Biodistribution of monoclonal antibody aggregates upon SC administration

Grzegorz Kijanka

Universiteit Leiden

MedImmune

Division of Drug Delivery Technology
Leiden Academic Centre for Drug Research (LACDR)

24th February 2015

Table of contents.

1. Protein aggregates - immunogenicity

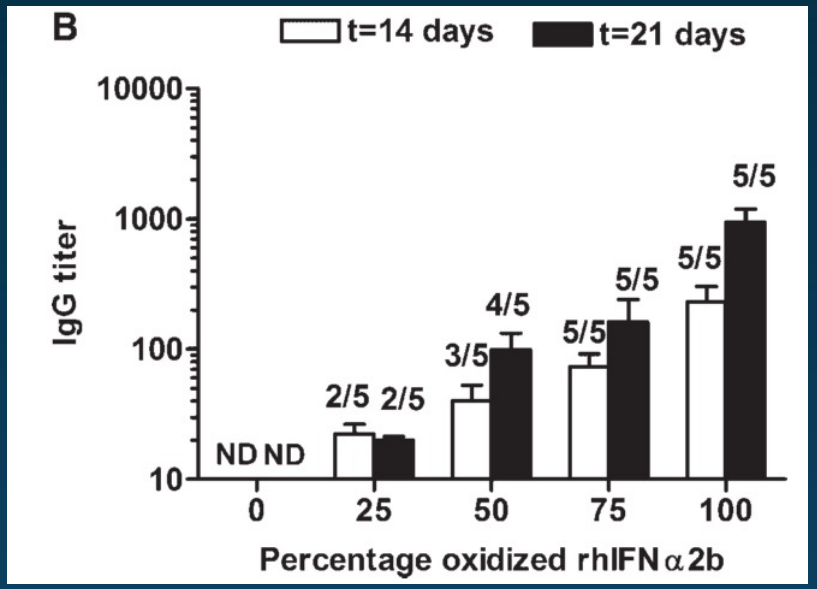
Immunogenicity

Product related factors:
- Origin
- Sequence
- PTMs
- Formulation
- Impurities (**aggregates**)
- ...

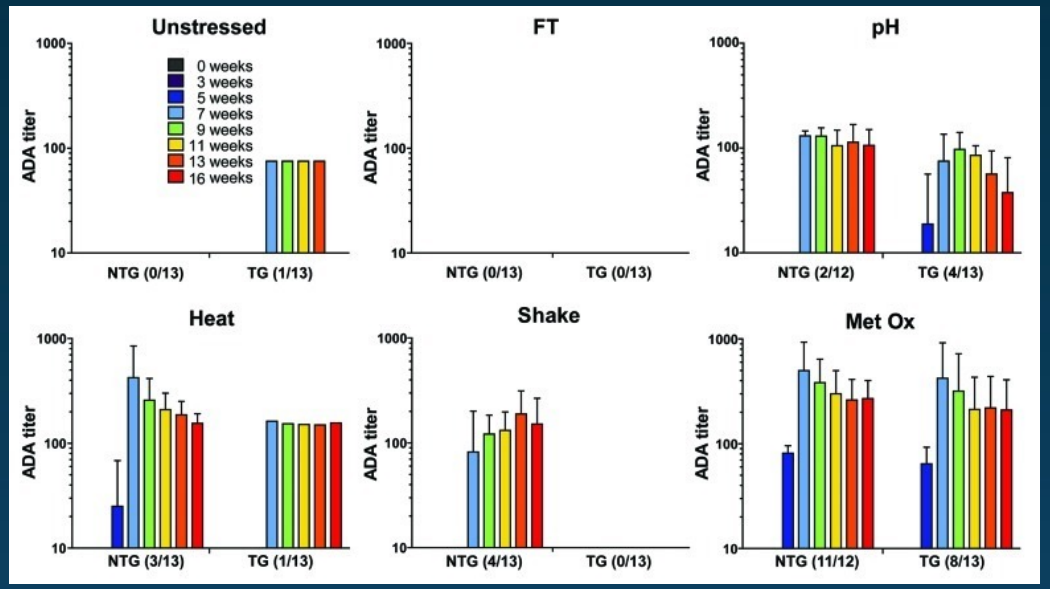
Therapy related factors:
- Dose
- Duration
- Aplication route
- Co-treatment
- ...

Patient related factors:
- Age
- Genetic background
- Disease
- Immunological state
- ...

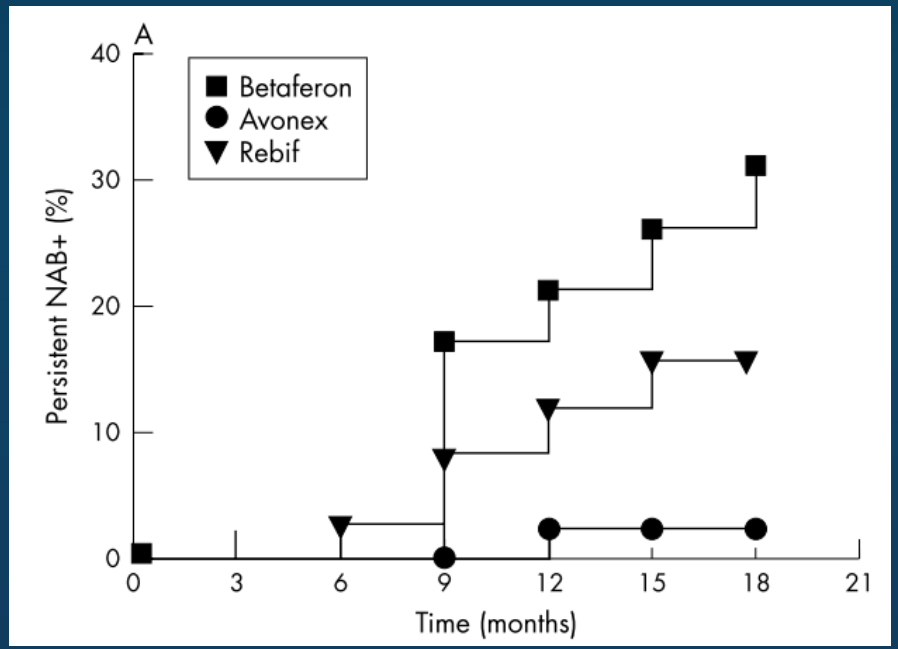
1. Protein aggregates - immunogenicity



Hermeling et al. J Pharm Sci. 2006



Filipe et al. MAb. 2012



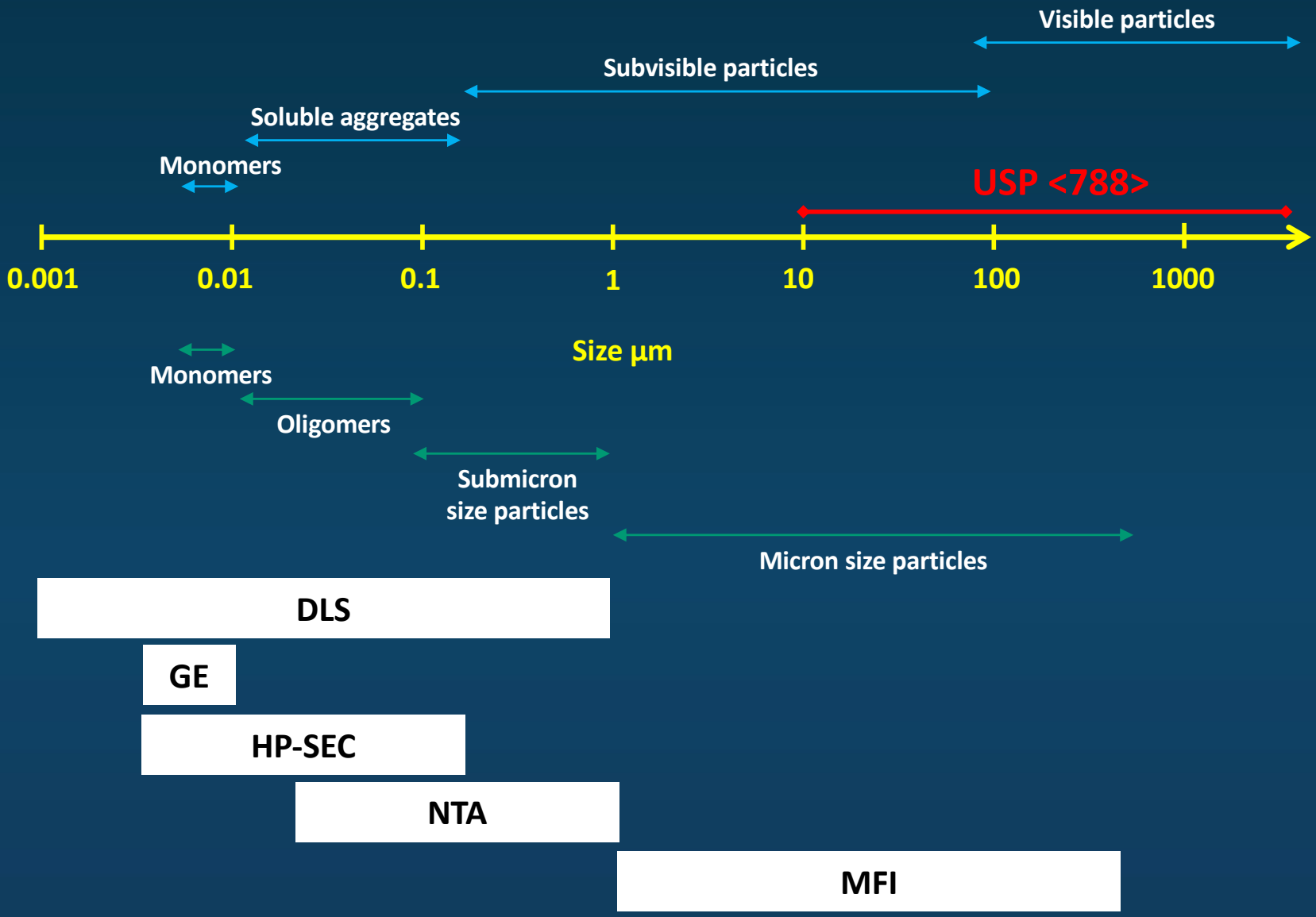
Bertolotto et al. J Neurol Neurosurg Psychiatry 2002

1. Protein aggregates

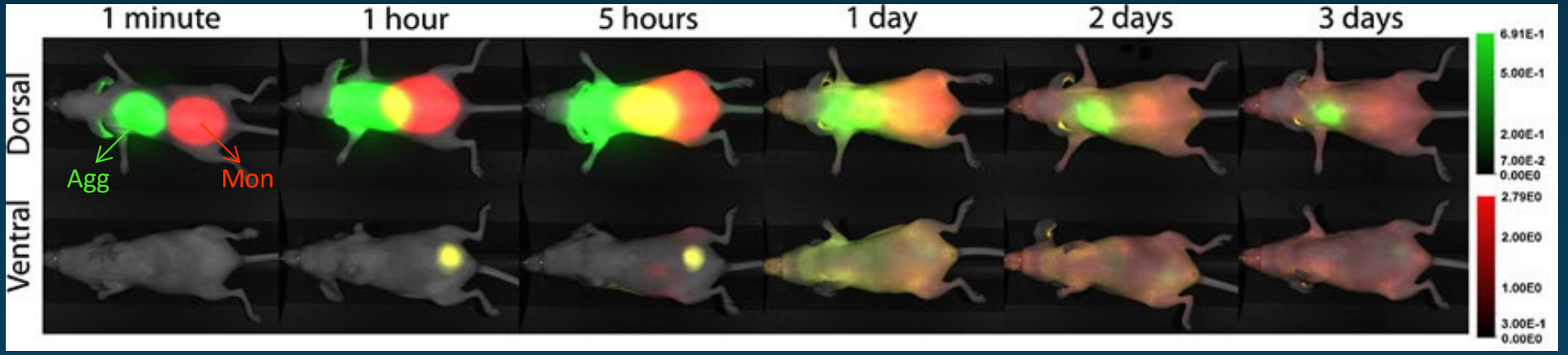
- “Protein aggregate”– assembly of protein molecules with higher MW than desired species

- Protein aggregates characterization:
  - **size**
  - morphology
  - secondary/tertiary structure
  - reversibility
  - covalent modifications
  - ...

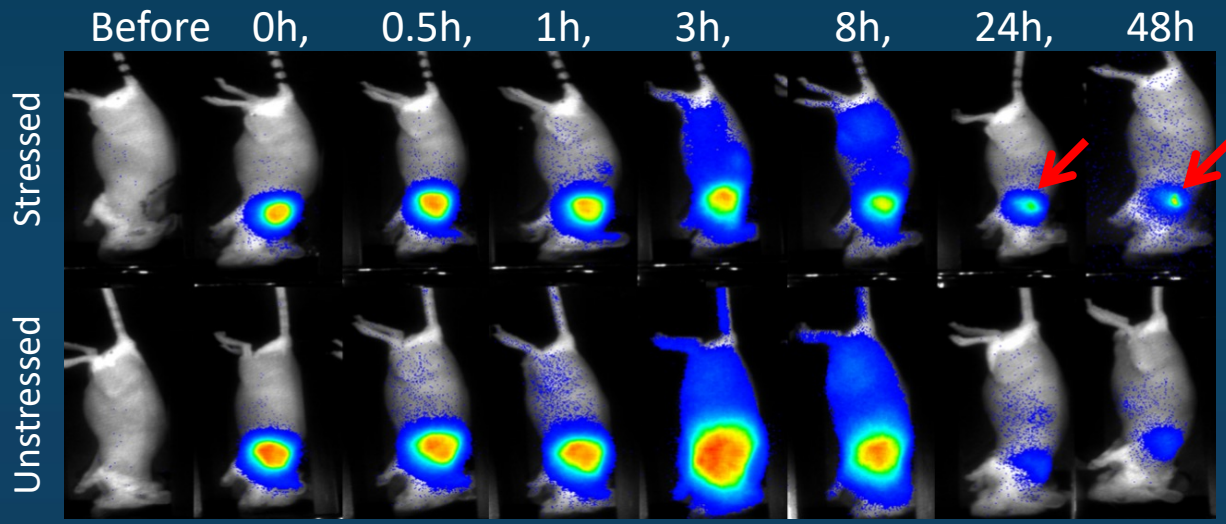
1. Protein aggregates

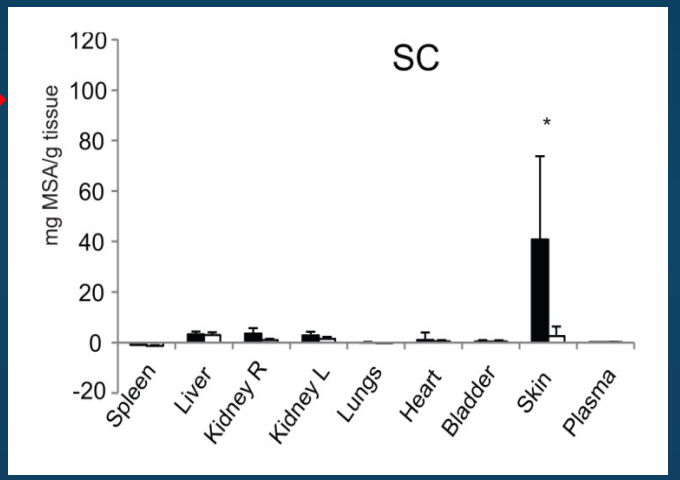


1. What happens with aggregates after injection?



Filipe et al. Pharm Res. 2014





Kijanka et al. PLOS 2014

1. What happens with aggregates after injection?

- Questions:
  - Which aggregates (dimers, oligomers, sub and/or micron size particles) contribute the most to altered disposition from injection spot?
  - How do different aggregates influence the biodistribution of protein?
  - Does the origin of protein (self/foreign) influence the biodistribution of aggregates?
  - Can (altered) biodistribution of aggegated protein increase the risk of immunogenicity?
  - Is it possible, by measuring the biodistribution, to select the most immunogenic size range of protein aggregates?

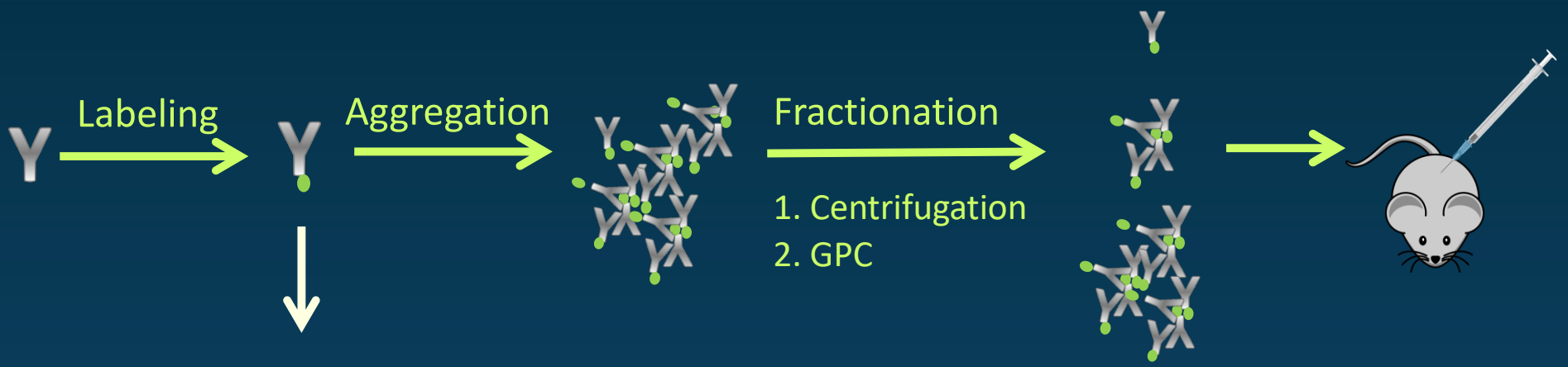
2. Aim of the project.

- To determine the biodistribution of different IgG's size species upon SC injection

3. Key materials

- Model proteins:
  - rhIgG1 (r347)
  - rmIgG1 (1A7)
- Animal model: SKH1 mice
  - Hairless strain
  - Immunecompetent
- Fluorescent dye: IR Dye800 CW
  - Fluorescence in near infra-red, good penetration through tissues
  - Very stable *in vivo*

3. *In vivo* experiments - overview



Characterization:
1) Degree of labeling
2) % of free dye (IRDye 800 CW)

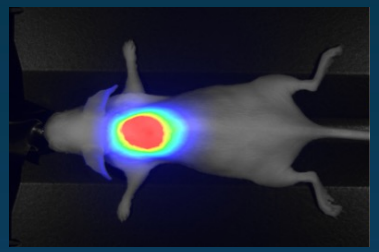
Characterization:
1) SEC
2) SDS-PAGE
3) DLS
4) NTA
5) MFI

Fractions:
1) Monomers
2) Monomers (stressed)
3) Soluble aggregates (oligomers)
4) Submicron size particles
5) Micron size particles

3. *In vivo* experiments - overview

Imaging

Euthanasia



Collecting organs / tissues

Biodistribution

1) SC injection
2) 50 µg of IgG (in 100 µl PBS)
3) 1A7 and r347

1) 1 hr, 24 hrs, 7 days

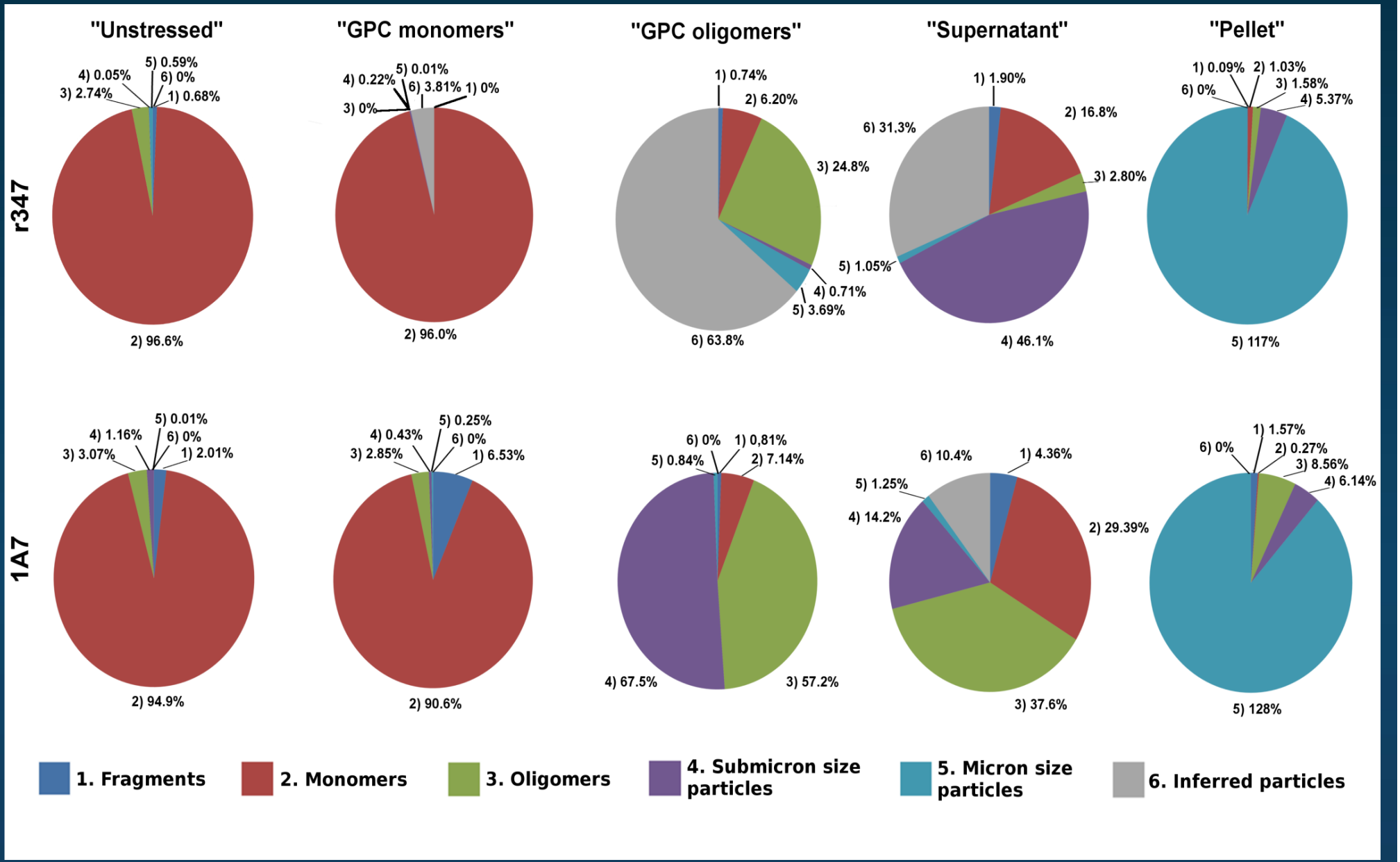
1) Tissues: blood, urine, muscle, skin (hind leg), skin (injection spot)
2) Organs: thymus, lung, heart, liver, kidney, spleen, (lymph nodes)

1) Ex vivo organs imaging
2) Quantitative biodistribution

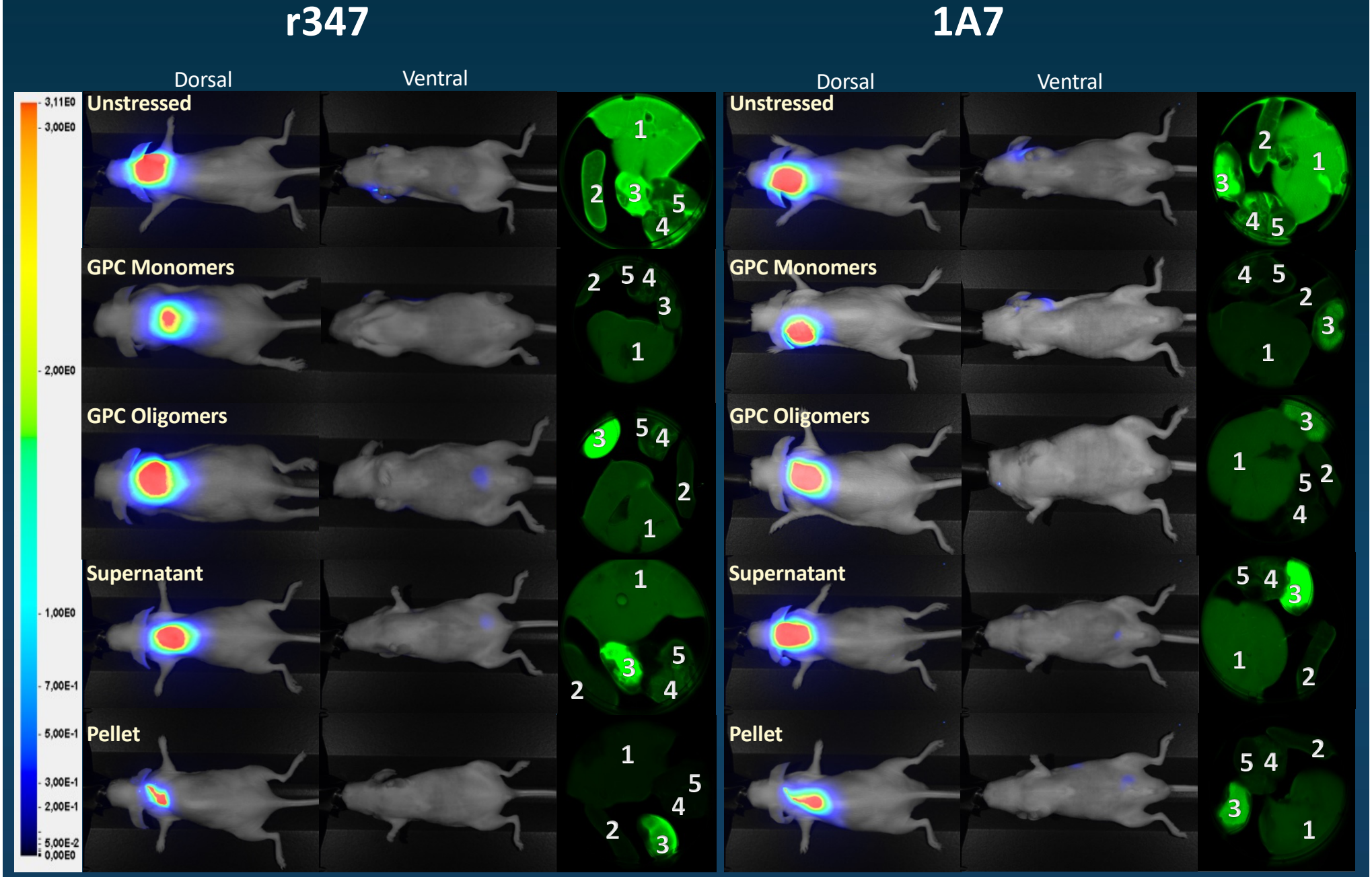
3. Aggregation and fractionation

- Final aggregation conditions
  - r347-IR Dye800CW conjugates 1mg/ml, pH=4.6, 63˚C, 1hr + 30min of stirring (700 rpm)
  - 1A7-IR DyeCW conjugates 1mg/ml, pH=4.6, 55˚C, 1hr + 30min of stirring (700 rpm)
- Fractionation via centrifugation (3000g, 10 min, RT)
  - “Pellet”: fraction enriched with micron size particles
  - “Supernatant”: submicron size particles
- Fractionation via GPC
  - Monomers subjected to stress conditions: “GPC Monomers ”
  - Oligomers: “GPC Oligomers”

4. Characterization of 1A7 and r347 fractions – mass balance

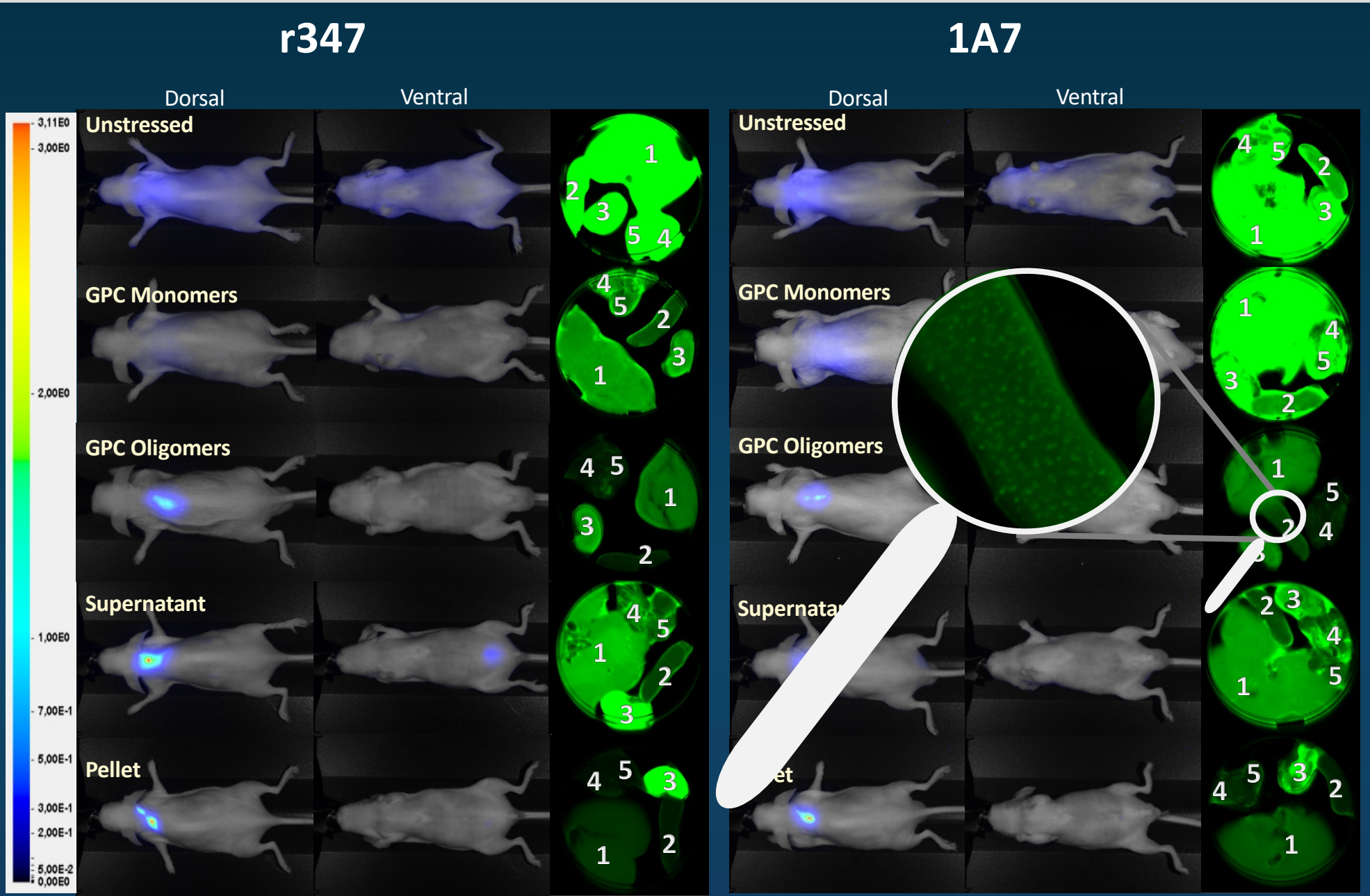


4. *In vivo* biodistribution – 1hr



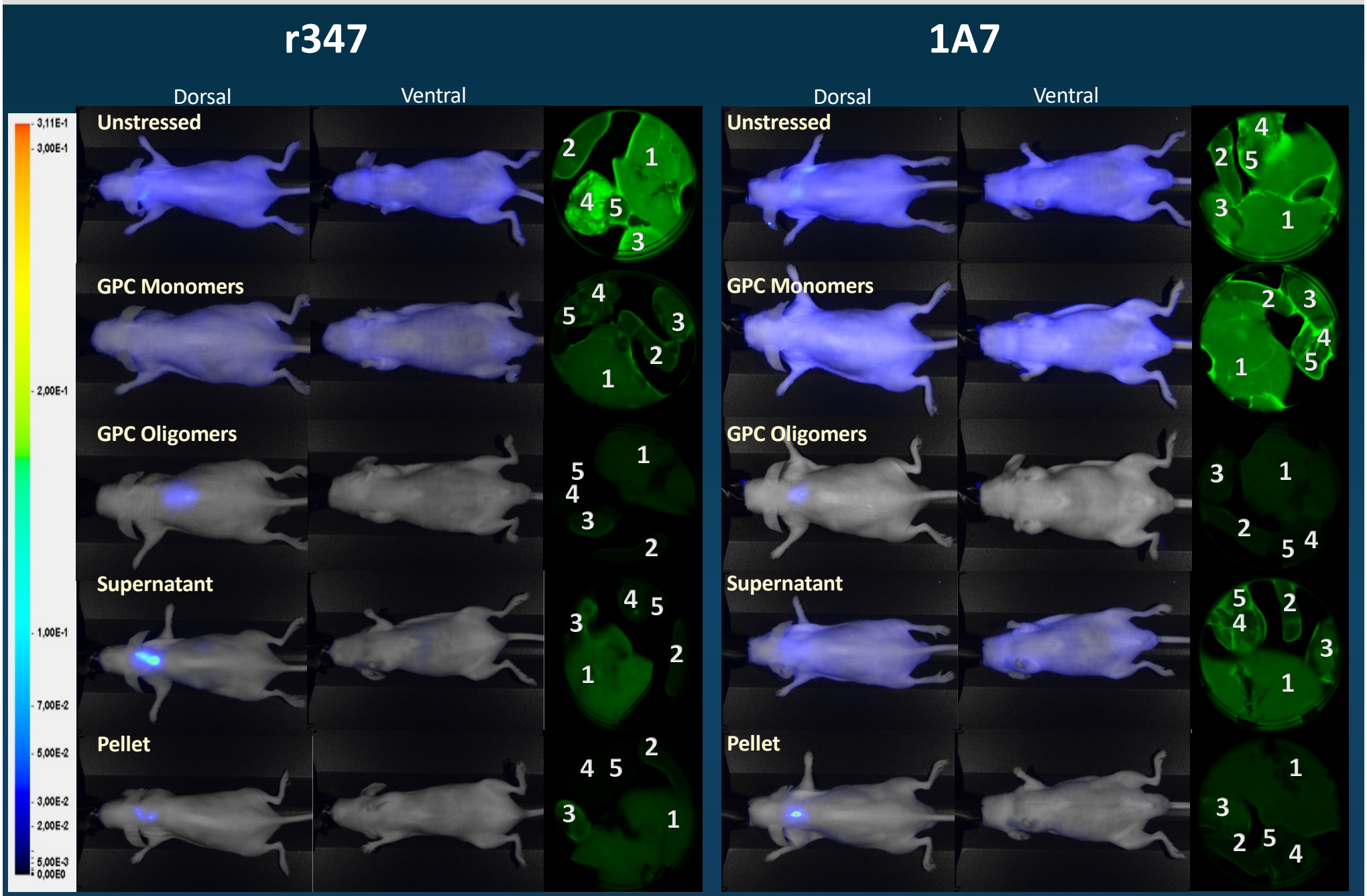
1) Liver, 2) Spleen, 3) Kidney, 4) Lung, 5) Heart

4. *In vivo* biodistribution – 24hrs



1) Liver, 2) Spleen, 3) Kidney, 4) Lung, 5) Heart

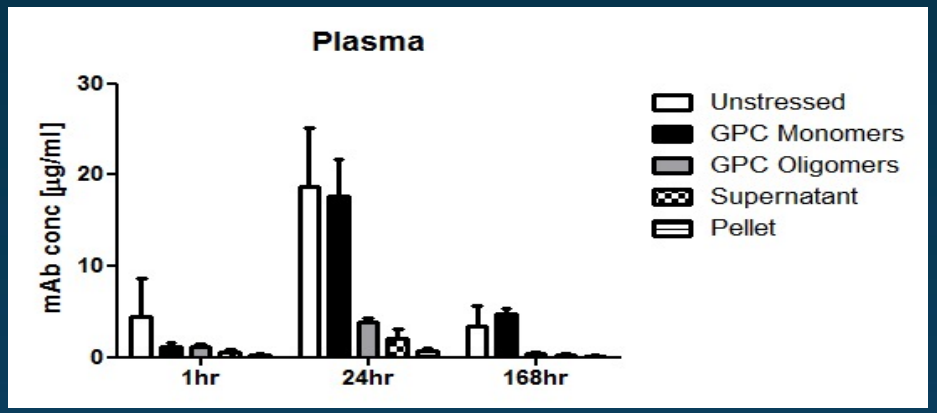
4. *In vivo* biodistribution – 7 days

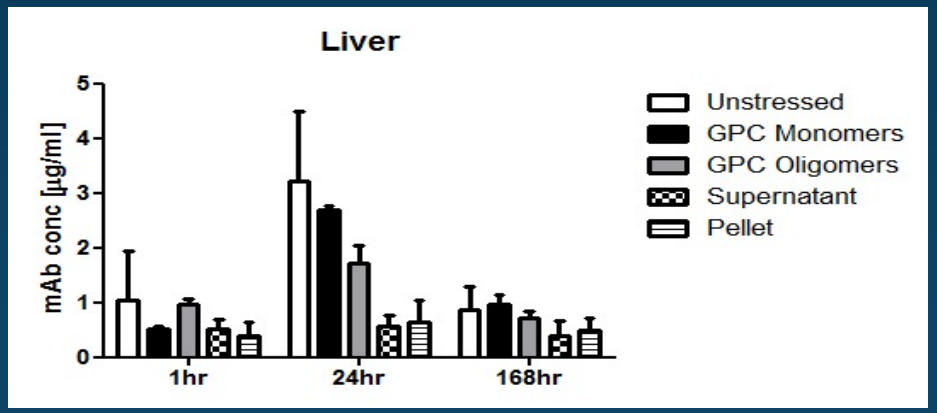


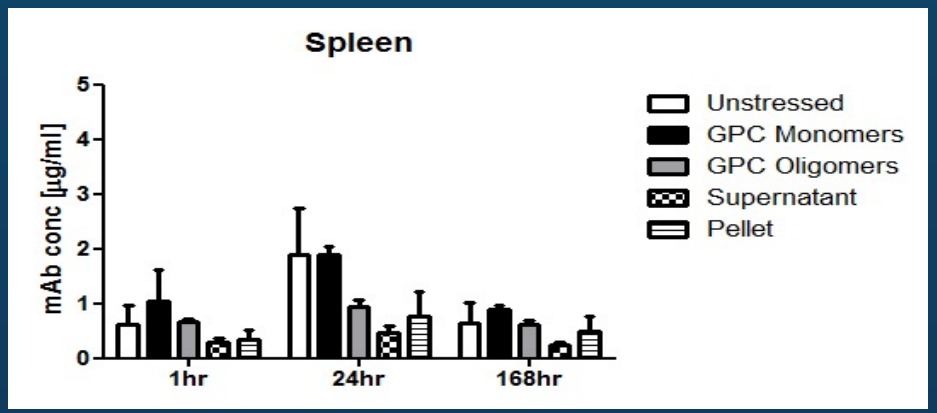
1) Liver, 2) Spleen, 3) Kidney, 4) Lung, 5) Heart

4. Quantitative biodistribution

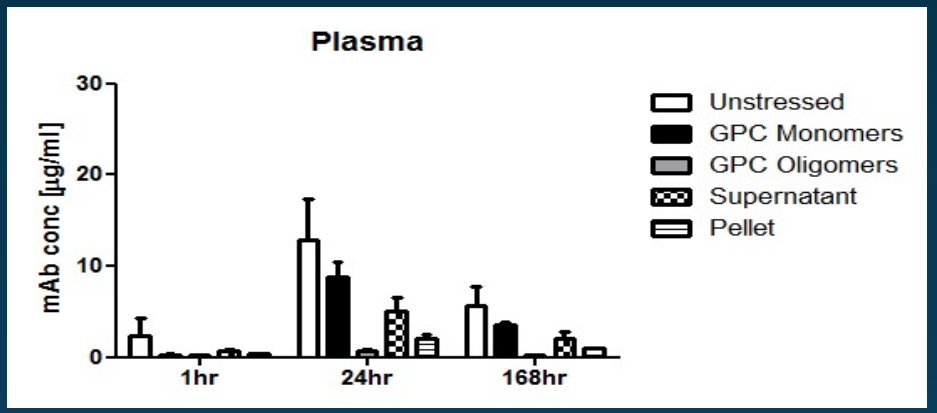
r347

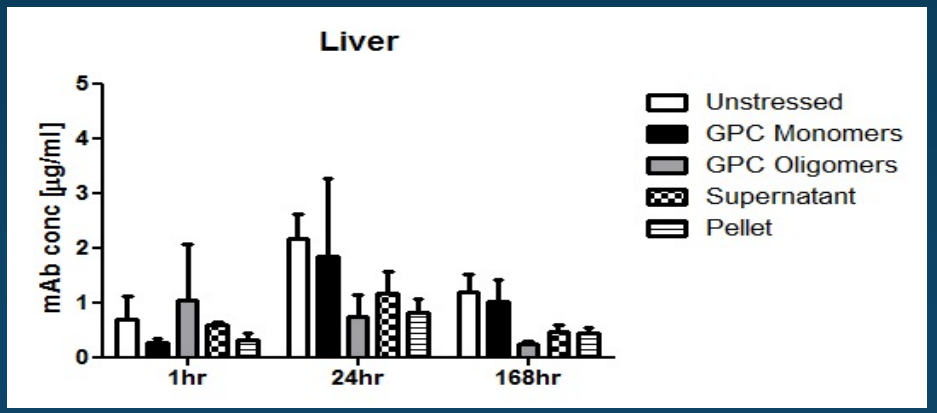


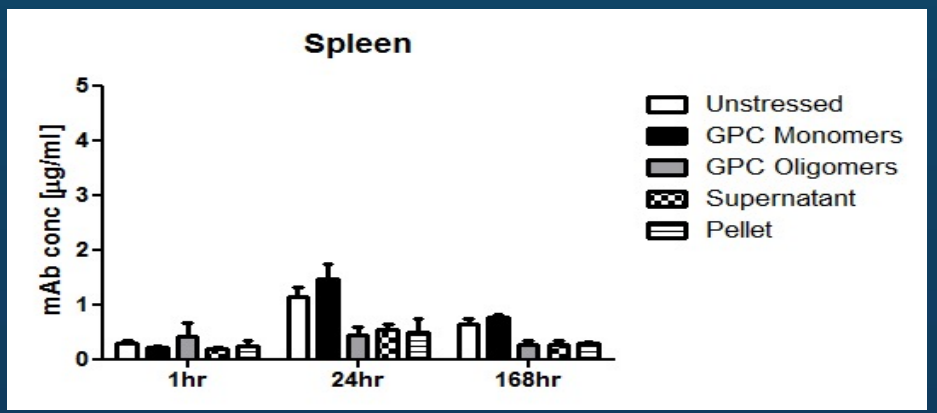




1A7







5. Conclusions

- Similar biodistribution of murine (1A7) and human (r347) antibody upon SC injection
- Monomeric antibodies, even subjected to stress conditions, nicely distribute within the whole body of animals
- Presence of aggregates (both sub micron size and micron size) alters biodistribution
- There is no specific tissue/organ in which aggregated antibodies accumulate (measurably)
- Fluorescent „dots” were detected in spleens and lymph nodes of some animals injected with „1A7 Oligomers”

6. Acknowladgements

- LACDR
  - Wim Jiscoot
  - Stefan Romerijn
  - Eleni Varypataki
- Med Immune
  - Jared Bee
  - Xu Liu
  - Kirsten Schneider-Ohrum
  - Robert Kubiak
  - Melissa Coughlin
  - Steven Bishop
  - Richard Remmele
  - Mark Schenerman
  - Srilatha Kuntumalla
  - Maria Andrea Miller
  - Norman Peterson
  - Wendy White
- LUMC
  - Clemens Löwik
  - Ivo Que

Thank you for your attention!

Imaging control