

CheckImmune

ADDED VALUE FOR YOUR CLINICAL DEVELOPMENT

EBF Autumn Focus Workshop

Challenging the Current Paradigm for ADA testing

21-22 September 2023

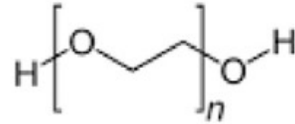
NH Malaga, Malaga, Spain

“Detection of anti-PEG antibodies in clinical studies”

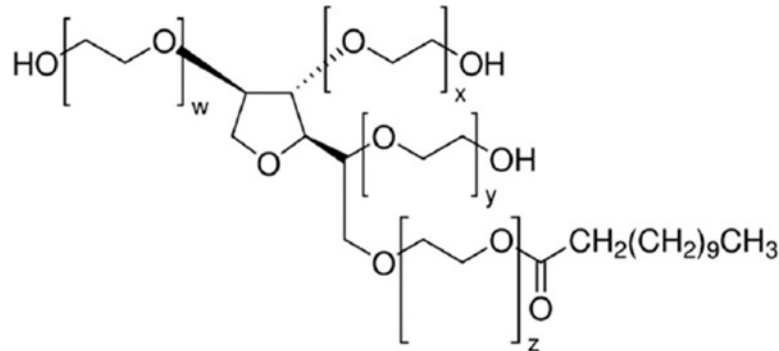
Toralf Roch – 21st September 2023



ethylene glycol



linear polyethylene glycol (PEG)



branched PEG

- Highly flexible linear or branched polymers
- 0.4-40 kDa MW range
- PEG is usually functionalized by different end-groups
- Polyethylene glycol (PEG) used as supplements in foods and cosmetic products
- Carrier in PEGylated protein-based drug
- In Lipo Nanoparticles (LNPs) as used in COVID-19 mRNA vaccines

How does polyethylene glycol stabilize proteins?

- Prevention of protein aggregation by forming a „protective layer“
 - Improvement of solubility by preventing protein aggregation
 - Protection against denaturation and increase in thermal stability by stabilizing the protein structure and preventing the formation unwanted folding intermediates
 - Increase of hydrodynamic diameter to reduce kidney uptake
- Enhancement of enzyme/protein activity

PEGylation can increase drug half-life

- Individuals suffering from haemophilia receive every two days a FVIII infusion
- Extended stability will strongly improve the quality of live

- mean half-life ($T_{1/2}$) of BAX 855 (PEGylated FVIII) compared with Advate (non-PEGylated FVIII) was 1.4- to 1.5-fold higher
 - Reduction of infusion numbers up to 30-35%
 - Reduction of annual bleeding rates (ABR)

Currently about 20 protein drugs containing PEG are clinically used

Trade names	Active Pharmaceutical Ingredient	Indication
Jivi®	60K-PEG recombinant Factor VIII antihemophilic factor	Hemophilia A
Palynziq®	2K-PEG-rhu-Phenylalanine ammonia-lyase, Pegvaliase-pqpz	Phenyl-ketonuria
Adynovate®	20K-PEG-Factor VIII Antihemophilic Factor VIII	Hemophilia A
Onivyde®	2K-PEG-Liposomal irinotecan hydrochloride trihydrate	Metastatic pancreatic cancer
Plegridy®	20K-PEG-Interferon beta-1a	Relapsing forms of multiple sclerosis.
Pegasys®	40K-PEG-interferon alpha-2	Hepatitis C and B
Sylatron™	12K-PEG-Interferon alpha 2b	Melanoma
Cimzia®	40K-PEG-Certolizumab	Rheumatoid arthritis, Crohn's disease, Axial spondyloarthritis and psoriatic arthritis
Mircera®	30K-PEG- erythropoietin (epoetin) beta	Anemia associated with chronic kidney disease
Macugen®	40K-PEG-anti-VEGF aptamer, Pegaptanib	Age-related macular degeneration
Adagen®	5K-PEG-adenosine deaminase (bovine),Pegademase	Severe combined immunodeficiency disease(SCID)
Doxil®/Caelyx®	2K-PEG-Liposomal doxorubicin HCl	Cancer
Oncaspar	5K-PEGylated L-asparaginase, Pegaspargase	Acute lymphoblastic leukemia
Krystexxa®	10K-PEG-Uricase, Pegloticase	Gout
Omontys®	40K-PEG-Erythropoietin-mimetic peptide,Peginesatide	Anemia associated with chronic kidney disease

- Pre-existing (natural) anti-PEG Abs in healthy humans

- 0.2% in 1984¹
- 25% in 2012²
- 40% in 2016³

Possible explanations: - Assay formats
- Cut off criteria
- Increased exposure to PEG

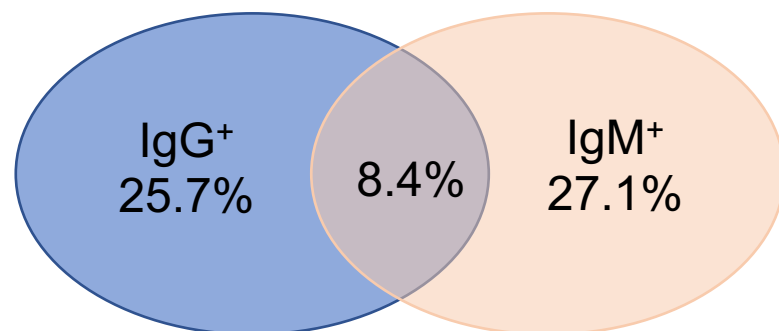
1) Richter, A. W. and Eva B. Åkerblom. "Polyethylene glycol reactive antibodies in man: titer distribution in allergic patients treated with monomethoxy polyethylene glycol modified allergens or placebo, and in healthy blood donors." *International archives of allergy and applied immunology* 74 1 (1984): 36-9 .

2) Ricardo P Garay, et al (2012) Antibodies against polyethylene glycol in healthy subjects and in patients treated with PEG-conjugated agents, *Expert Opinion on Drug Delivery*, 9:11, 1319-1323, DOI: 10.1517/17425247.2012.720969

3) Bing-Mae Chen, et al *Analytical Chemistry* 2016 88 (21), 10661-10666 DOI: 10.1021/acs.analchem.6b03109

High prevalence of anti PEG antibodies in healthy individuals

1504 healthy individuals.1)



- Anti-PEG IgM levels remain stable and are independent of age
- Anti-PEG IgG incidence and level decrease with increasing age
- Anti-PEG IgG/M antibodies are more prevalent in females than in males
- Data from 2022 (post covid 19 pandemic) confirmed the data obtained in 2016 ^{1,2}

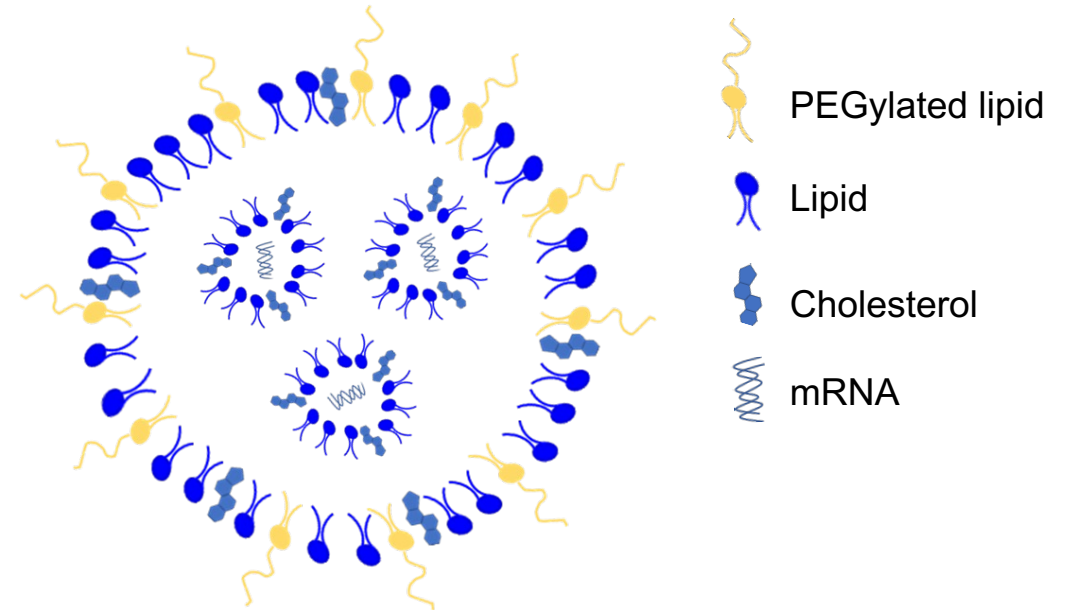
1) Bing-Mae Chen, et al, Measurement of Pre-Existing IgG and IgM Antibodies against Polyethylene Glycol in Healthy Individuals, *Analytical Chemistry* **2016** 88 (21), 10661-10666

2) Yi Ju, et al. Anti-PEG Antibodies Boosted in Humans by SARS-CoV-2 Lipid Nanoparticle mRNA Vaccine *ACS Nano* 2022 16 (8), 11769-11780 et al DOI: 10.1021/acsnano.2c04543

3) Bing-Mae Chen, et al, Polyethylene Glycol Immunogenicity: Theoretical, Clinical, and Practical Aspects of Anti-Polyethylene Glycol Antibodies *ACS Nano* 2021 15 (9), 14022-14048

Anti-PEG antibodies were boosted by COVID19 vaccines

- BNT162b2 (BioNTech) and mRNA-1273 (Moderna) induced Anti-PEG IgG and IgM
- mRNA-1273 induced higher anti-PEG levels than BNT162b2 and more vaccinated individuals were found positive
- Higher dose of mRNA-1273 could explain the higher anti-PEG Ab levels



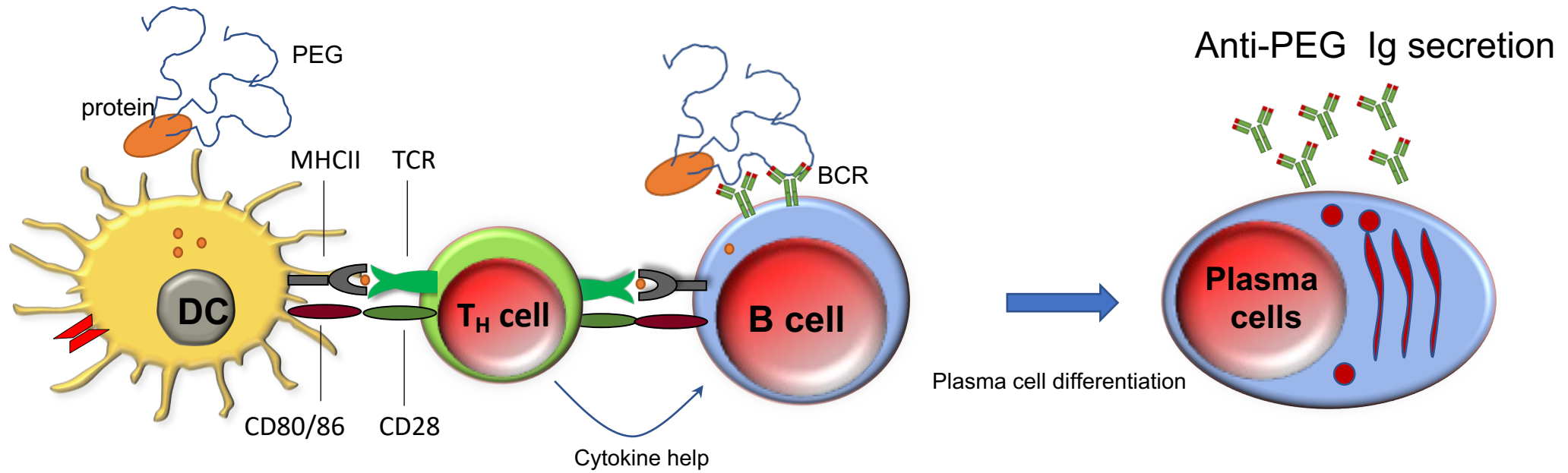
mRNA-1273 (Moderna)

polyethylene glycol [PEG] 2000 dimyristoyl glycerol

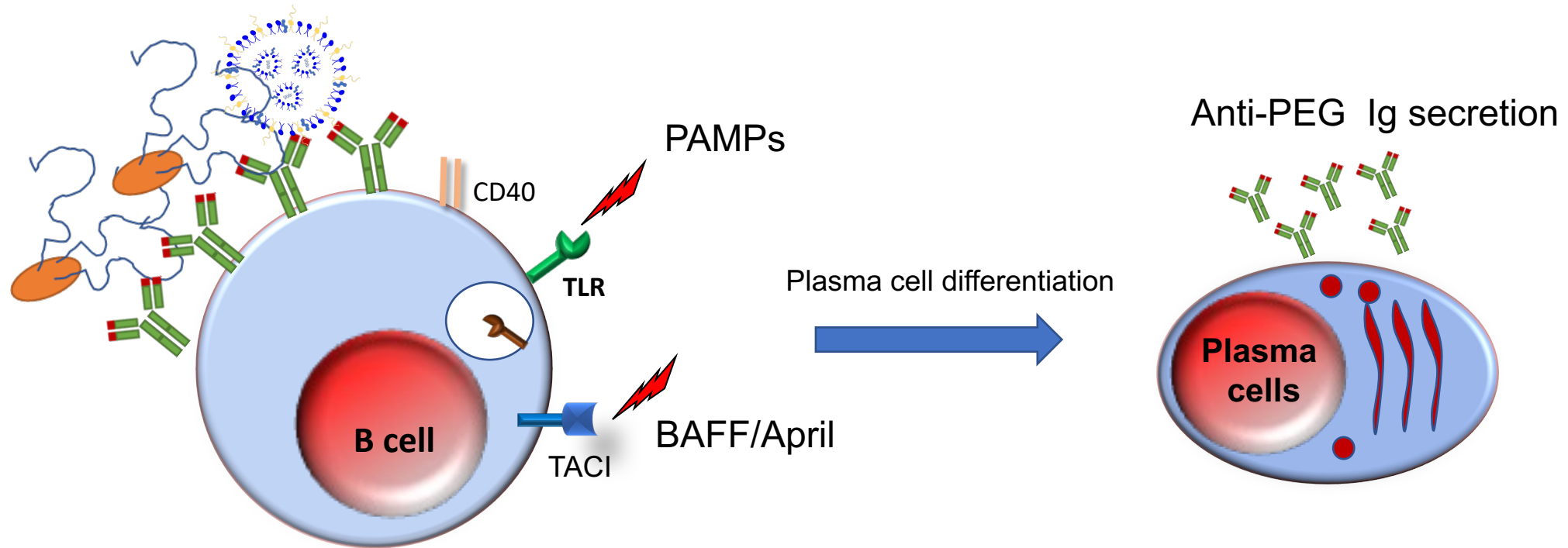
BNT162b2 (BioNTech)

2 [(polyethyleneglycol)-2000]-N,N-ditetradecylacetamide

Thymus (T) dependent generation of anti-PEG antibodies



Thymus-independent (TI) generation of anti-PEG antibodies



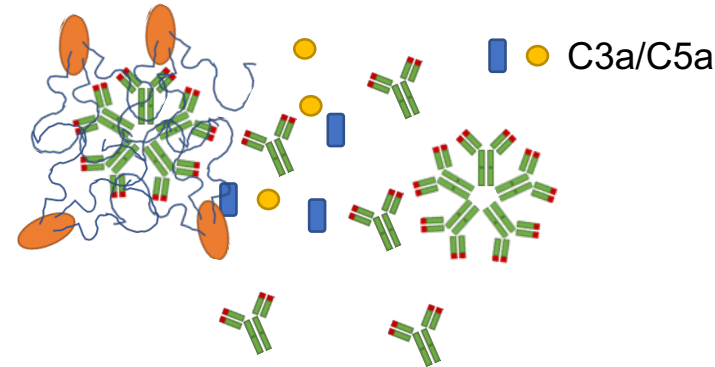
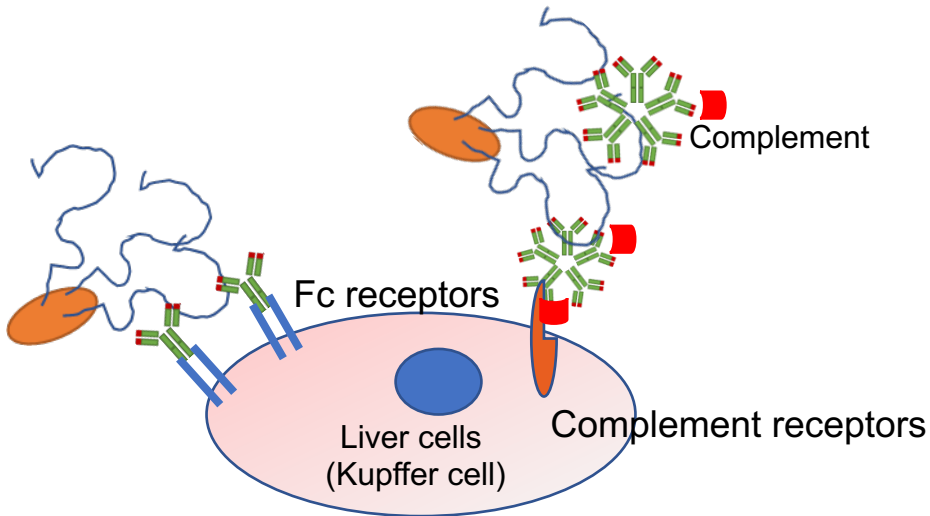
PEGylated liposomes, nanoparticles, nucleic acids, and LNP as well as human and non-human proteins can trigger T-dependent and T-independent immune reactions, resulting in formation of anti-PEG Ig secretion.

As more „foreign“ the protein drugs are as higher the risk of high anti-PEG responses.

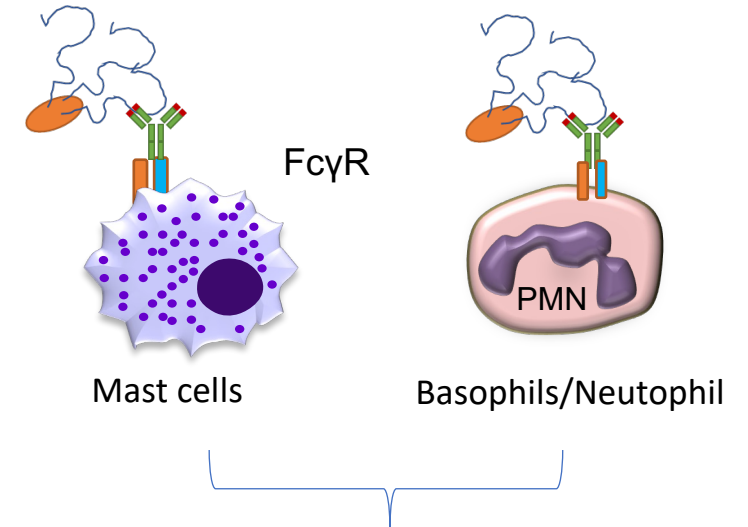
Anti-PEG IgG/M can have immunological adverse consequences

Anti-PEG IgG/M PEG complexes can activate the complement cascade

Accelerated blood clearance (ABC phenomenon)



Hypersensitivity reactions



Histamin, Serotonin,
platelet-activating factor, cytokines
→ facial swelling, headache, chest
tightness, hypothermia, hypotension

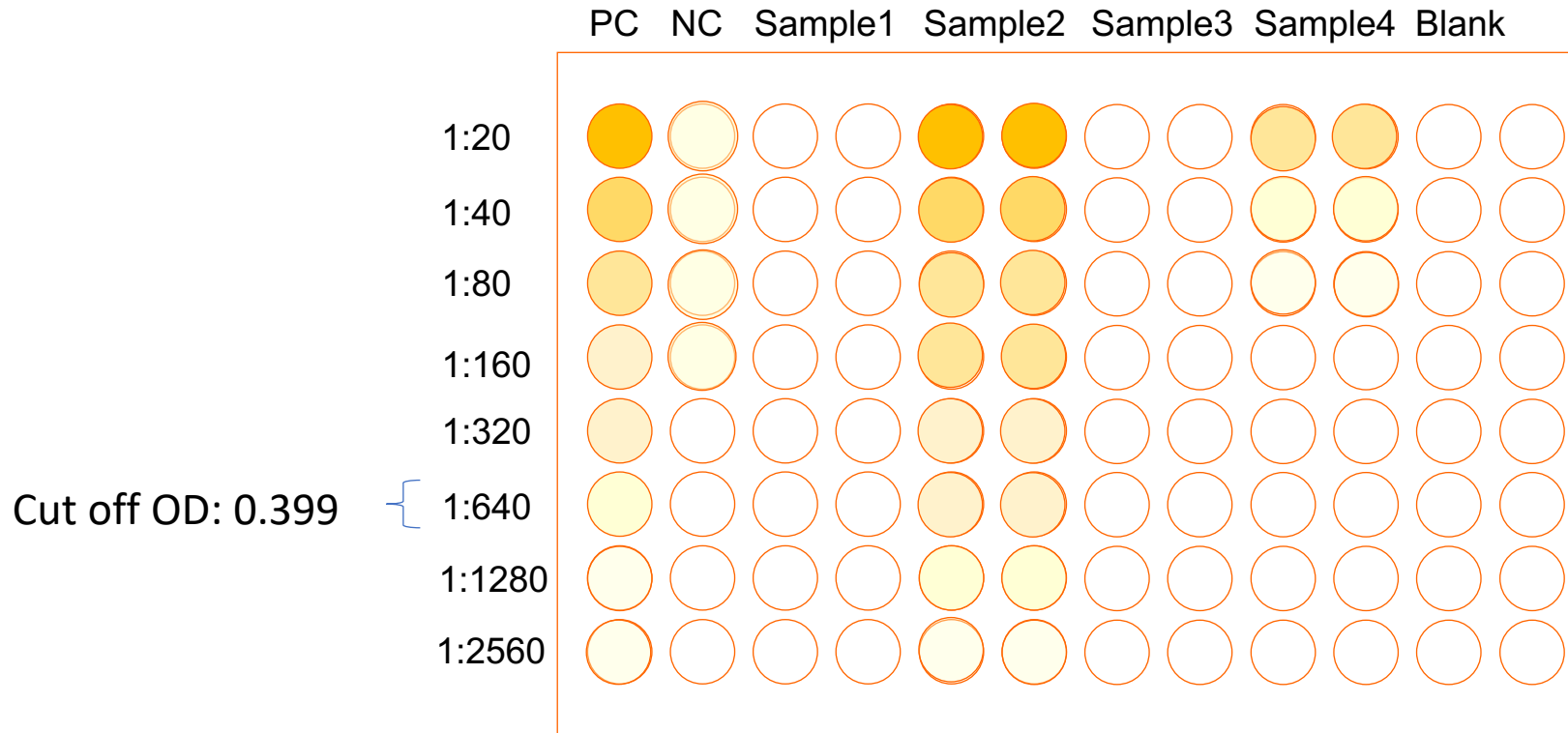
Liver clearance anti-PEG IgG/M PEG complexes:

- Increase of drug clearance
- Decreasing the drugs bioavailability
- Therapeutic efficacy can be reduced

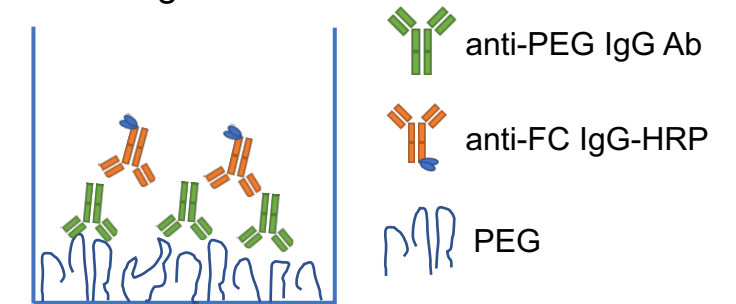
How can we minimize the immunogenicity of PEG?

- structural modification of the PEG
- replacing methoxy PEG with other functional groups, such as amino (-NH₂), carboxyl (-COOH) and hydroxyl (-OH)
- using alternative polymers, such as polyvinyl alcohol, polyvinyl-pyrrolidone or polyacrylamide

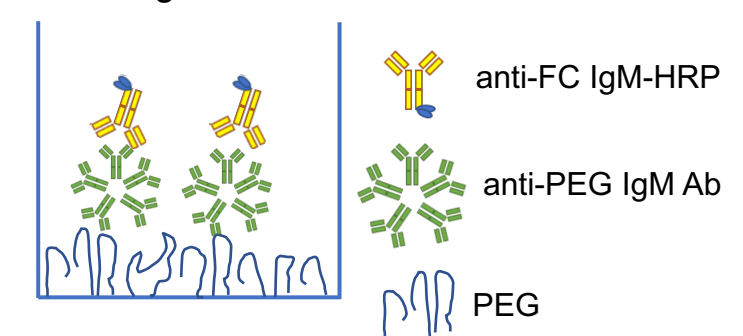
Screening



anti-PEG IgG detection



anti-PEG IgM detection

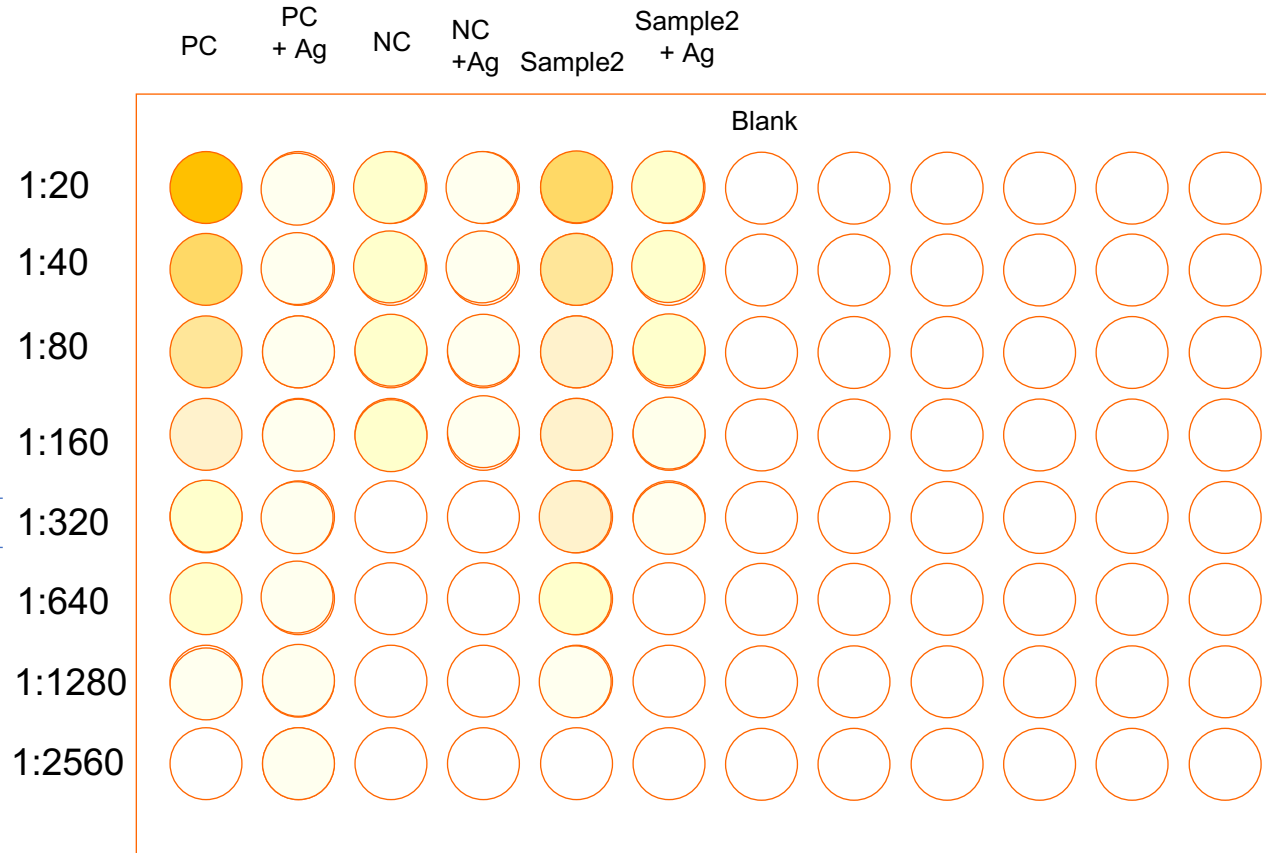


Cut off determination: - 160 HD analysed in a 1:20 dilution with and without antigen
- The 95% percentil of the competition values = cut off

Negative control (NC): plasma of HD (untreated donors)
Blank: PBS/2%BSA
Positive control (PC): Pool of positive donors

Anti-PEG Ab initial screening followed by a confirmatory assay in case the screening was positive

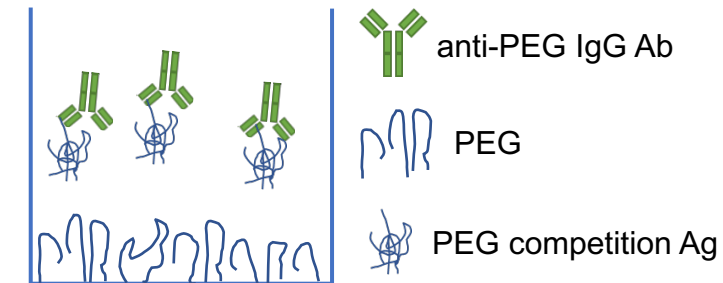
Confirmation



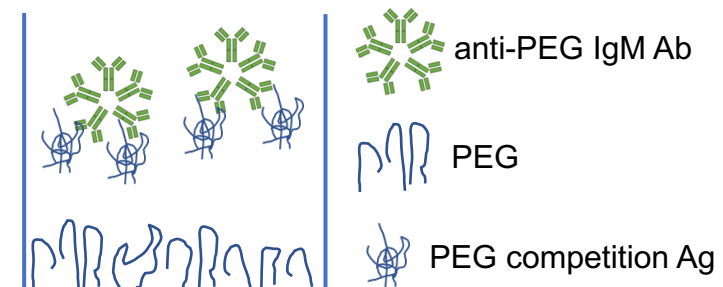
Cut off OD: 0.399

Samples + Ag > 2 titer steps below -AG → specific → positive donor

anti-PEG IgG competition



anti-PEG IgM competition

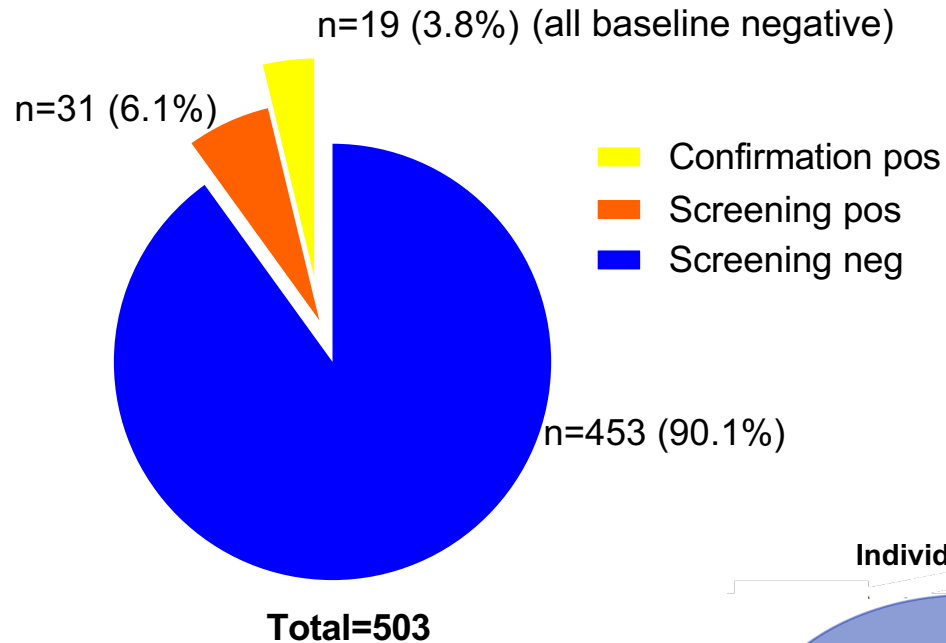


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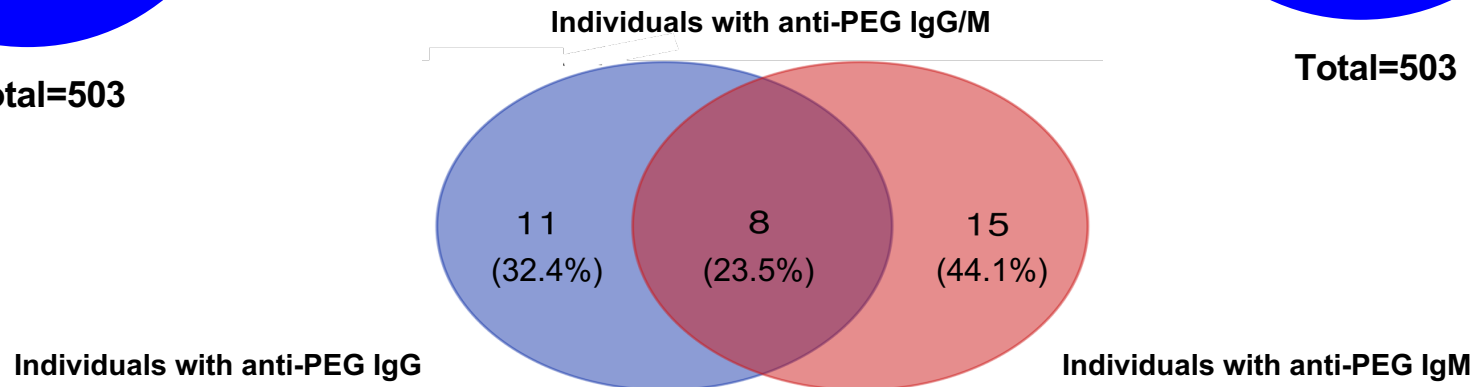
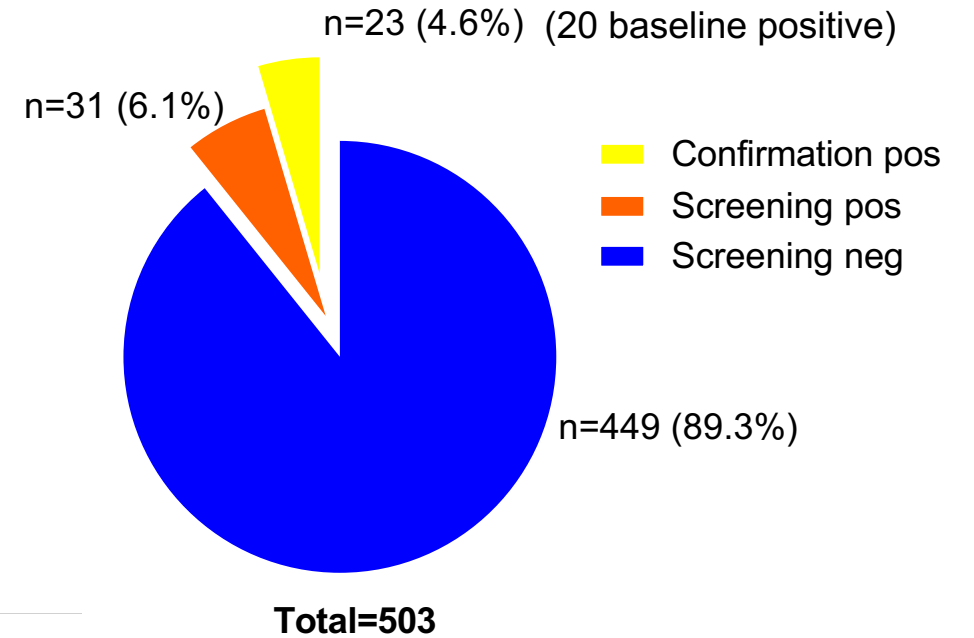
Negative control (NC): plasma of HD (untreated donors)
 Blank: PBS/2%BSA
 Positive control (PC): Pool of positive donors
 Ag: PEG

Cross-sectional analysis of anti-PEG antibody occurrence during therapeutic applications of a PEGylated protein drugs

Anti-PEG IgG



Anti-PEG IgM



- Passive hemagglutination
- Western blot analysis
- Acoustic membrane microparticle (AMMP®) technology
- Surface Plasmon Resonance (SPR)
- Flow cytometry

- Limited sensitivity and quantifiability
- Hard to validate
- Expensive hardware

- ELISA-based methods

Commonly used and relatively easy to validate

Summary

- PEGylation can increase the half-life of protein-based drugs
- A high proportion of healthy humans shows pre-existing anti-PEG Ab
- Continuous PEG exposure can increase the level of anti-PEG IgG
- Anti-PEG Ig can induced unwanted immunological reaction

- Is a broad screening of anti-PEG IgM/IgG really needed, since we all are under permanent exposure of PEG?
- What about CHO and HAMA (human anti-mouse antibody) ELISA?
- Should we better rely, (establish and validate) on inhibitory (functional) assays?
- Continuous PK screening more useful than ADA?

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