



“Challenges in developing an LC-MS/MS assay for the quantification of Pegasys; what are we measuring?”

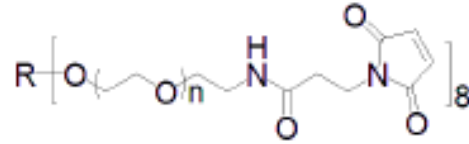
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Artwork from Reflections Art in Health

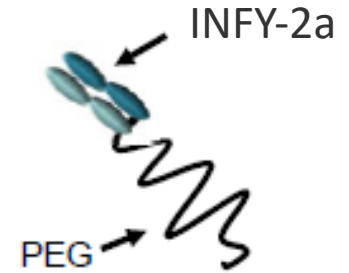
Background: Pegasys

- **PEG (40kDa) + interferon alpha 2a (INF α 2a : 20kDa)**

Polyethylene Glycol (PEG)
8 arm, ~40,000 Da "core"



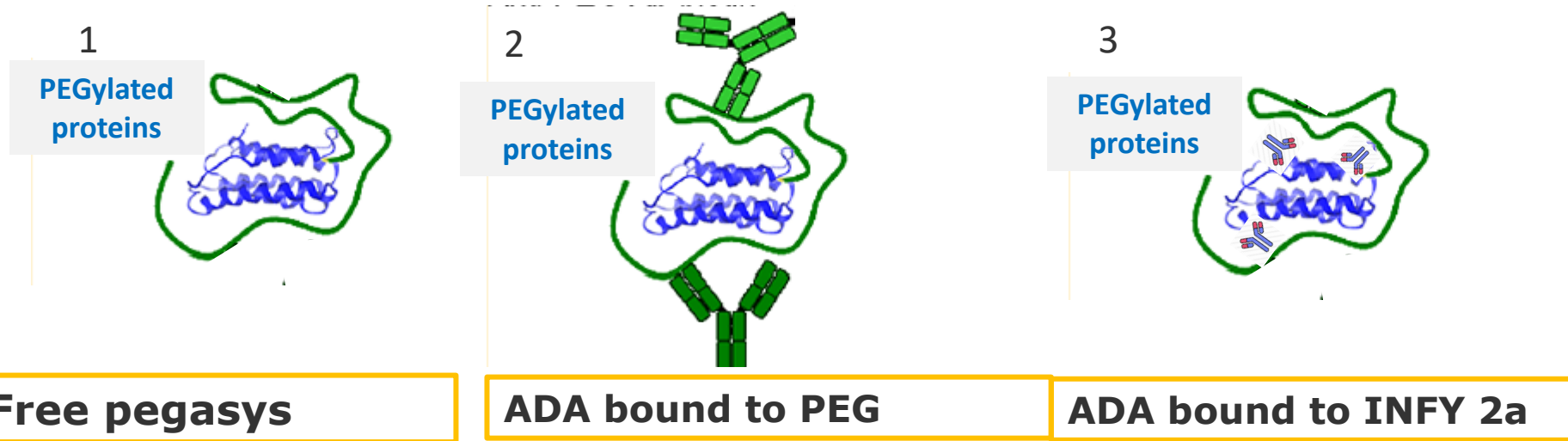
R = Tripentaerythritol core structure



- Covalent linkage of PEG to proteins
 - ✓ Minimize proteolytic cleavage = increase **half-life (x3)** ↓ **in injection frequency**
 - ✓ Mask immunogenic sites and or epitopes (undesired)
- **Possible anti-drug antibody (ADA) to:**
 - ✓ **INF α 2a**
 - ✓ **PEG portions**

Free and ADA bound Pegasys

- PD is driven by free circulating pegasys and not ADA bound pegasys
- Possible Scenarios in monkey serum



Possible Analytical Approaches:

1. ELISA based method:

- Validated assay not longer available; no critical reagents
- Very limited or no information of the assay design**
- Measures “free” or total pegasys? **Depends on assay design**

2. LC-MS based method – total serum crash with organic solvent

- Measures total pegasys

3. Hybrid-LC-MS based method – Immunoprecipitation

- Measures “free” pegasys in serum? **Depends on assay design**
- Choice of capture antibody is important**

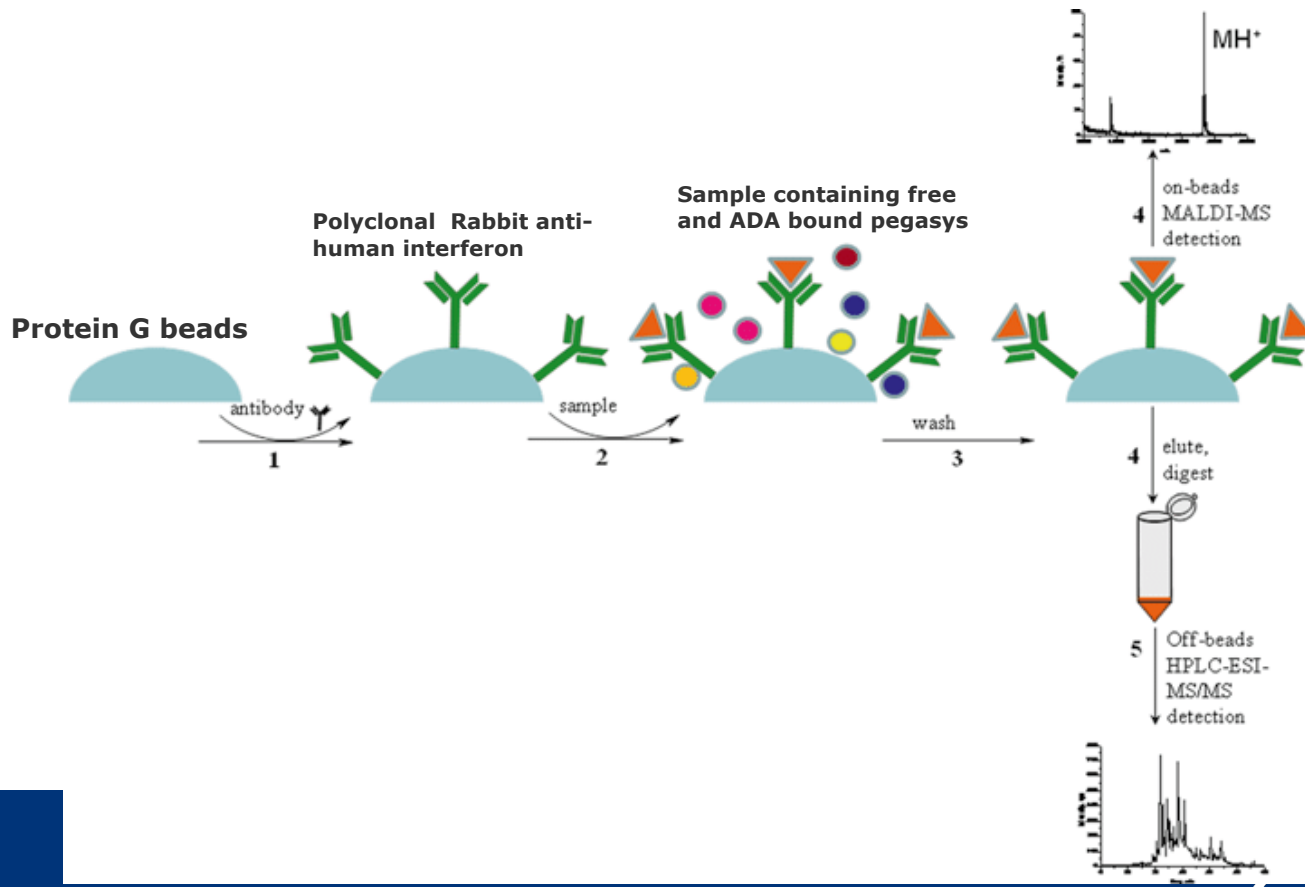
Hybrid LCMS assay for “Free Pegasys”

1. Method development and validation @ PRA Health Sciences:

- Capture antibody = anti-interferon AB
- Polyclonal Rabbit anti human interferon antiserum
- Monkey and Human serum as target matrix
- Analytical range:
 - ✓ Monkey serum = 10 to 1000 ng/ml;
 - ✓ Human serum = 1 -100 ng/ml

- Acceptance criteria – Ligand binding assay: set at 20% (25% for LLOQ)
- Identification of Unique surrogate peptide**

Hybrid LCMS assay for Free Pegasys



Lessons learnt

1. Incurred samples:
 - More ADA present in day 92 samples than on day 29
 - Higher ADA titer (indirect assessment) on day 92 could be related to continuous dosing of pegasys?
2. ADA-PEGASYS complex, rapidly cleared from circulation?

Acknowledgement

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Thank You!

Q&A

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