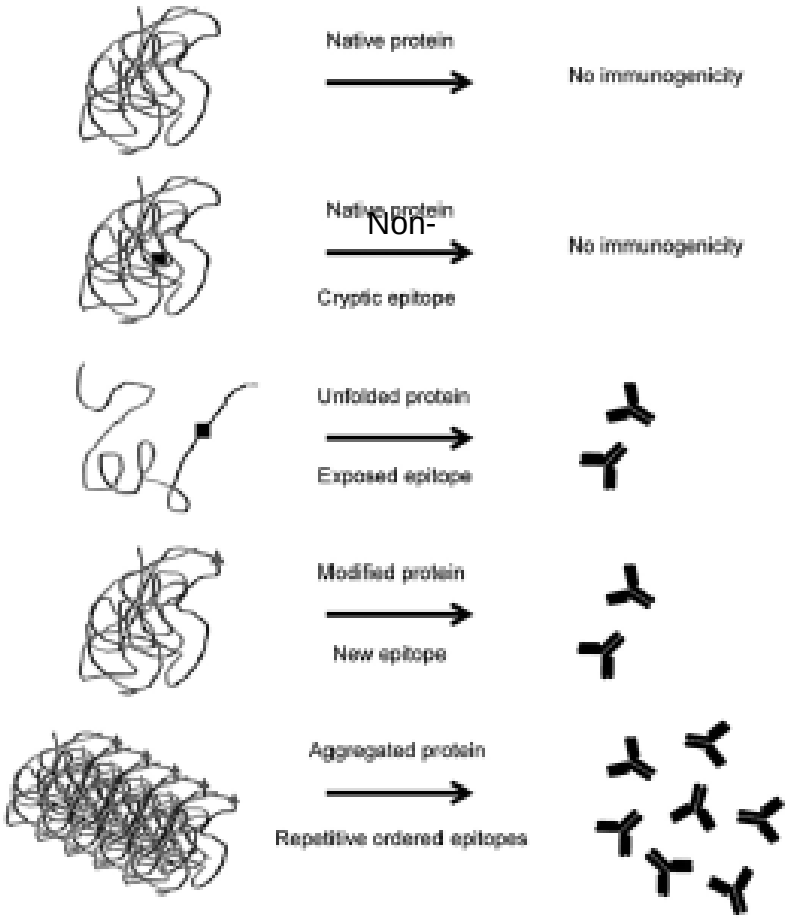
Immunogenicity of biopharmaceuticals



Small molecules escape immune surveillance

Immunogenicity of mAbs predom. vs. Fc (isoforms of amino acids/glycans)

Anti-Drug-Antibodies: shorter T½, lack of efficacy; worst case cross react with endogenous proteins.

Aggregates greatly enhance immunogenicity.

Beers van MMC et al. Biotechnology Journal (2012).
Krämer I. J Endocrinol Invest (2008).

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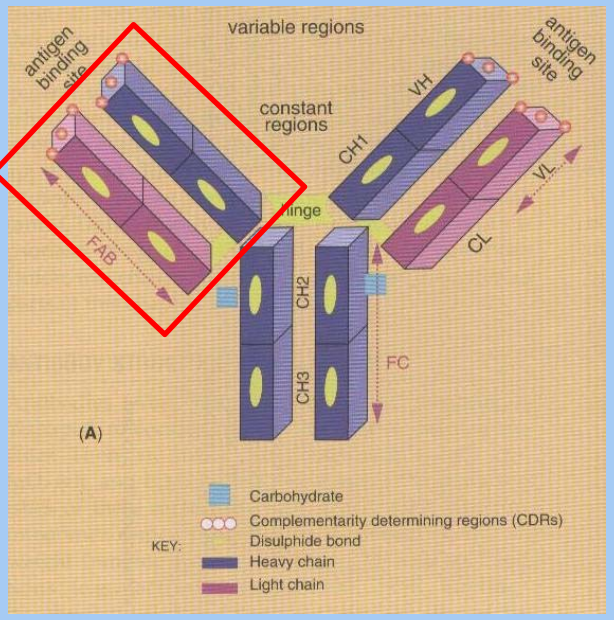
Abciximab (ReoPro)

- Inhibits platelet aggregation by binding to the platelet GPIIb/IIIa rec.
- Indication: percut. coronary intervent.



Abciximab was developed as a Fab for the following reasons:

- short t½ (20-30 min) is for this indication an advantage: easy to titrate
- Fc is not needed: cytotoxic functions CDC, ADCC unwanted
- A missing Fc reduces immunogenicity of abciximab
- Abciximab – without Fc (glycosylated!) – is produced by E.coli

Crommelin DJA, Sindelar RD (eds.). Pharmaceut. Biotechnol., p. 314, 2008

Newsome BW, Ernstoff MS. Br J Clin Pharmacol 2008;66:6-19

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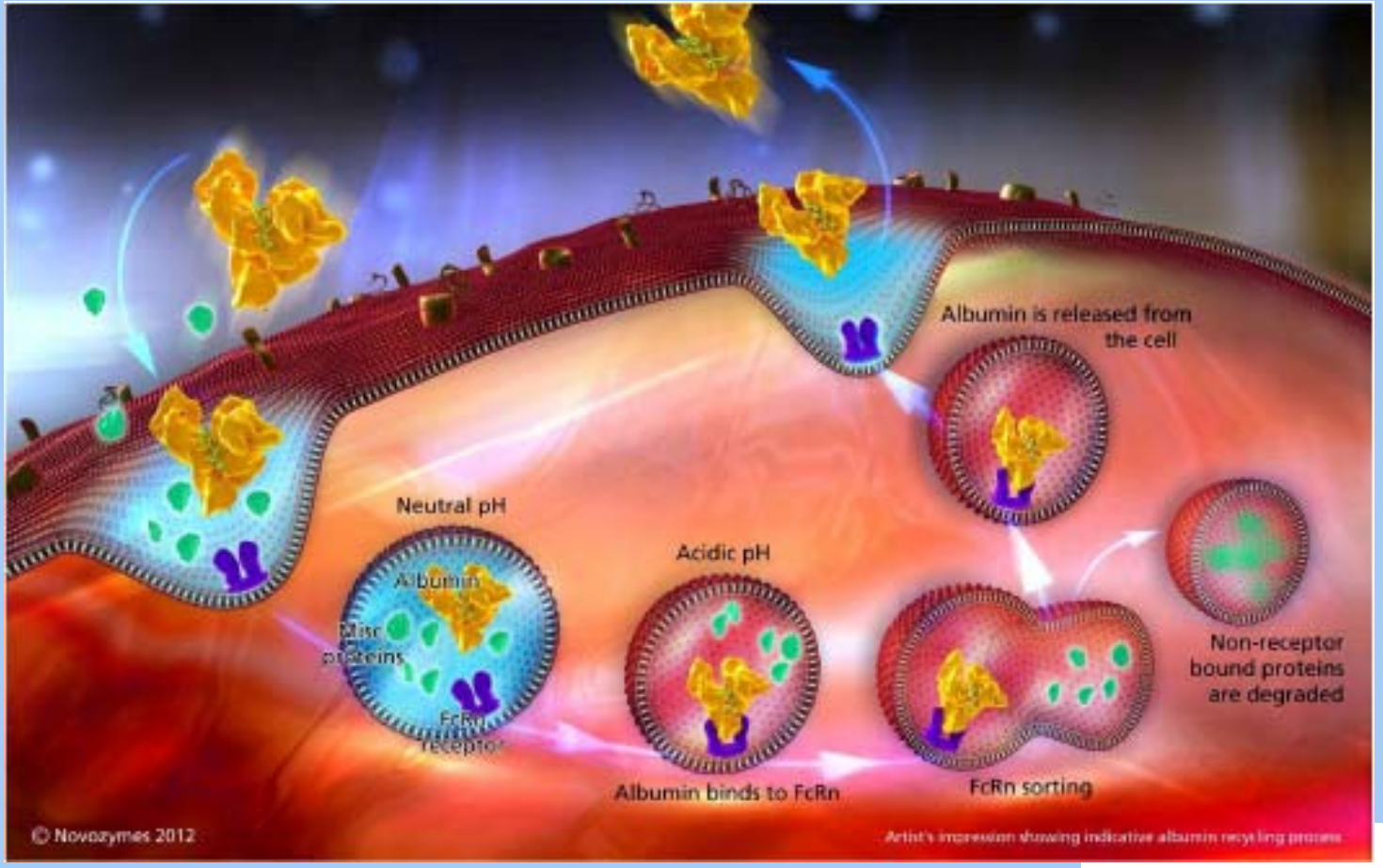
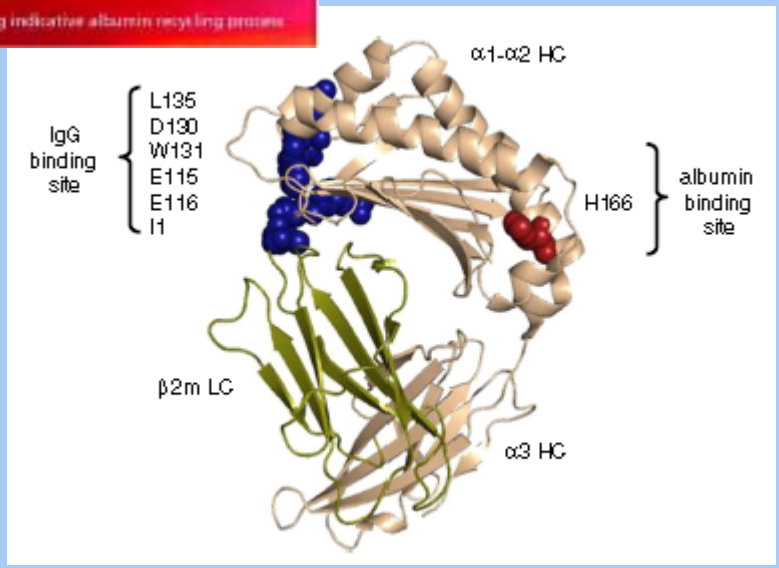
PEG-ylation

product	actief	dosering	top/dalspiegel ratio	bijwerkingen
(Roferon-A®)	interferon-α 2a	3x /wk	>40	
(Pegasys ®)	m-PEG-Interferon α 2a	1x /wk	1½	
(Intron A®)	interferon-α 2b	3x /wk	>40	
(PEG Intron®)	interferon-α 2b	1x /wk	6	
Oncaspar®	PEG-asparaginase 2000-2500 IU/m²	1x /2-4wk		(antilichaam-vorming) 5-18%
Paronal®	E coli-asparaginase 6000 IU/m²	3x /wk		(antilichaam-vorming) 45-75%
Neulasta®	pegfilgrastim			(neutropenie, koorts) 9%
Neupogen®	filgrastim			(neutropenie, koorts) 18%

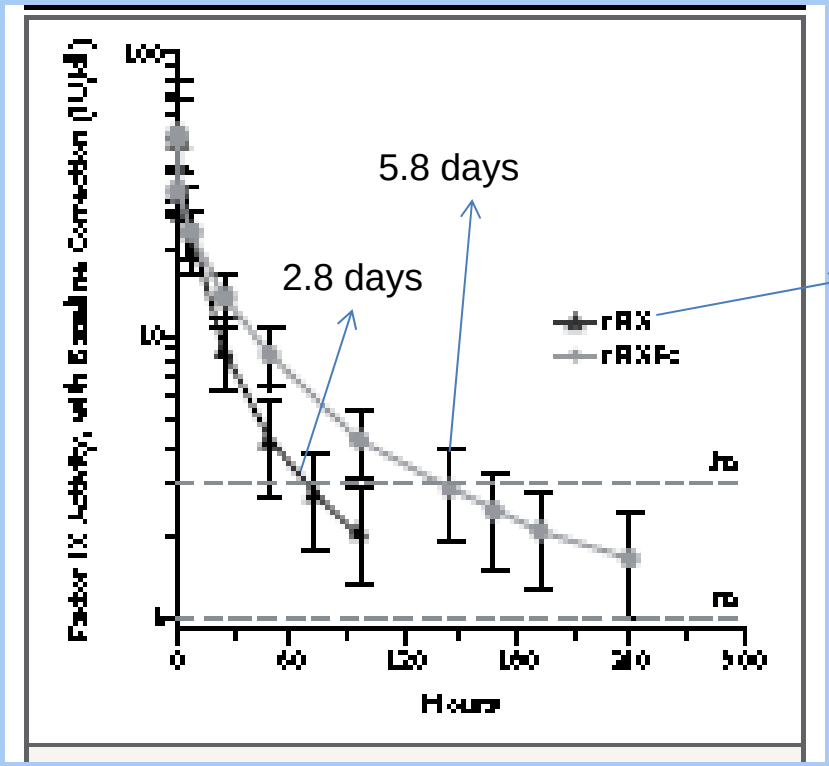
Wafelman AR in summary book Anselmus Colloquium 2008.





Sleep D et al. Biochim. Biophys. Acta (2013).

Phase III non-randomized clinical trial rec factor IX Fc fusion protein in Hemophilia B



1-2x daily dosing

<3%: moderate hemophiliaB

<1%: severe hemophiliaB

Conclusion: prophylactic rFIXFc, 1/wk or /2wk: low annualized bleeding rates in hemoph.B patients.

Powell JS et al. New Engl J Med (2013).

Phase III double-blind randomized clinical trial of balugrastim vs. pegfilgrastim

Balugrastim is the result of genetic fusion of r-HSA and G-CSF

Table 2 Incidence and Duration of Severe Neutropenia and Incidence of Febrile Neutropenia in Cycle 1 (Double-Blind Phase)

Parameter	Pegfilgrastim 6 mg	Balugrastim 40 mg	*P* Value (95% CI)
PP Population			
N	148	150	–
Incidence of severe neutropenia, n (%)	87 (58.8)	87 (58.0)	.907 (–11.98-10.41)
Mean DSN, d (SD)	1.0 (1.08)	1.1 (1.13)	(–0.13-0.37)
Incidence of febrile neutropenia, n (%)	4 (2.7)	2 (1.3)	.446

Table 3 Absolute Neutrophil Count Nadir, Time to Absolute Neutrophil Count Nadir, and Time to Recovery in Cycle 1 (Per-Protocol Population)

	Double Blind			Balugrastim 40 mg	
Parameter	Pegfilgrastim 6 mg	Balugrastim 40 mg	*P* value (95% CI)[b]	Open Label	All
N[a]	148	150	–	77	227
ANC nadir × 10^9/L, mean (SD)	0.8 (1.04)	0.8 (1.17)	.763 (–0.21-0.29)	0.8 (1.01)	0.8 (1.12)
Time to ANC nadir, d, mean (SD)	6.7 (3.33)	6.8 (2.90)	.963 (–0.69-0.73)	6.5 (2.32)	6.7 (2.71)
Time to ANC recovery (≥1.5 × 10^9/L), d, mean (SD)	2.1 (0.96)	2.0 (0.94)	.259 (–0.37-0.10)	1.9 (0.88)	1.9 (0.92)

Conclusion: comparable safety, noninferiority with regard to duration of severe neutropenia (DSN)

Volovat C et al. Clin Breast Cancer (2014).

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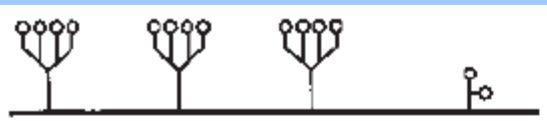
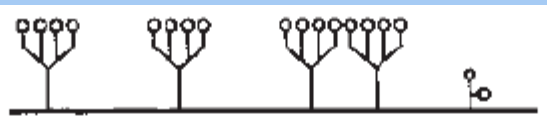
Hypersialylation:
novel erythropoetin stimulating protein (NESP)



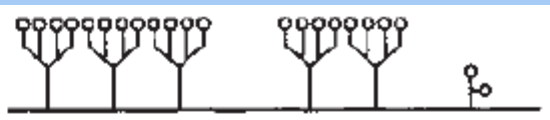
rHuEPO
3 N-linked carbohydrate chains

- Up to 14 sialic acids
- 30 400 Daltons
- ~40% carbohydrate
- pI ~4.0



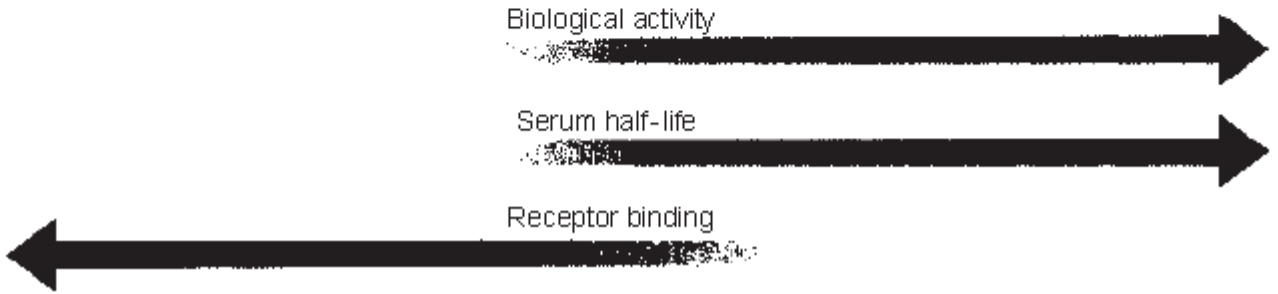
4 N-linked carbohydrate chains

- Up to 18 sialic acids
- ~33 750 Daltons
- ~46% carbohydrate
- pI ~3.65



NESP
5 N-linked carbohydrate chains

- Up to 22 sialic acids
- 37 100 Daltons
- ~51% carbohydrate
- pI ~3.3



Egrie JC, Browne JK. Br J Cancer 2001;84(Suppl.1):3-10.

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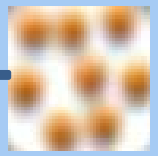
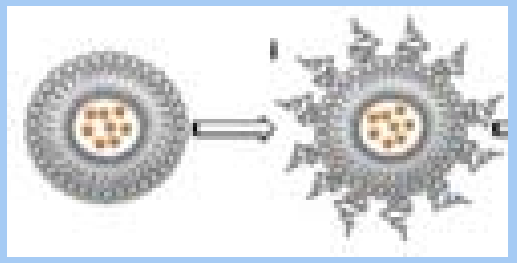
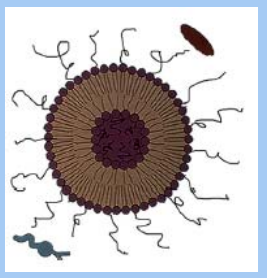
From DOX to liposomal DOX to PEG-liposomal DOX…

Cardiotoxicity





Doxil ® superior over doxorubicin in Kaposi's sarcoma Northfelt et al. J Clin Oncol (1998)



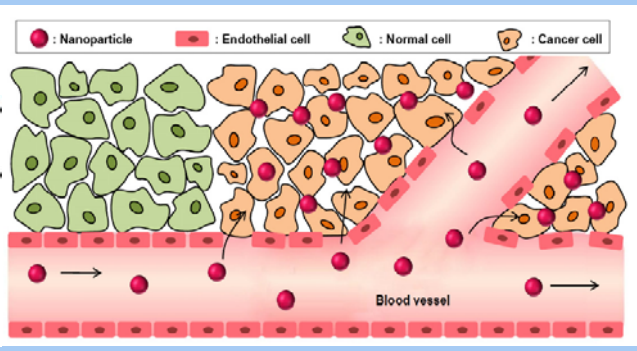
Facilitates breakdown by MPS

Opsonized

EPR

(PEG)liposomes are designed with d= 50-100 nm

Organ	Physiological Structure	Estimated Pore Size (nm)
Capillary	Fenestrated (diaphragmed) (endocrine glands)	6–12
	Fenestrated (nondiaphragmed) (kidney glomerulus)	10–15
	Discontinuous/leaky	30–180
Heart	Left ventricle microvessels	5



Koshkaryev A et al. Adv Drug Deliv Rev (2013).
Nam J et al. Adv Drug Deliv Rev (2013).
Barenholz Y. J Controlled Release (2012).
Kraft JC et al. J Pharm Sci (2014).

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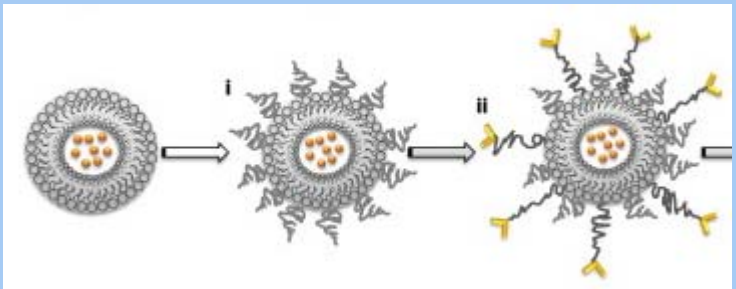
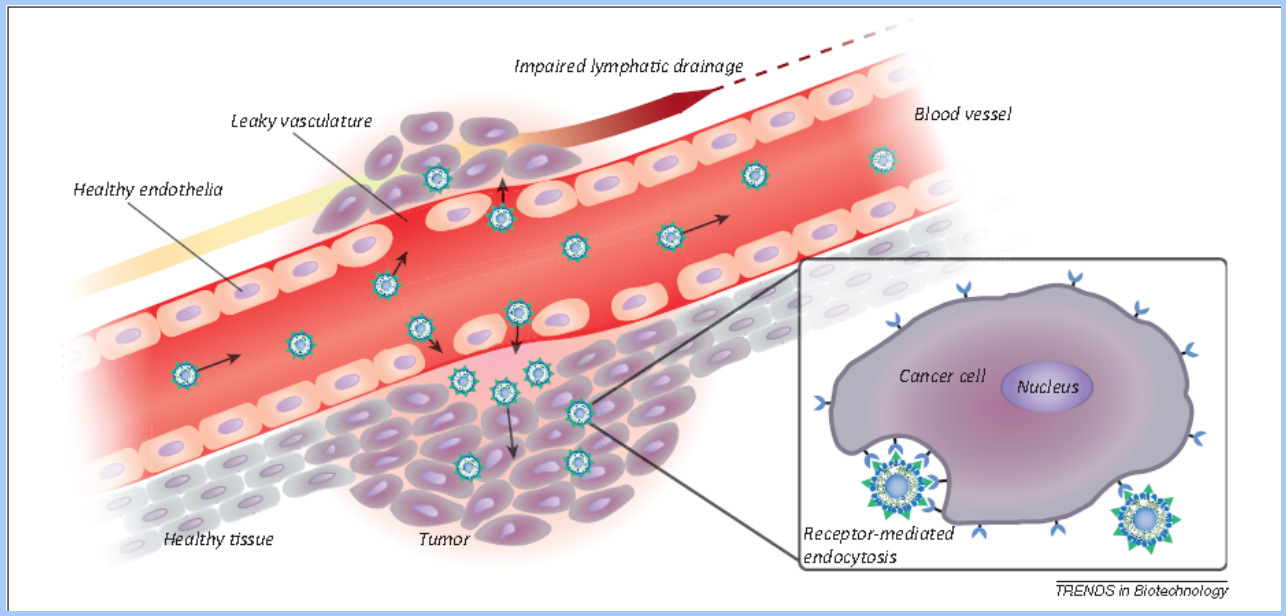
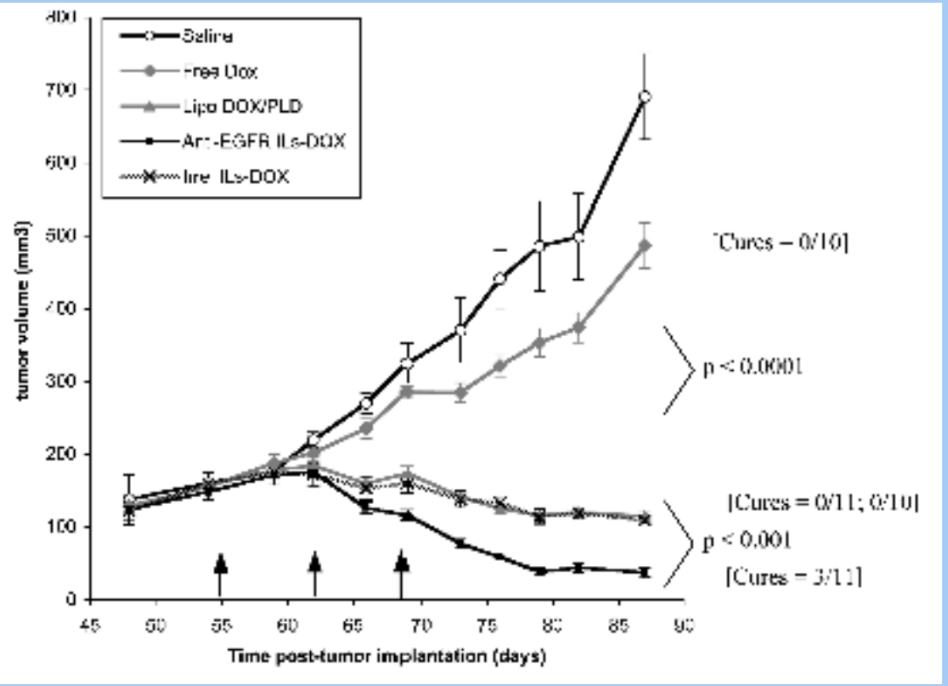
…to targeted PEG-liposomal DOX (cytostatic)





Koshkaryev A et al. Adv Drug Deliv Rev (2013).
Noble GT et al. Trends in Biotechnology (2014).

Pre-clinical (tumor xenografted mice) EGFR-targ. immunolipos.



Fab' of cetuximab

First-in-man open label phase I clinical trial

Well tolerated up to 50 mg/m²; clinical activity was recorded

Mamot Ch et al. Cancer Res (2005).
Mamot Ch et al. Lancet Oncology (2012).

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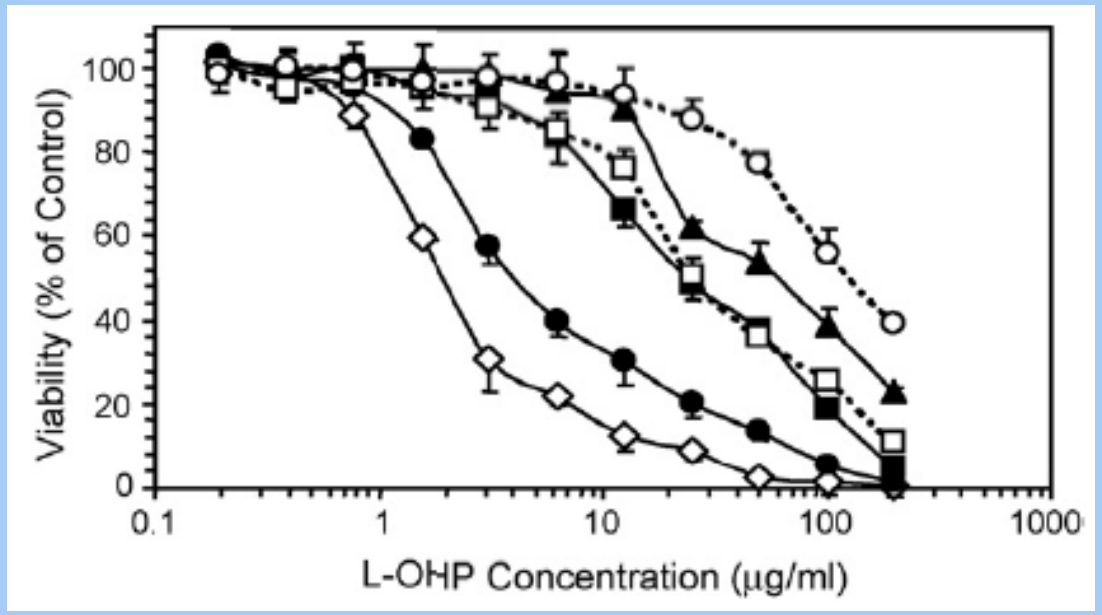
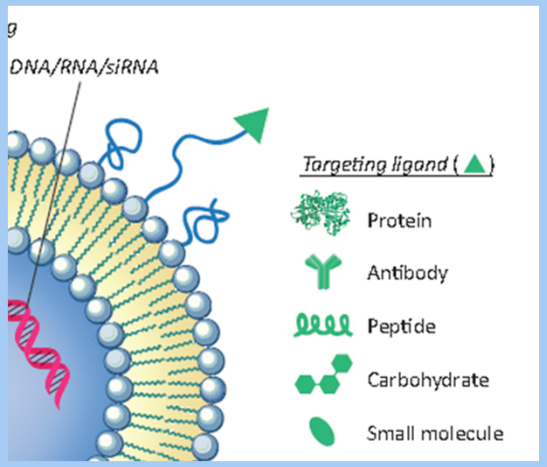
Pre-clinical (tumor-bearing mice) oxali in transferrin-PEG-lip

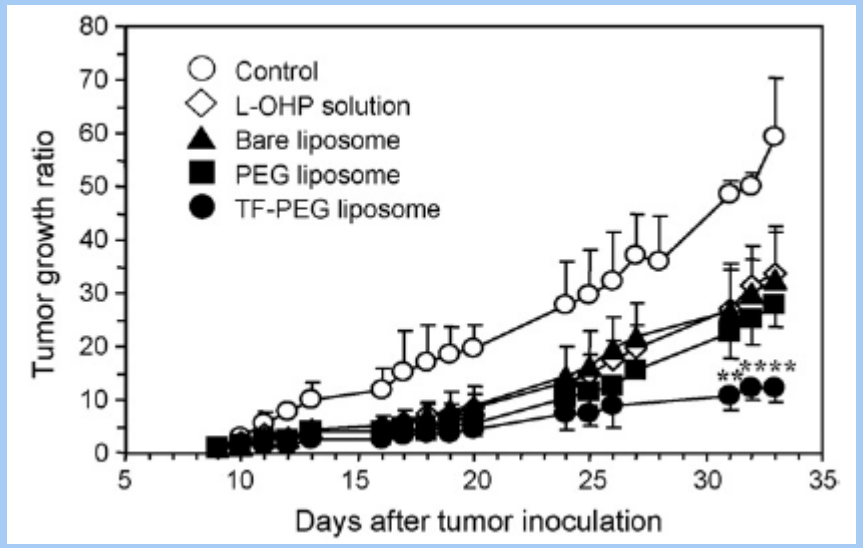


In vitro

Squares = PEG-lip(oxali)
Circles = tf-PEG-lip(oxali)
Black = without excess tf
Open = excess tf, competition

→ transferrin receptor-mediated internalization of tf-PEG-lip, *not* PEG-lip





In vivo

Suzuki R et al. Int J Pharm (2008).
Noble GT et al. Trends in Biotech (2014).

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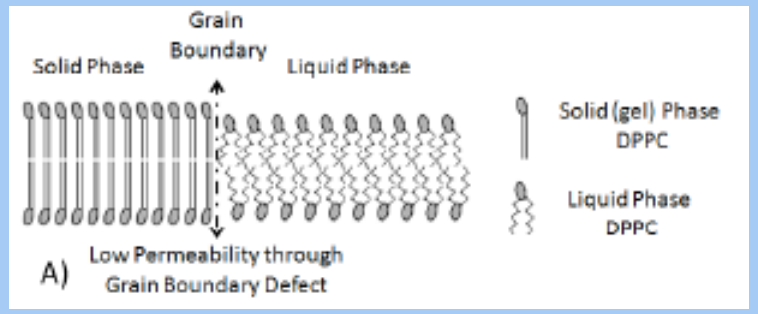
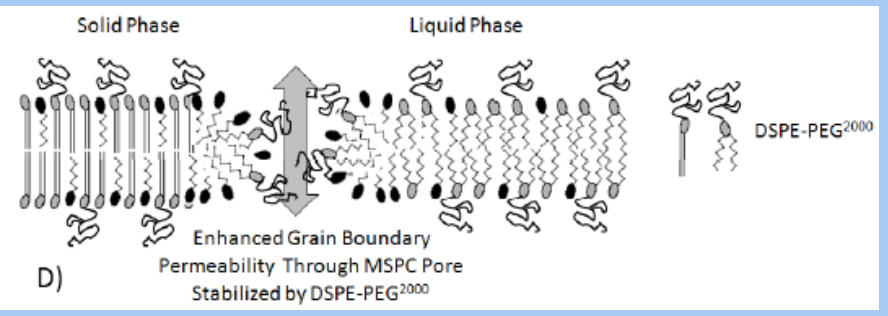
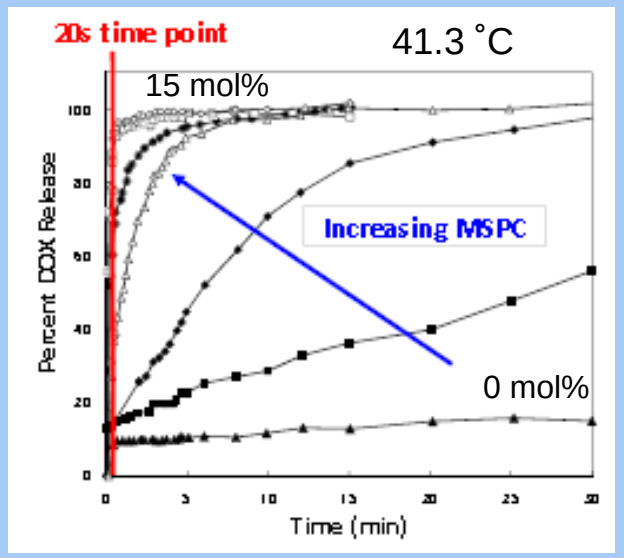
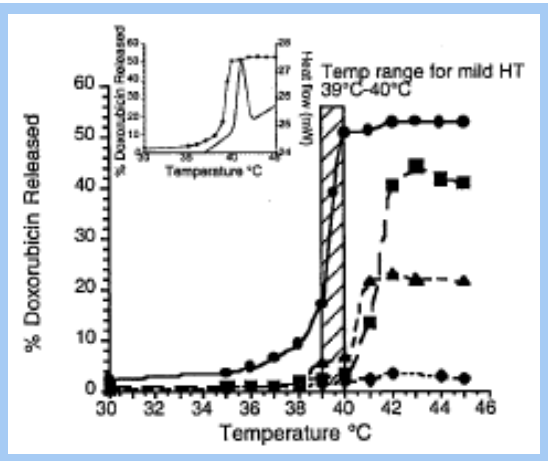
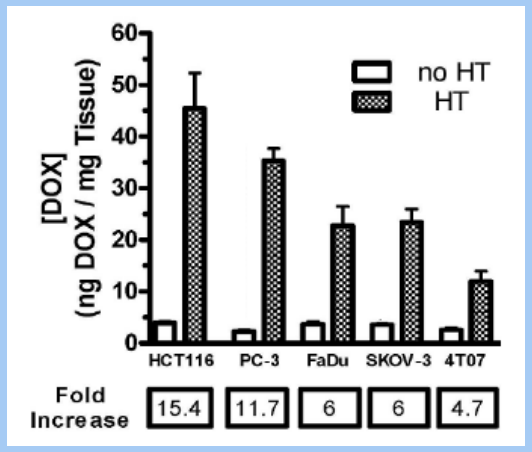
MR-Imageable temperature-sensitive (HIFU) liposomes

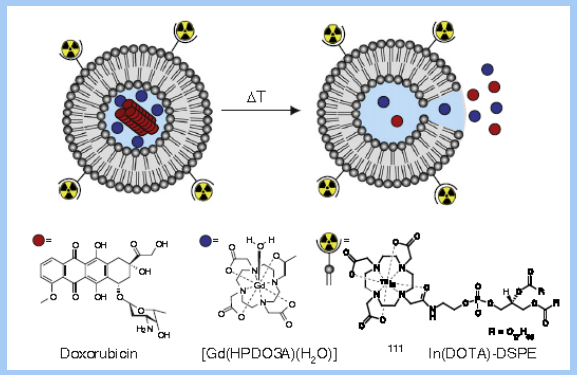












Landon C et al. Open Nanomedicine Journal (2011).
Needham D et al. Cancer Res (2000).
Smet de M et al. J Controlled Release (2013).

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