AstraZeneca

Relevance of protein aggregation in the lung

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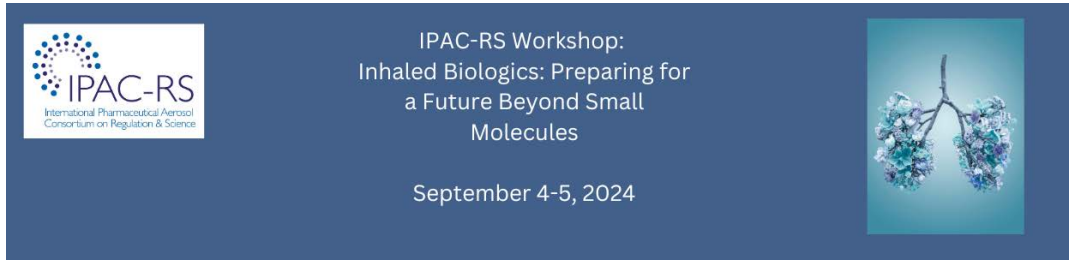
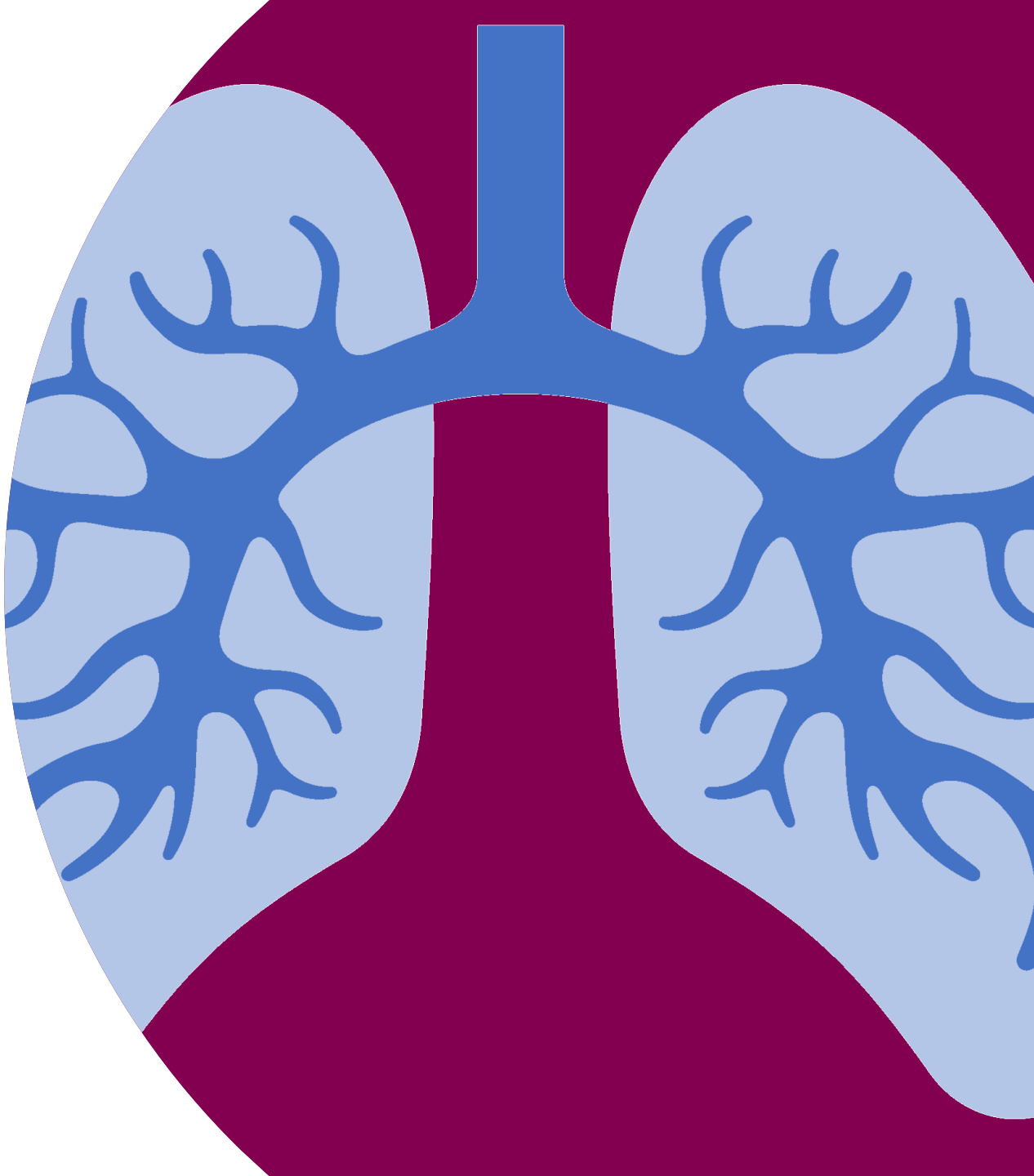




Presentation outline

1. Considerations and framework for risk assessment & review of current state knowledge
2. Basis of risk mitigation strategy and perspectives on relevant regulatory guidance
3. Towards bio-relevant aggregate testing for IBs

Relevance of protein aggregates in the lung: Safety & Efficacy

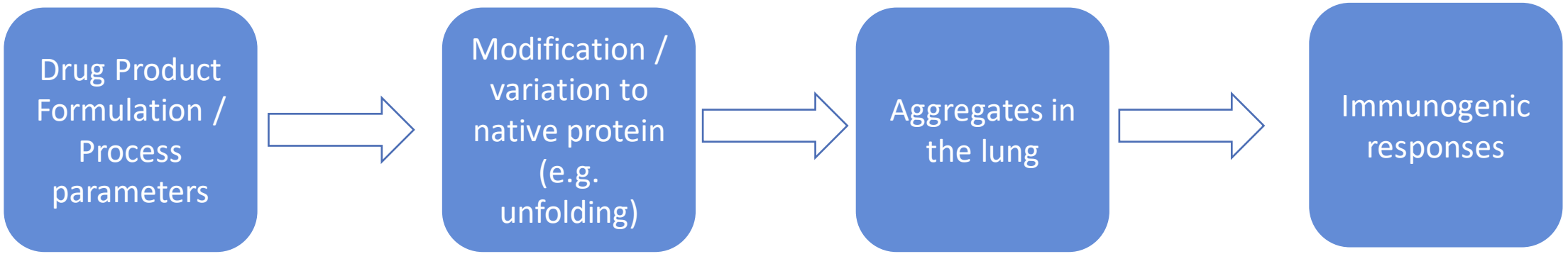
Safety:

1. Does aggregates of IBs risk inducing or augmenting immunogenic responses? ***Today´s focus***

Efficacy:

1. In principle, aggregated protein would be less likely to interact with drug target and could (if dominating drug species in vivo) accelerate clearance, thus reducing drug exposure and effect
2. If immunological responses to aggregates (above) include *neutralizing* or *drug-clearing* anti-drug anti-bodies (ADAs), these may reduce exposure to active drug.
3. Drug (aggregate) induced inflammatory lung phenotype would work against any therapeutic anti-inflammatory effects e.g. in the treatment of asthma.

Causal sequence in consideration for *drug product quality-related* aggregation risk



Objectives for Drug Product Quality

Primary: *Minimize variations* in 'aggregation potential', to ensure *consistency* in treatment response

Secondary: Minimize potential for aggregation to the extent possible by formulation/process design

Immunogenicity to protein therapeutics is likely determined by multiple factors

Protein identify

- Potential immunogenic T-cell epitope content in the protein's primary amino acid sequence

Treatment regimen

- Dose, dose frequency, treatment duration
- **Dose route:** Higher immunogenicity via subcutaneous route compared to intravenous has been recognized but debated[1]

Product factors

- Glycosylation pattern
- Host cell protein and/or DNA
- Other impurities such as aggregates
  - **Impact of aggregate size, type and quantity not fully clarified**:
    - Micron-sized particles with partly preserved protein structure have been recognized as potentially more immunogenic than smaller aggregates or aggregates of fully denatured protein[1]
    - Others have suggested sub-micron (100-1000 nM) are more immunogenic[2]

[1]Jiskoot et al, J Pharm Sci 105 (2016) 1567-1575

[2]Kijanka et al, J Pharm Sci, 107 (2018) 2847-2859

Well-established link between aggregates in drug product and immunogenicity

Non-clinical example[3]

- Human gamma-globulin administered to mice with varying levels of aggregates
- Faster 'immune elimination' of aggregated human gamma-globulin

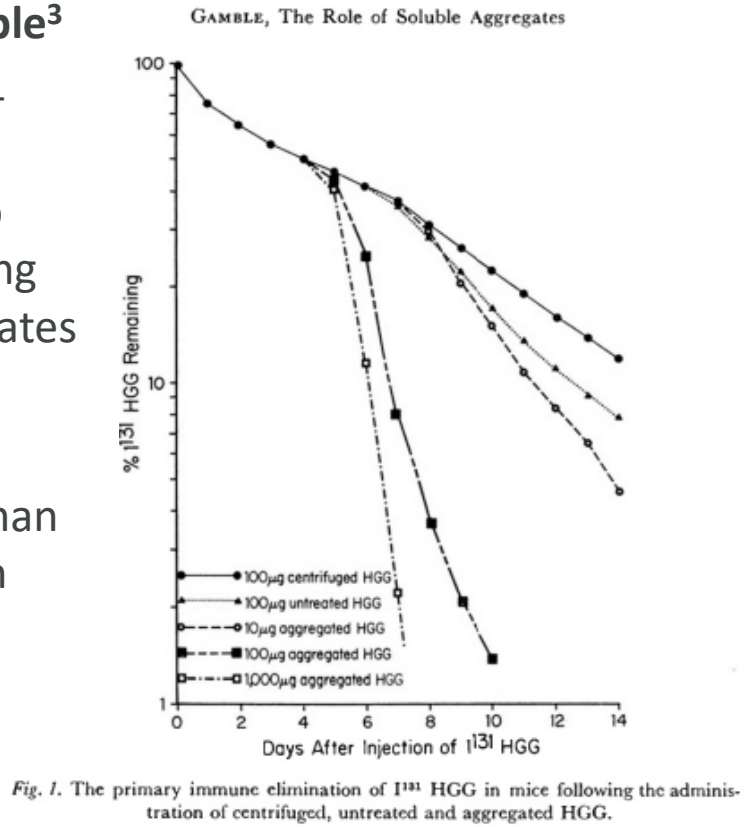


Fig. 1. The primary immune elimination of I^{131} HGG in mice following the administration of centrifuged, untreated and aggregated HGG.

Clinical observations

Collated in review by Rosenberg[4]

- In the 1950s, intravenous **immune globulin** preparations containing substantial aggregated material triggered severe hypersensitivity
- More *durable* antibody response **to human growth hormone** treatment of the product with 50-70% aggregated protein, compared to that with 10%
- Treatment with human recombinant IL-2 formulated as 27-mer is associated with ADA in 80-100 % of patients

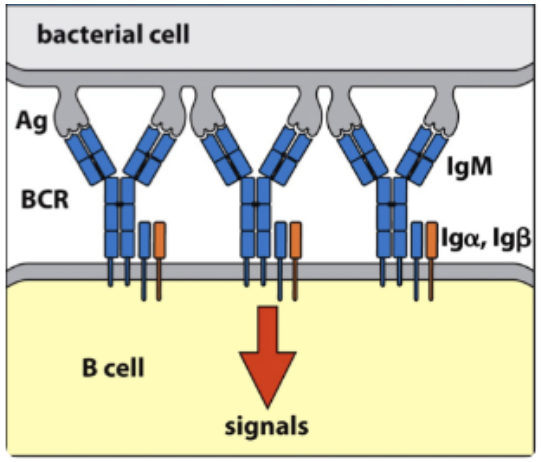
[3]Gamble, Int Arch Allergy Appl Immunol. 1966;30(5):446-55.

[4]Rosenberg, The AAPS Journal 2006; 8 (3) Article 59

Immunogenicity of aggregates can relate to uptake by dendritic cells and ability to cross-link B-cell receptors

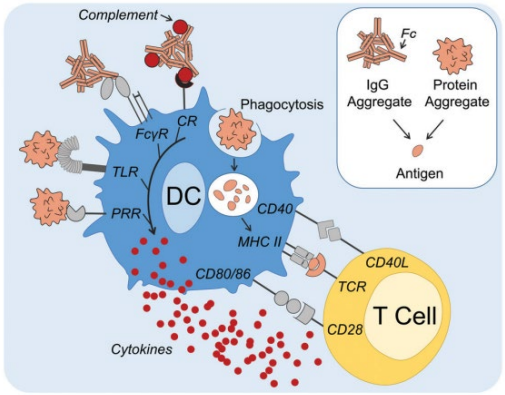
Cross-linking of B-cell receptor

- B-cells activate when the multiple B-cell receptors are simultaneously bound
- An immune response evolved to respond to virus and bacteria
- Aggregates may have several adjacent epitopes and therefore trigger this response



Dendritic cell uptake

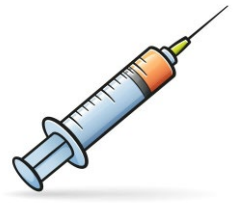
- Antigen-presenting cells such as dendritic cells (DC) are involved in creating T-cell response
- Aggregation enhances uptake via pattern recognition receptors
- Activated T-cells can provide help with B cells and drive high affinity antibody responses → persistent ADA responses



Lundahl et al., *RSC Chem. Biol.*, 2021, 2, 1004–1020

Causal sequence in consideration for aggregation risk

Aggregates dosed → Immunogenic responses

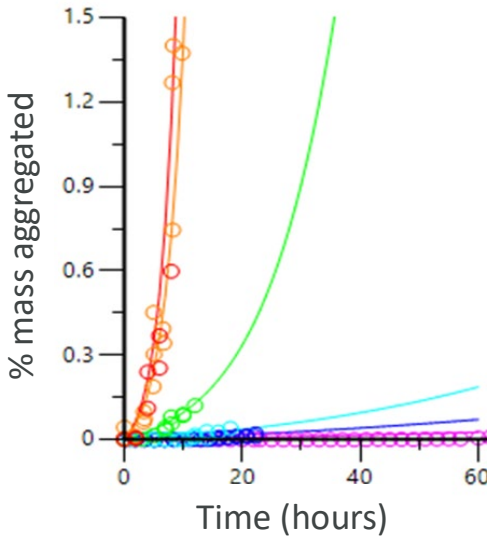


Drug Product Formulation / Process parameters → Modification to native protein (e.g. unfolding) → Aggregates in the lung → Immunogenic responses

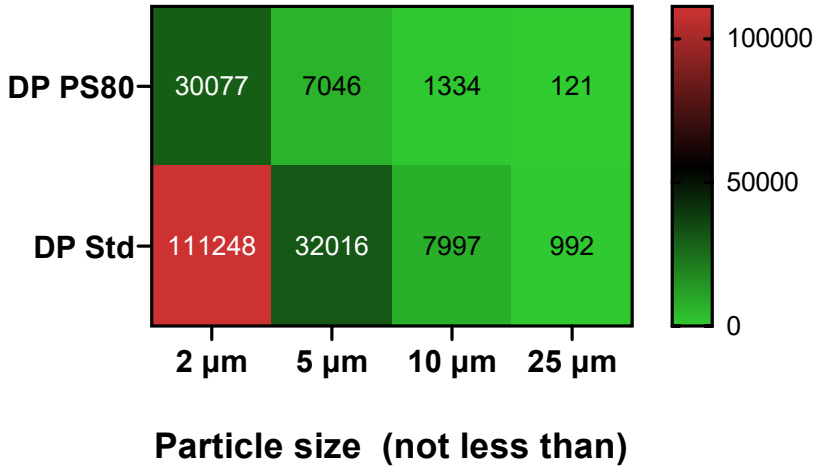
Modification to native protein (e.g. unfolding) → Aggregates in vitro

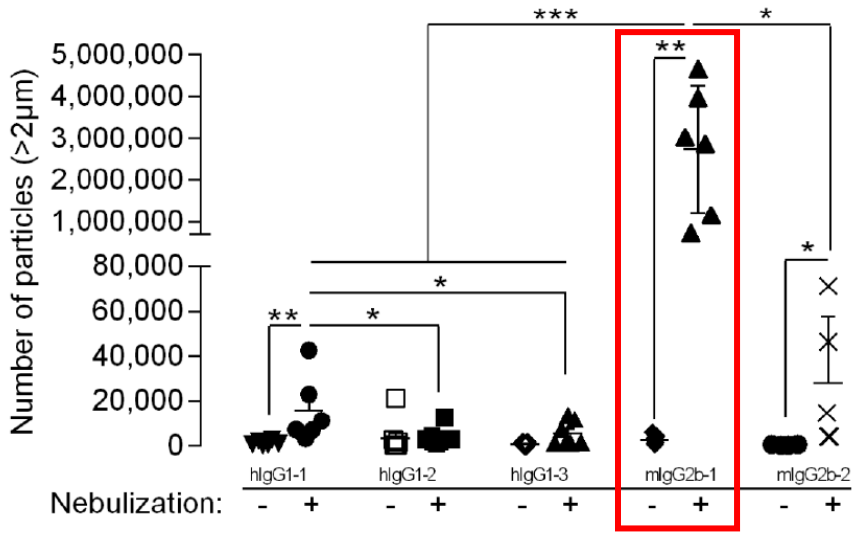
Aggregates from inhaled products can be *created* and studied in vitro – but does not prove presence in the lung

Dynamic Light Scattering



Particle count/mL by Microflow Imaging





Drug A, concentration dependent drug substance aggregation at 37°C

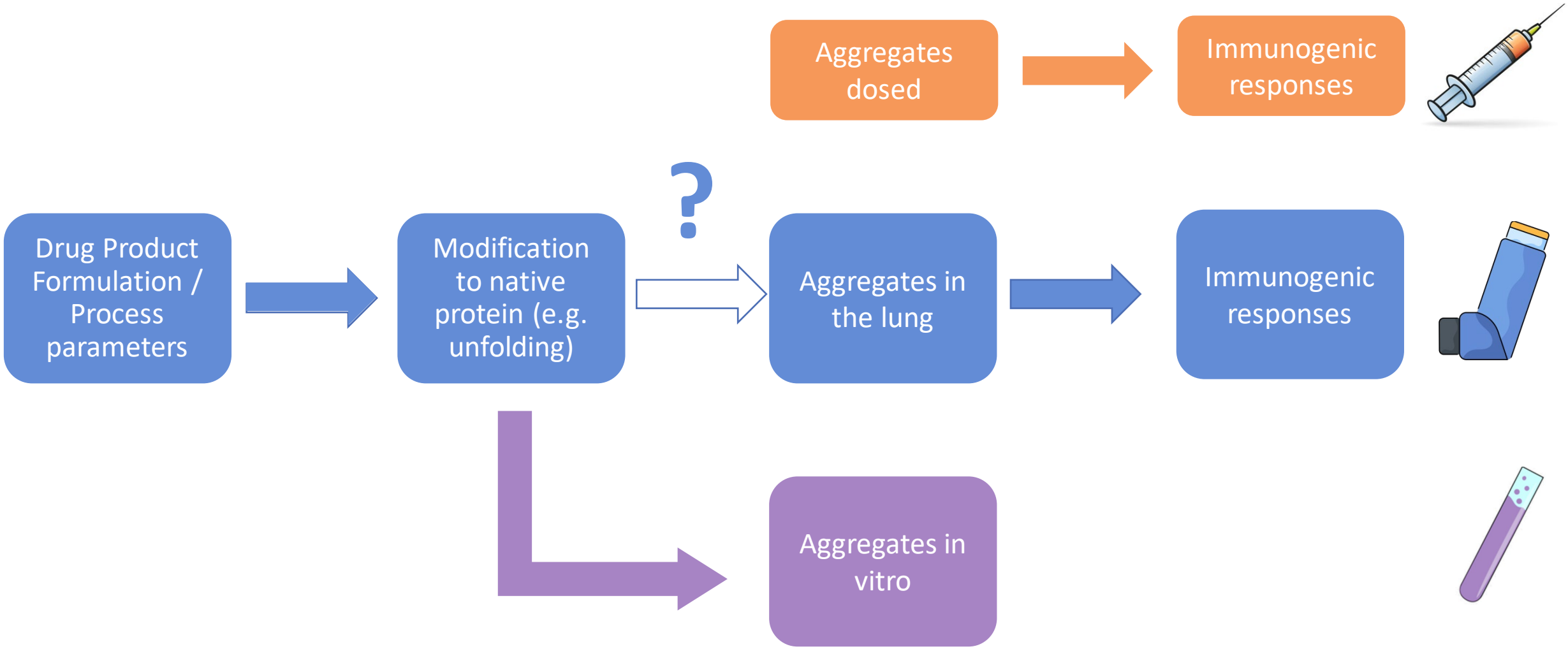
NIP-228, spray-dried and dissolved in water

mIG2b-1, nebulized and collected

[6]Mahri et al 2024, submitted

Secher et al., Pharmaceutics 2022, 14, 671.

Causal sequence in consideration for aggregation risk



Micron-sized aggregates are not delivered to the lung by inhalation and unlikely to form directly upon deposition

1. Any orally inhaled particle larger than ~10 um would deposit in the mouth/throat and not enter the lung. Even smaller particles are effectively filtered off by the nose in animal studies.
2. Formulation particle size is too small and contains too little protein to account for micrometer sized aggregates
    - **Case of nebulizer**: Droplet size of 3 µm and 50 mg/mL gives as total protein content equivalent to only ~1 µm
    - **Case of DPI**: Primary particle size of 2 µm and 10 % drug load to only ~1 µm. Protein largely dispersed in soluble matrix.
3. Monomer dominates the drug mass in in vitro tests, even for most 'sub-optimal' formulations

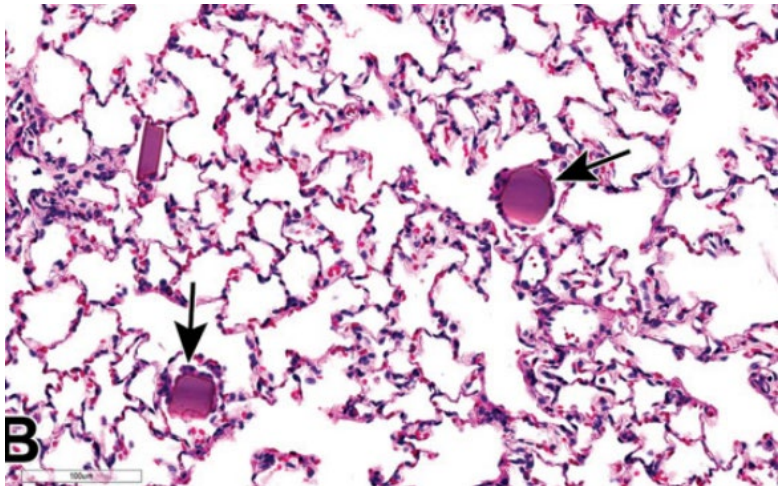
The risk we are considering is that aggregates form *in situ*, e.g. by induction from

- Exposure to lung micro-environment e.g. lipophilic surfactant layer in the lung periphery
- And/or protein-unfolding induced by formulation and/or delivery process (within scope of DP quality)

Studies indicating presence of drug aggregates in the lung have **not** used inhalation of therapeutic dose for local effect

Example 1

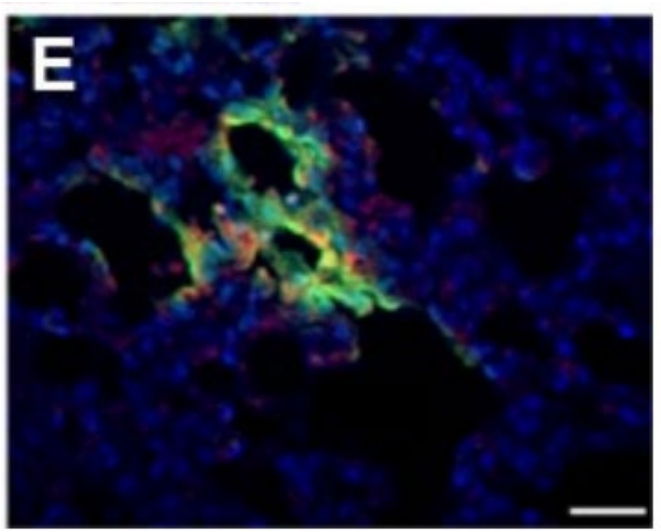
High dose inhaled **relaxin-A** for *systemic* treatment resulting drug-related crystalline material in the rat but not monkey



[4]Thierry et al., Toxicologic Pathology 2021, Vol. 49(2) 286-295.

Example 2

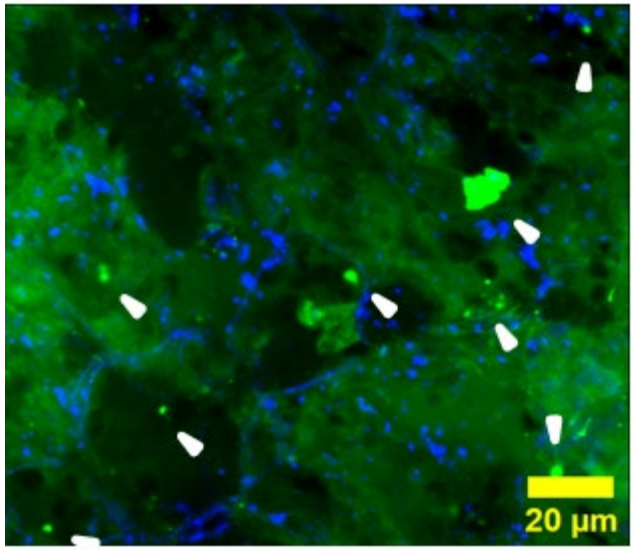
High dose inhaled **insulin** (for *systemic* treatment) resulting in drug-related fibrillar aggregates in mice



[5]Lasagna-Reeves, Endocrinology, October 2010, 151(10):4717–4724

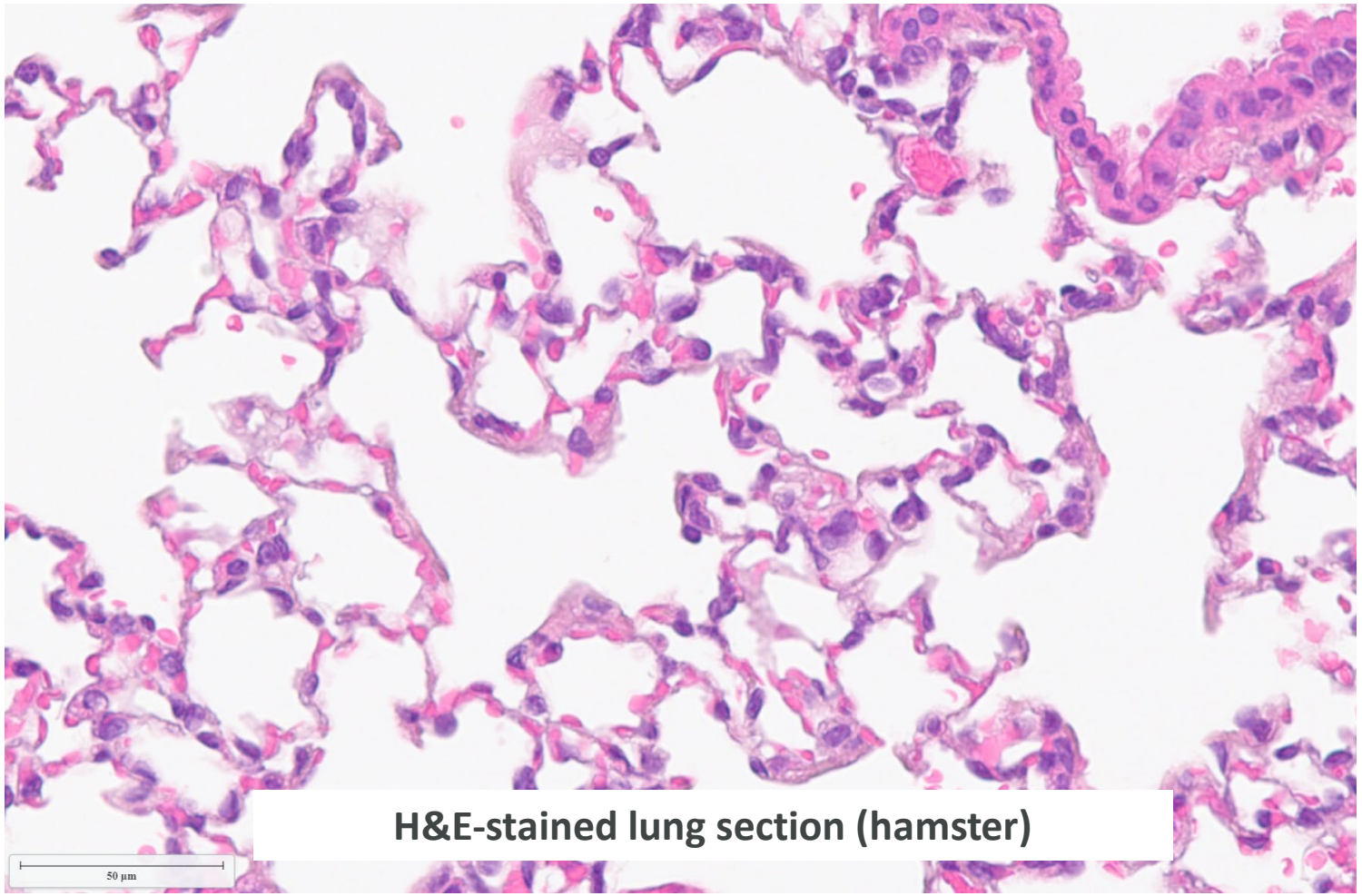
Example 3

High *dose intratracheally insufflated* **labelled NIP-228** mAb reveals non-macrophage associated 'spots'

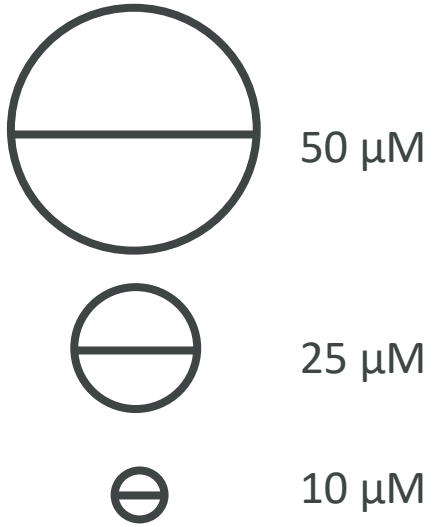


[6]Mahri et al 2024, submitted

AstraZeneca´s experience: µm-sized SVPs from spray-dried IBs (as observed in vitro) are not seen in vivo



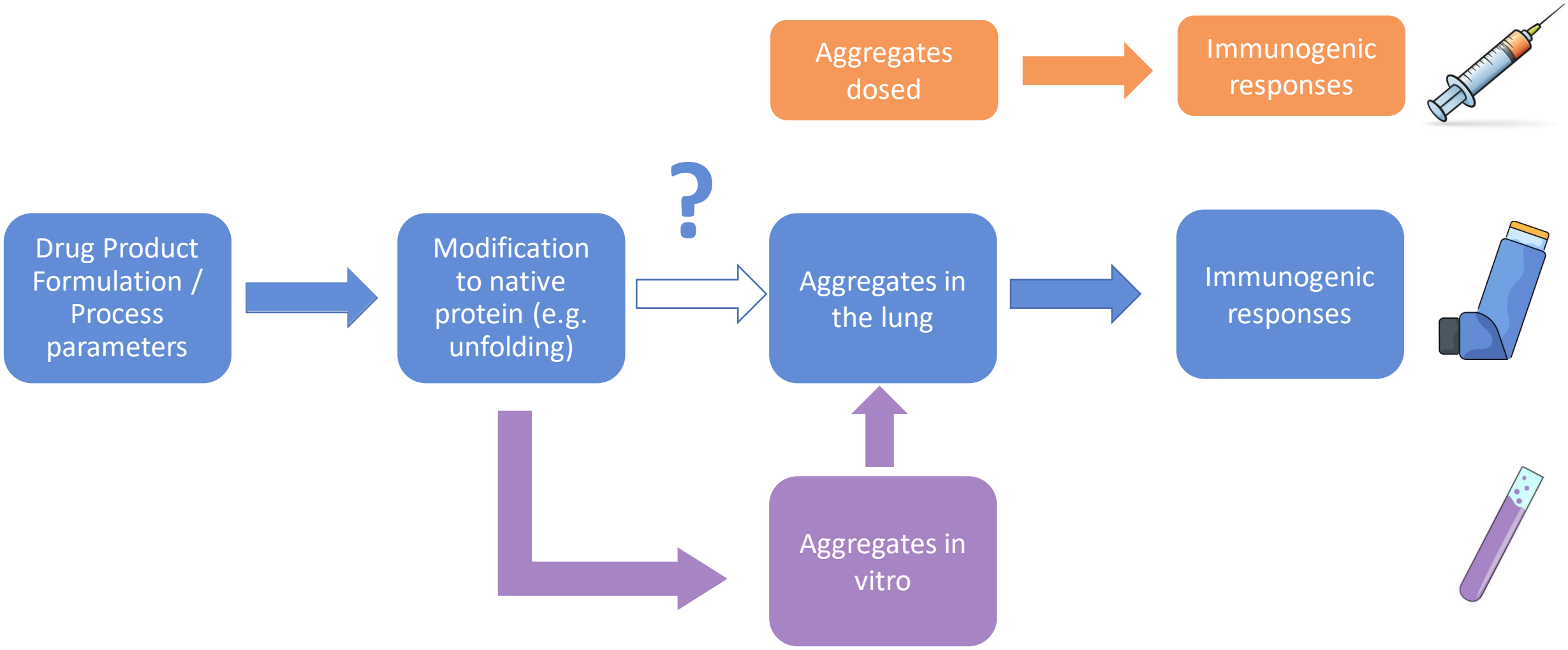
H&E-stained lung section (hamster)



NOTEs

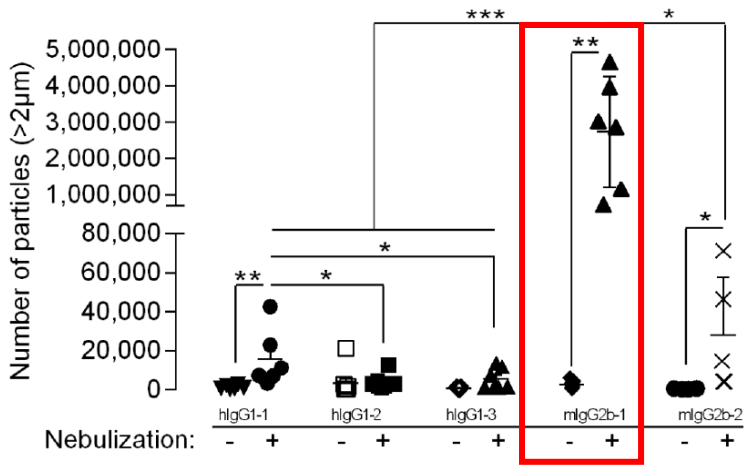
- Smaller aggregates may still be present
- IHC staining for drug protein generally reveals accumulation in macrophages

Causal sequence in consideration for aggregation risk



Antibody aggregates dosed to the mouse lung by intratracheal instillation can alter cellular homeostasis

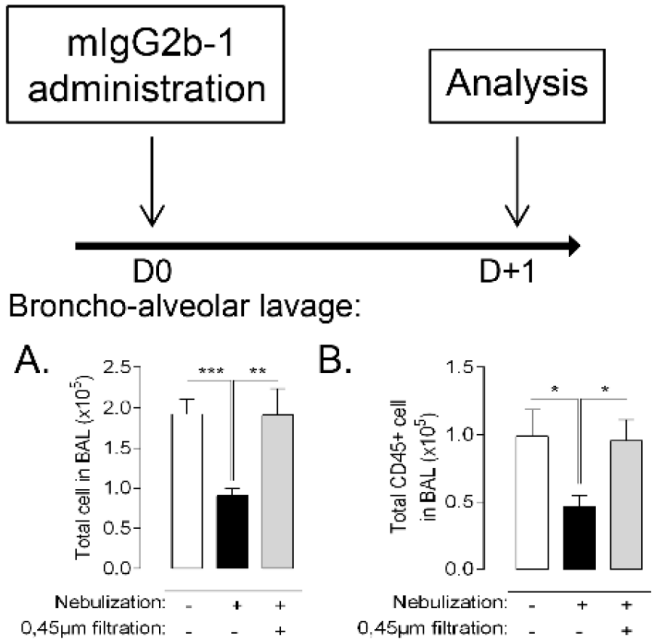
Characterization of Ab aggregates in collected nebulized aerosol



Antibody	2–5 µm	5–25 µm	>25 µm
hIgG1-1	84.3	14.7	1.1
hIgG1-2	78.4	21.1	0.4
hIgG1-3	56.8	42.8	0.4
mIgG2b-1	67	32.3	0.7
mIgG2b-2	57.8	40.7	1.6

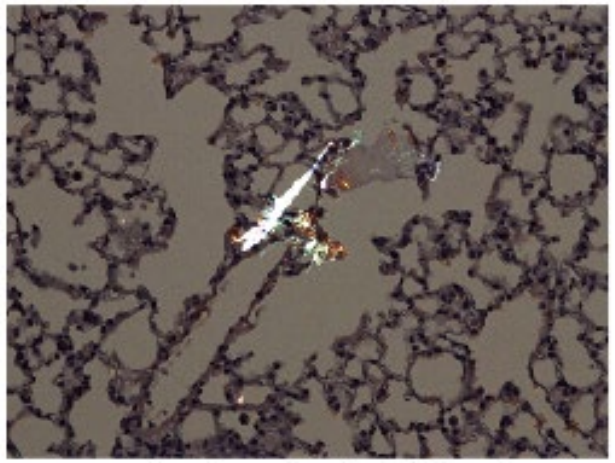
Secher et al., Pharmaceutics 2022, 14, 671.

Aggregate-containing dose solution reduces lung cell count*



*IgG aggregates, produced during nebulization, also induced a dose-dependent activation of human monocyte-derived dendritic cells in vitro

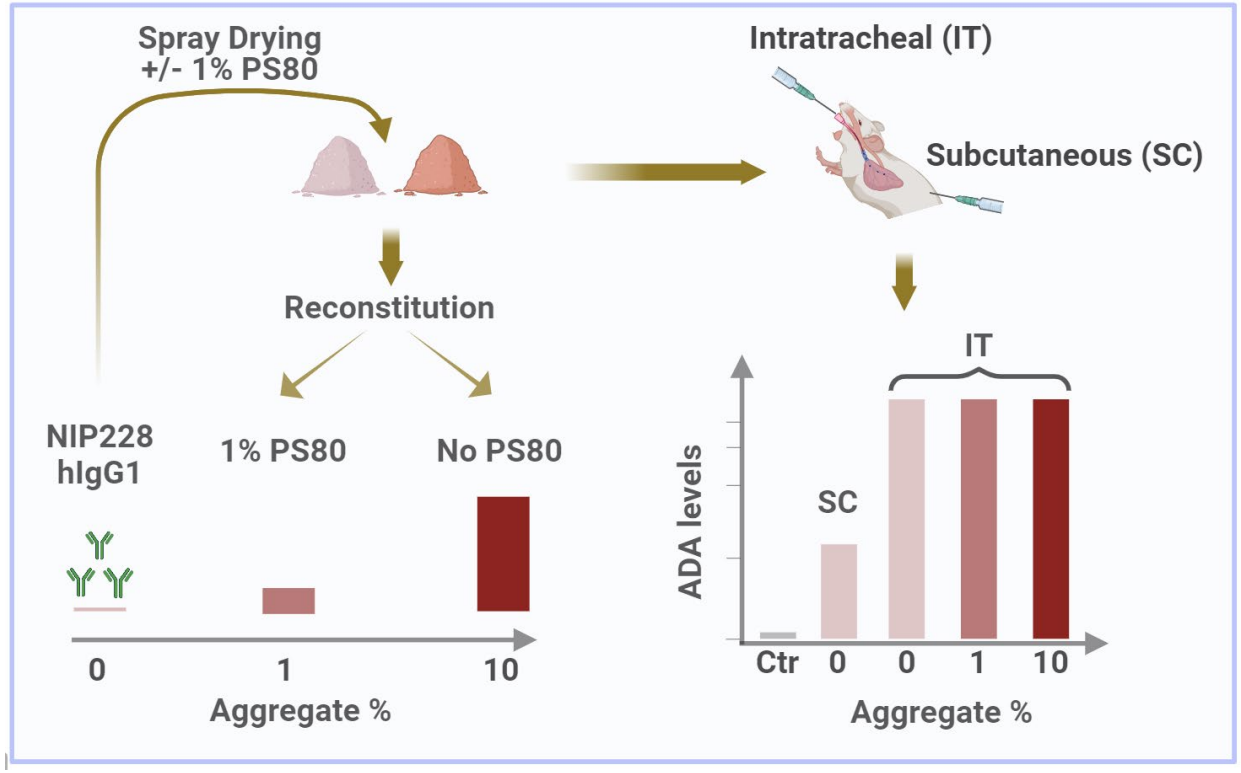
Aggregate (>10 µm) visualized by apple-green birefringence



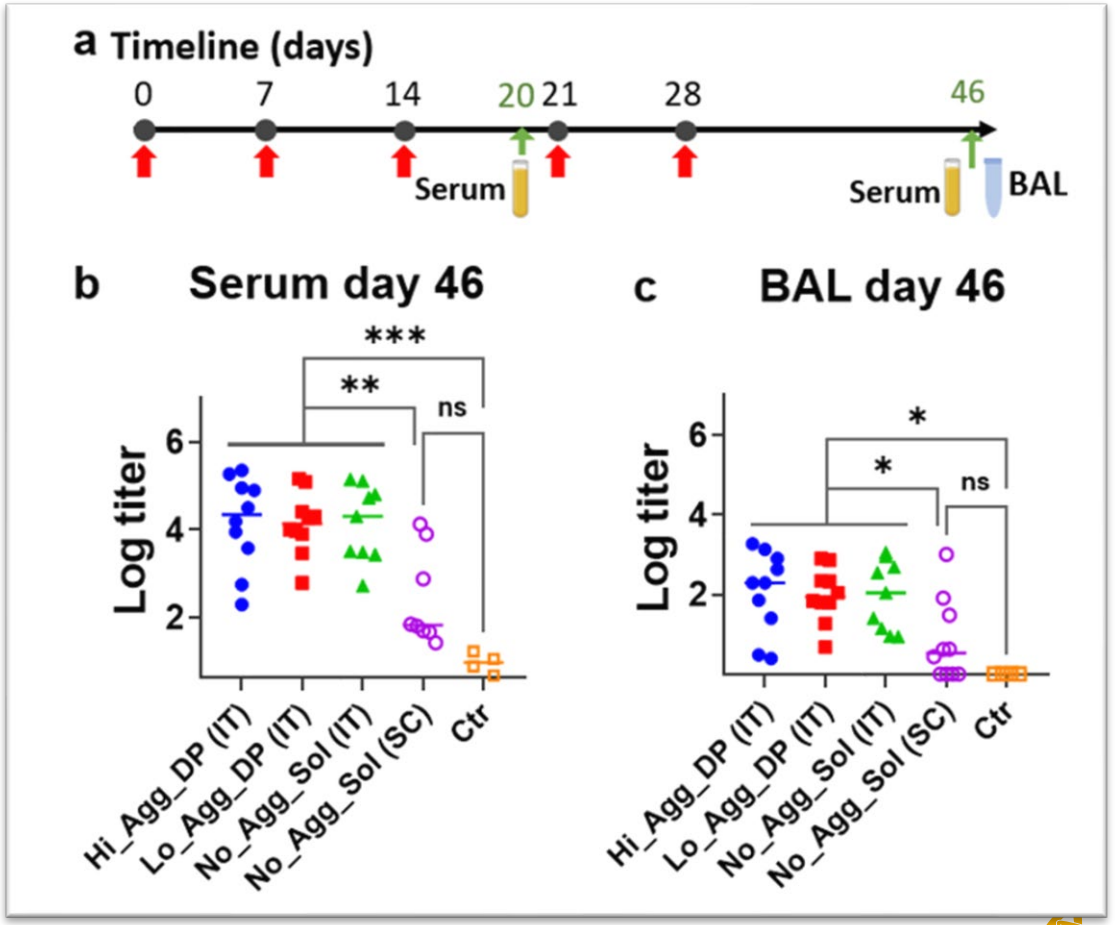
nebulized mIgG2b-1

Mouse ADA response to hNIP-228 is higher following dosing to lung - no correlation with in vitro aggregates

Graphical abstract in Mahri et al



Anti-drug antibody response

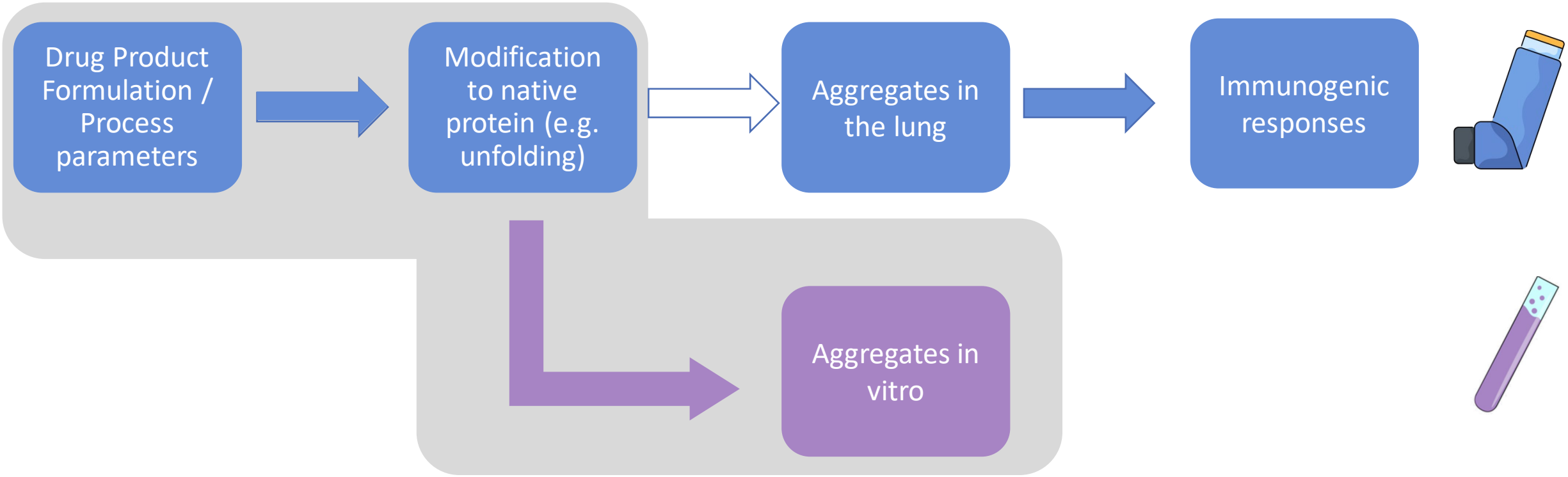


[6]Mahri et al 2024, submitted

Presentation outline

1. Considerations and framework IB risk assessment & review of current state knowledge
2. Basis of risk mitigation strategy and perspectives on relevant regulatory guidance
3. Towards bio-relevant aggregate testing for IBs

Aggregate risk mitigation by in vitro particle quantification as *a potential marker of native protein modification*



Perspectives on regulatory guidance

1. Aggregates/particles from in vitro tests is minimized in formulation development in the spirit of the FDA guidance of immunogenicity stating that: "*It is critical for manufacturers of therapeutic protein products to* ***minimize protein aggregation to the extent possible****. Strategies to minimize aggregate formation should be developed as early as feasible in product development*" (https://www.fda.gov/media/85017/download)

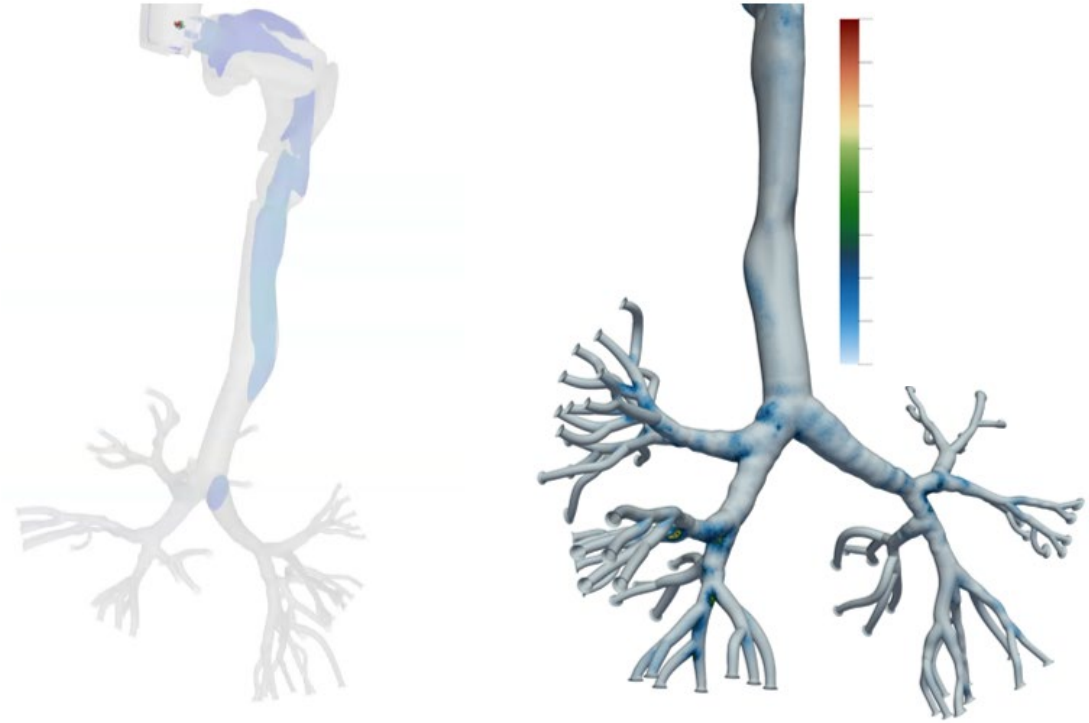
2. We assign ***no generically applicable limits for aggregates*** since formation aggregates in in vitro tests cannot be seen to resemble aggregation process in the lung. Furthermore, there exists no empirical in vitro – in vivo correlation.

3. Available regulatory guidance on particulate matter in medicinal products (e.g. USP 788) are not applicable to SVPs (and related) since these aggregates are not delivered (nor possible to inhale).

Presentation outline

1. Considerations and framework IB risk assessment & review of current state knowledge
2. Basis of risk mitigation strategy and perspectives on relevant regulatory guidance
3. Towards bio-relevant aggregate testing for IBs

Computational fluid dynamics and physiologically based biopharmaceutical modeling (PBBM) helps to identify relevant conditions for in vitro testing



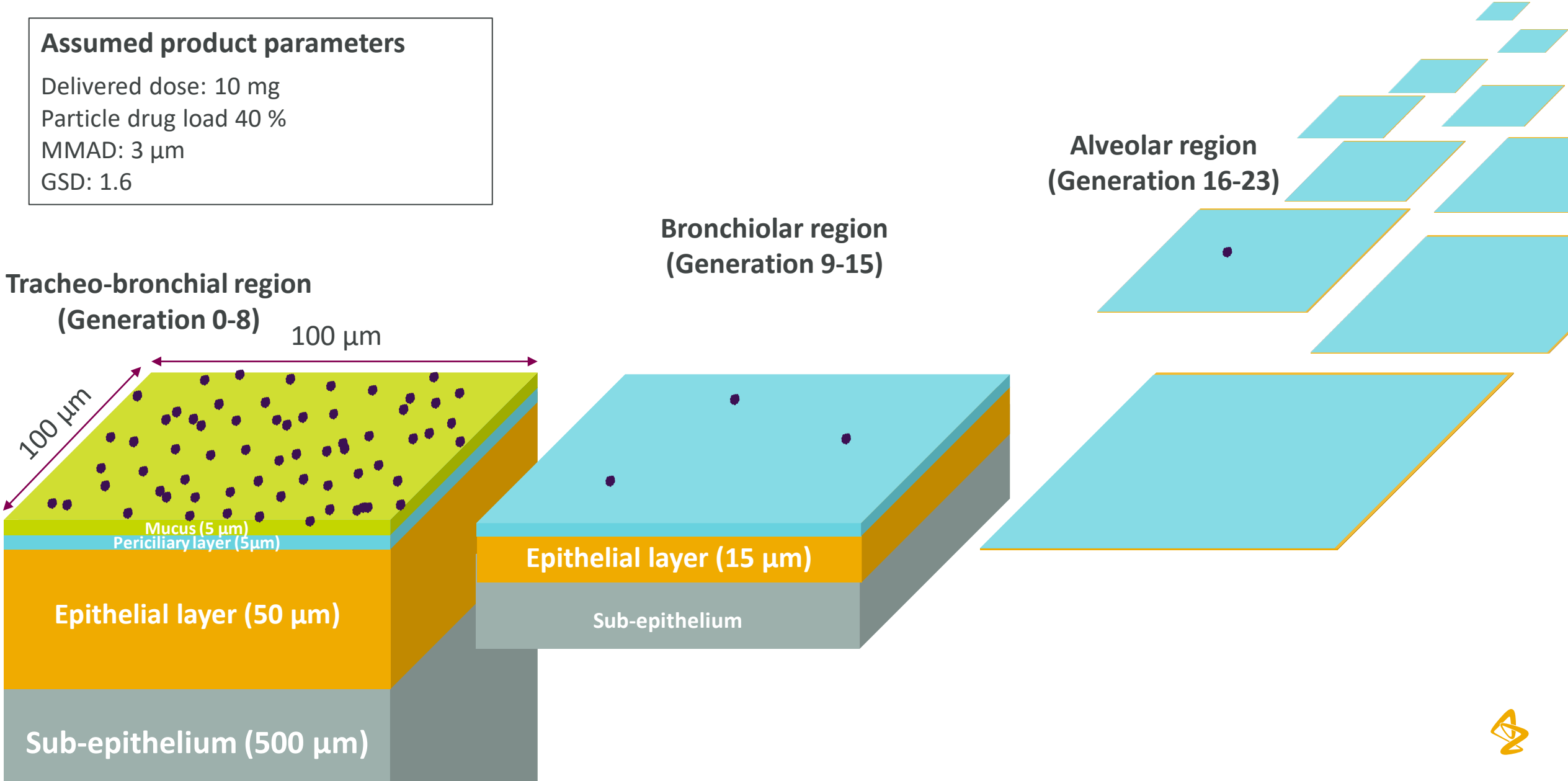
Key insights

1. Most regions of the lung would see very low concentrations of protein
2. It is far in between deposition sites of drug product particles in most lung regions
3. Higher concentrations are only seen in deposition hotspots, where any aggregates would be expected to be mucociliary cleared

PBBM-predicted deposition (averaged per lung region)

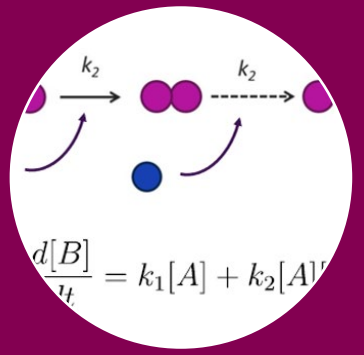
Assumed product parameters

Delivered dose: 10 mg
Particle drug load 40 %
MMAD: 3 µm
GSD: 1.6



Case study of semi-mechanistic modelling of aggregation in vivo

In vitro **dynamic light scattering**



Mathematical model of aggregate kinetics

Inhalation PBPM model

Risk assessment /guide further experimentation

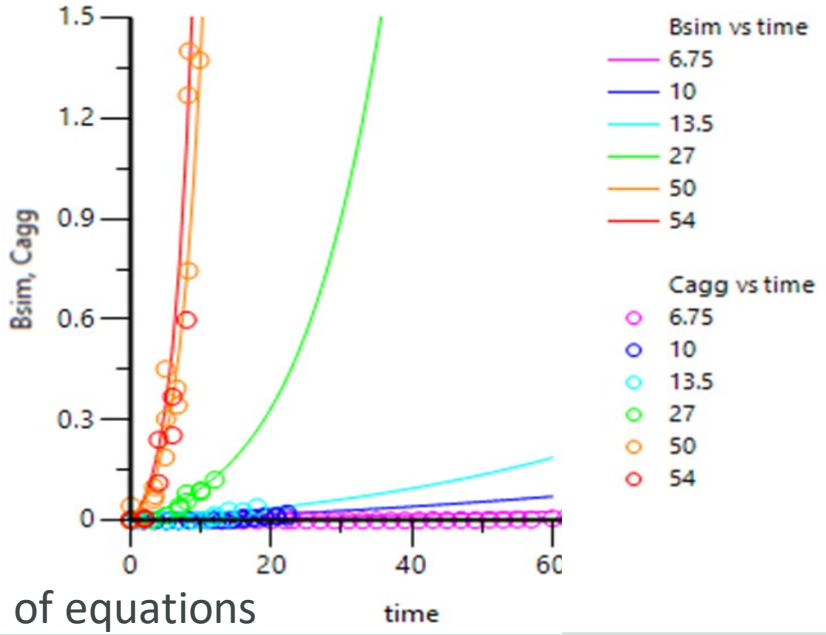
Model of aggregation of drug A

A: Monomer

B: Nuclei and oligomer

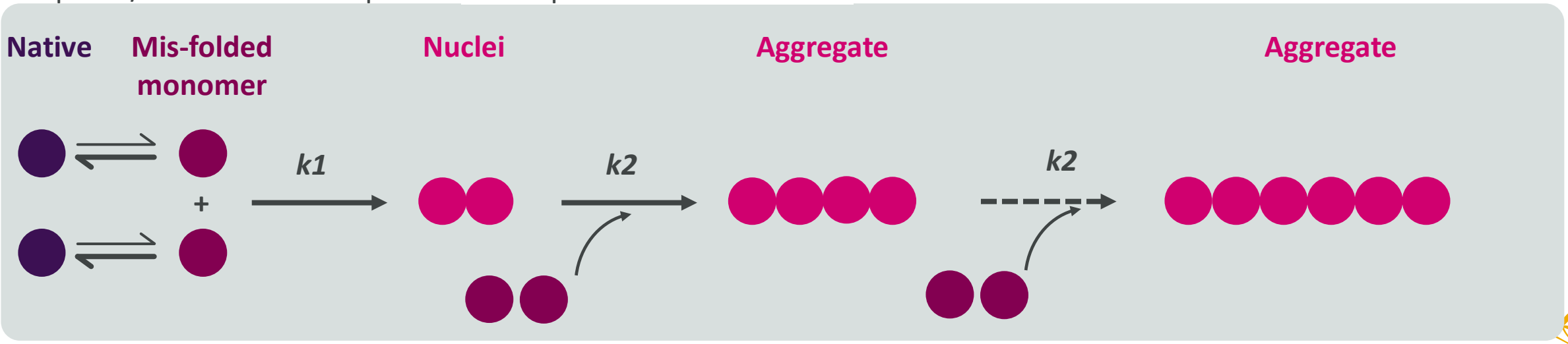
$$\frac{d[A]}{dt} = -k1[A]^2 - k2[A]^2[B]$$

$$\frac{d[B]}{dt} = k1[A]^2 + k2[A]^2[B]$$

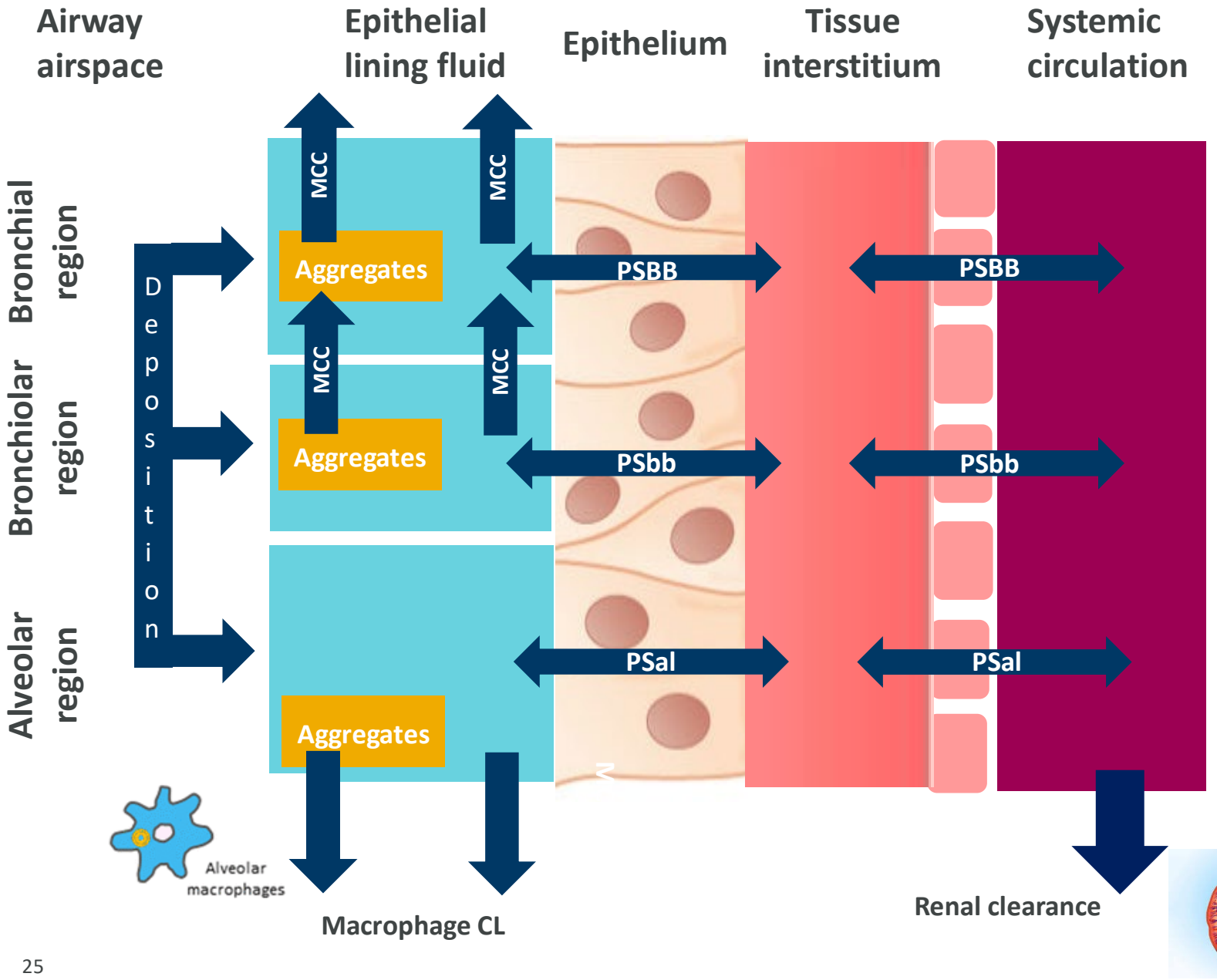


	Estimate	% CV
k_1	8.1 E-6	15
k_2	1.3 E-4	4

Graphical/mechanistic interpretation of equations



A PBBM model to simulate protein aggregation in the lung

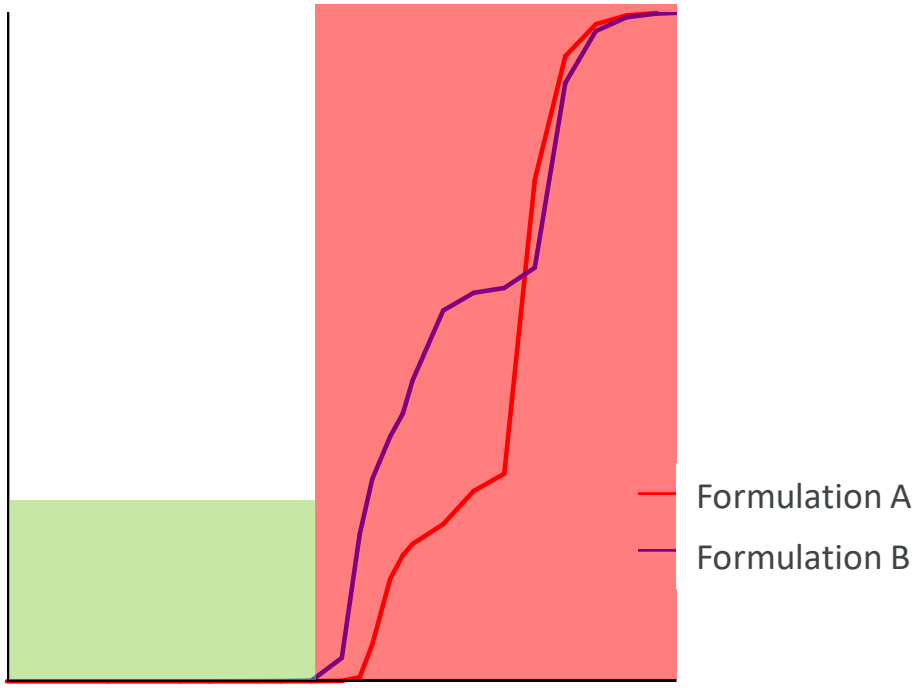


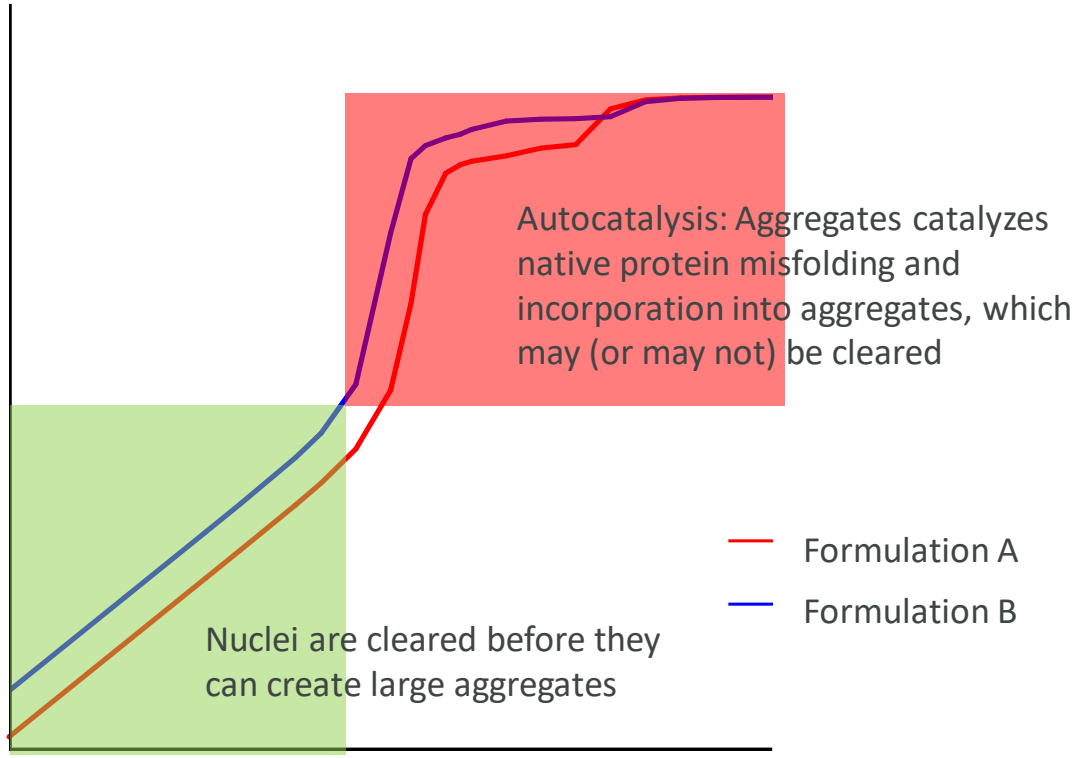
Technical

Key input and assumptions

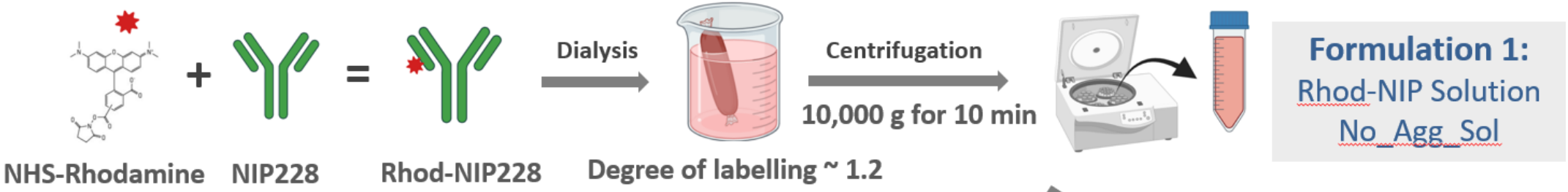
- Aggregation from DLS data/model
- Deposition from LungSIM model
- Predicted systemic PK
- Aggregate and monomer assumed to have equivalent disposition in the ELF.
- Parameter fitting to align the model to predicted plasma PK

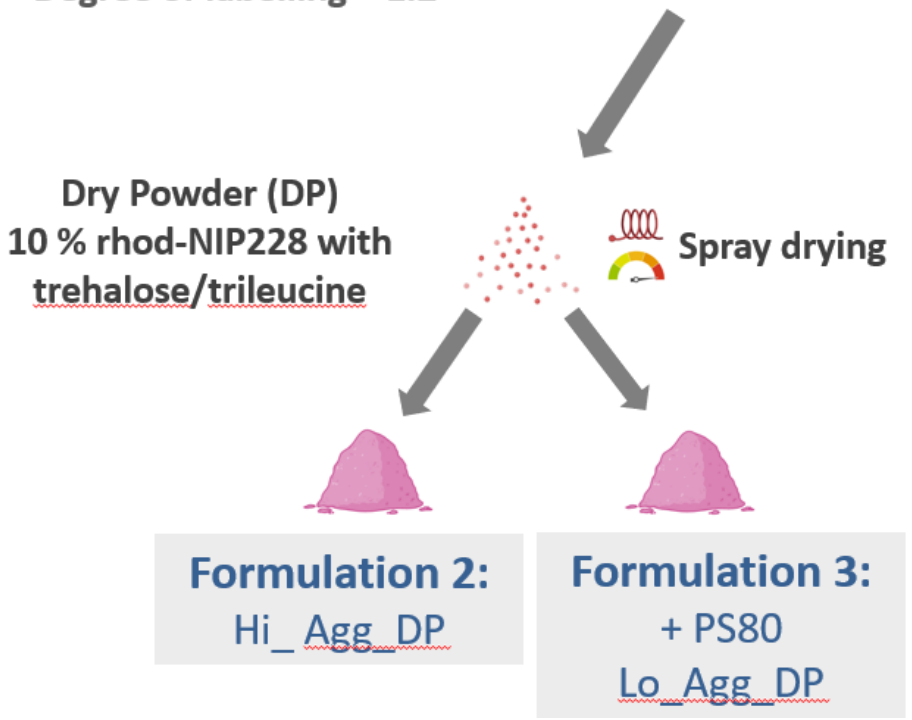
The model predicts the critical dose above which aggregation can be expected to be prominent





Platform methodology with labelling enabling in vivo and in vitro imaging

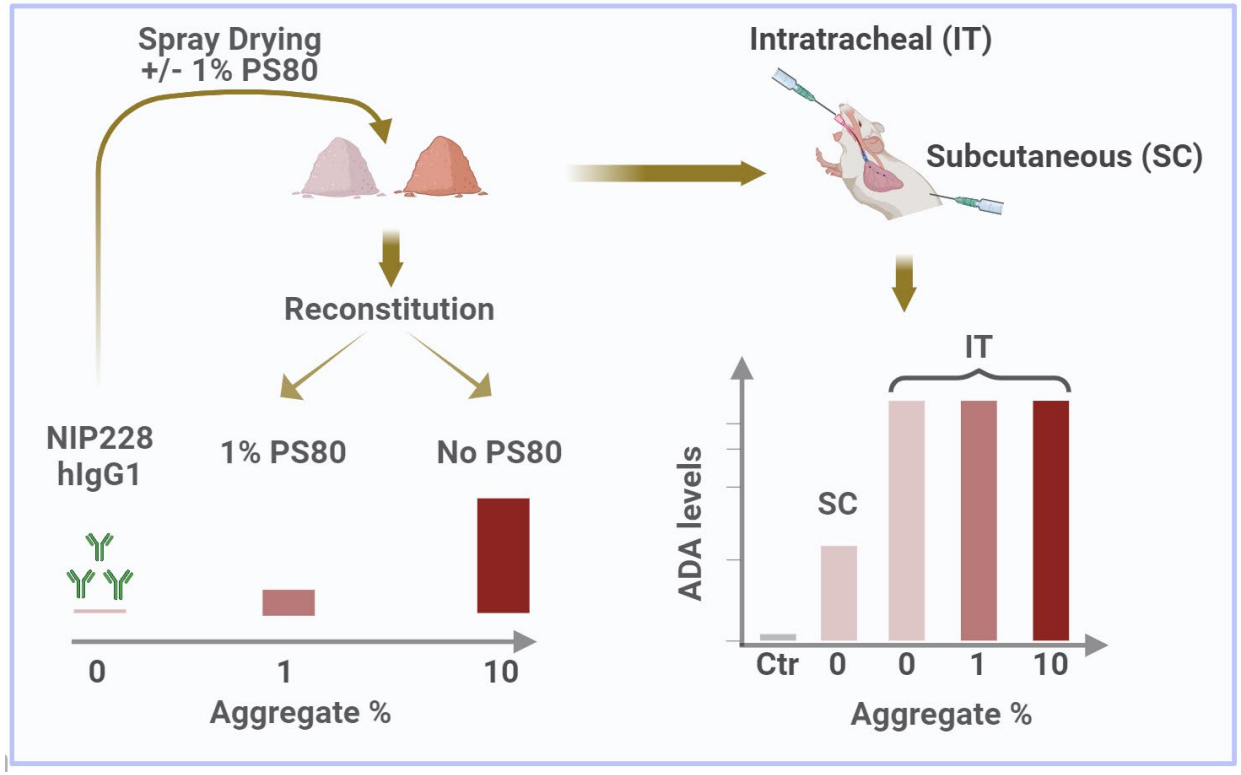




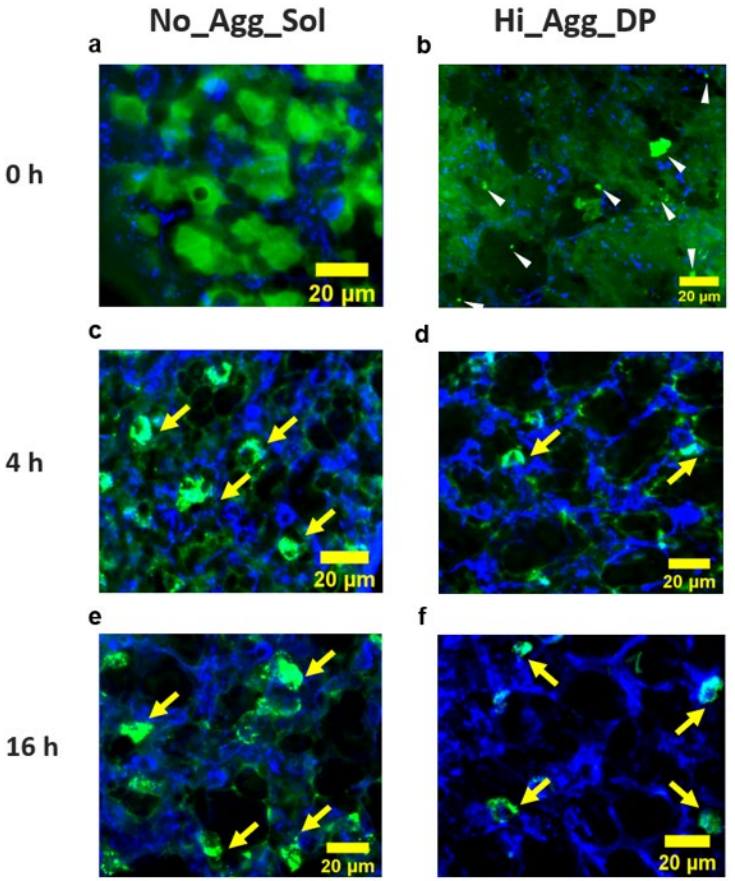
[6]Mahri et al 2024, submitted

Labelled hNIP-228 by intratracheal insufflation suggests some aggregates in the lung however needs confirmation with real inhaled delivery

Graphical abstract in Mahri et al



Confocal imaging



Ongoing collaboration with AZ and Rita Vanbever is looking at a labelled protein delivered by nasal inhalation

[6]Mahri et al 2024, submitted

Formulation-dependent clearance of labelled drug material from deposition sites on bronchial epithelial cells quantified by live imaging

Experimental setup for aerosol delivery

Fig. 1A

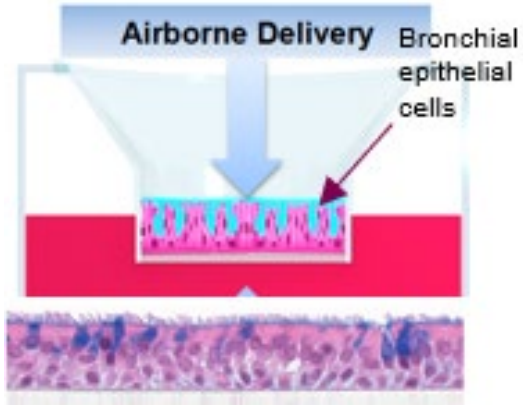
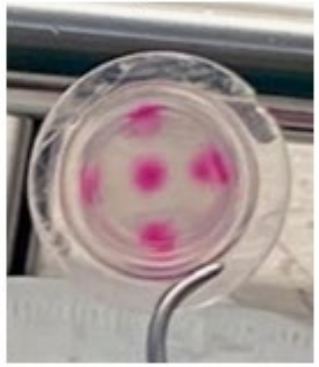


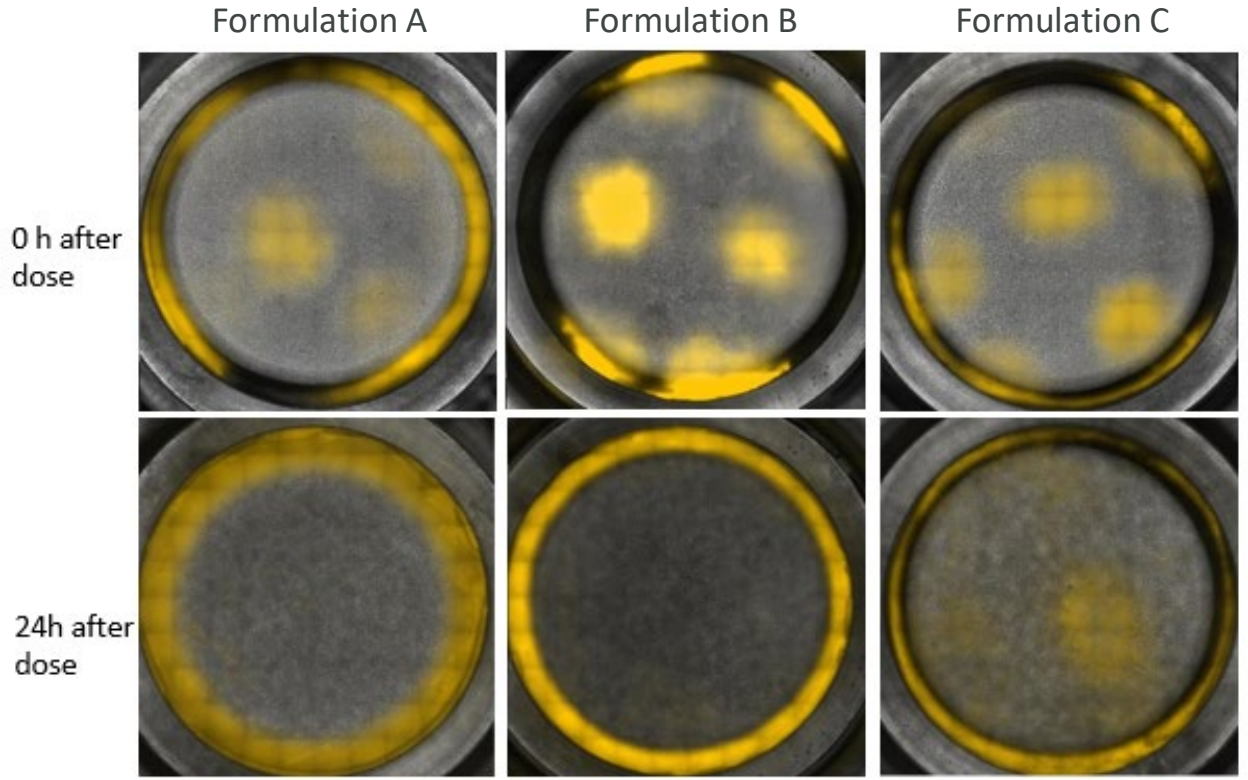
Fig. 1B

Fig. 1C

Fig. 1D



Differentiated clearance of drug related material (Rhodamine label) from center of well – potentially related to aggregation



Summary of presentation

1. Links between aggregate levels and immune responses have been established for the systemic route, but less so for the inhaled route.
2. Presence of protein aggregates of aerosolized products in the lung at therapeutic dose levels has not been demonstrated, however remains a possibility. C.f. injectables where detected aggregates in the solution are *de facto* delivered.
3. Aggregate levels from in vitro tests are minimized in formulation development in the spirit of the FDA guidance of immunogenicity
4. *Bio-relevant* testing of drug aggregates (in vitro, in vivo and in silico) should consider a relevant concentration range, aerosolized (properly dispersed) drug material as well as the micro-environments of the lung.

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