Nonclinical approaches to study shedding of gene therapy vectors

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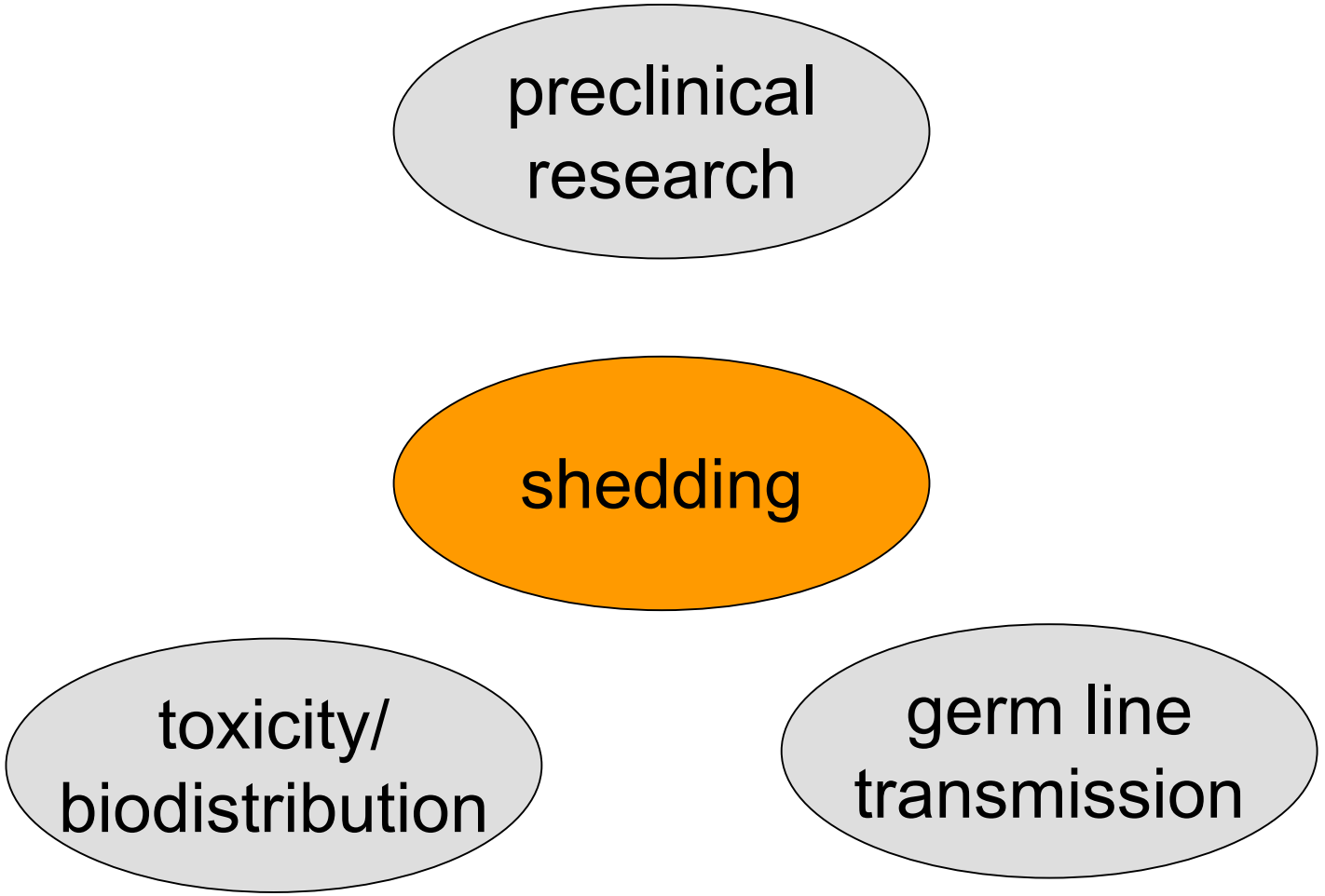
Shedding as preclinical issue



Excreta

- Urine
- Faeces
- Saliva
- Semen
- Breast milk

- Plasma and/or blood
- CSF

- Sputum
- Swabs
  - Nasal
  - Conjunctival
  - Vaginal/cervical
  - Urethral
  - Rectal
  - Buccal
  - Skin

Analytical methods

- QPCR:
  - Biodistribution (tissue samples)
  - Titration of shed vector sequences (excreta)
  - Infectiousness (*in vitro* binding to cells, titration by QPCR or replication center assay)
- Transgene expression *in vitro*
  - Due to generally low titers difficult to assess
  - Suitable cell lines required

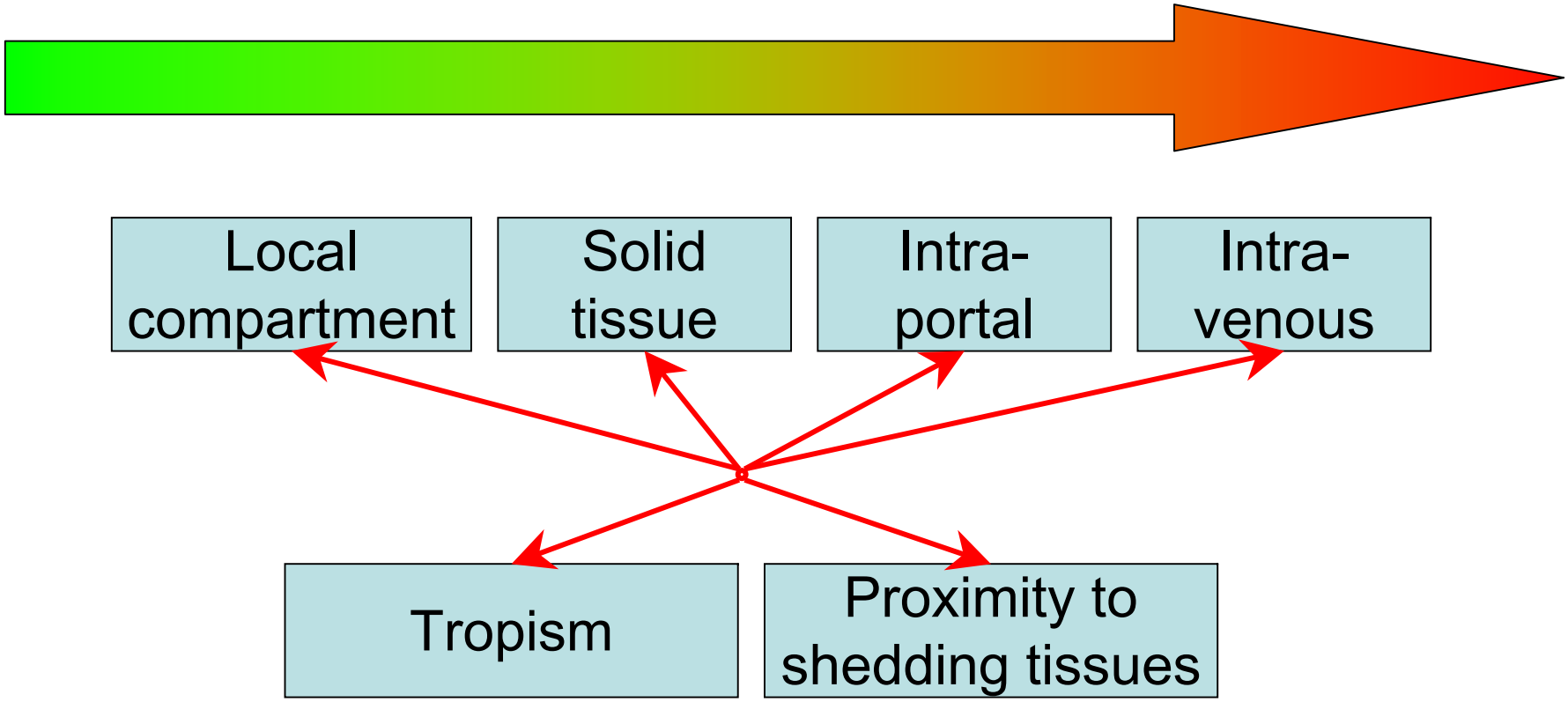
Analytical methods (2)

- Safety studies generally GLP-compliant
  - For shedding no established services provided by CROs yet (except for QPCR)
  - Risk assessment of shedding can so far only be based on less well established study designs and/or methods
- Determination of infectiousness complicated by matrices (inhibition, toxicity)
- Cross contamination!

Expected extent of shedding

DNA	Viral vector	DNA	Viral vector	Viral vector
Non-integrating	Non-integrating	Integrating	Integrating	Integrating
No replication	Replication deficient	No replication	Replication deficient	Replication competent

Risk of shedding



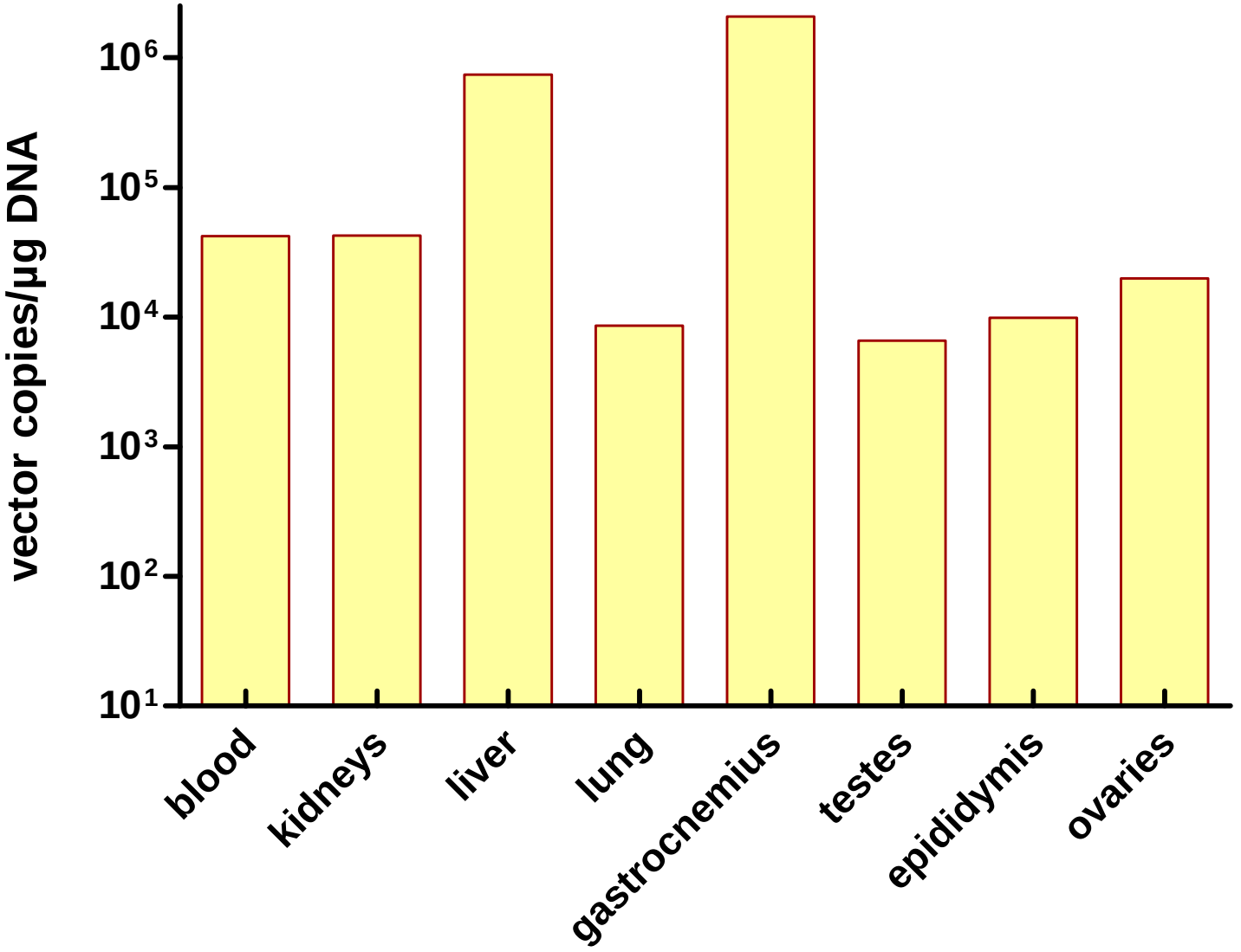
Routes of administration

- Route of administration and tropism determine extent and route of shedding
  - intravenous administration, depending on tropism distribution to relevant tissues
  - bladder and prostate tumours
  - brain

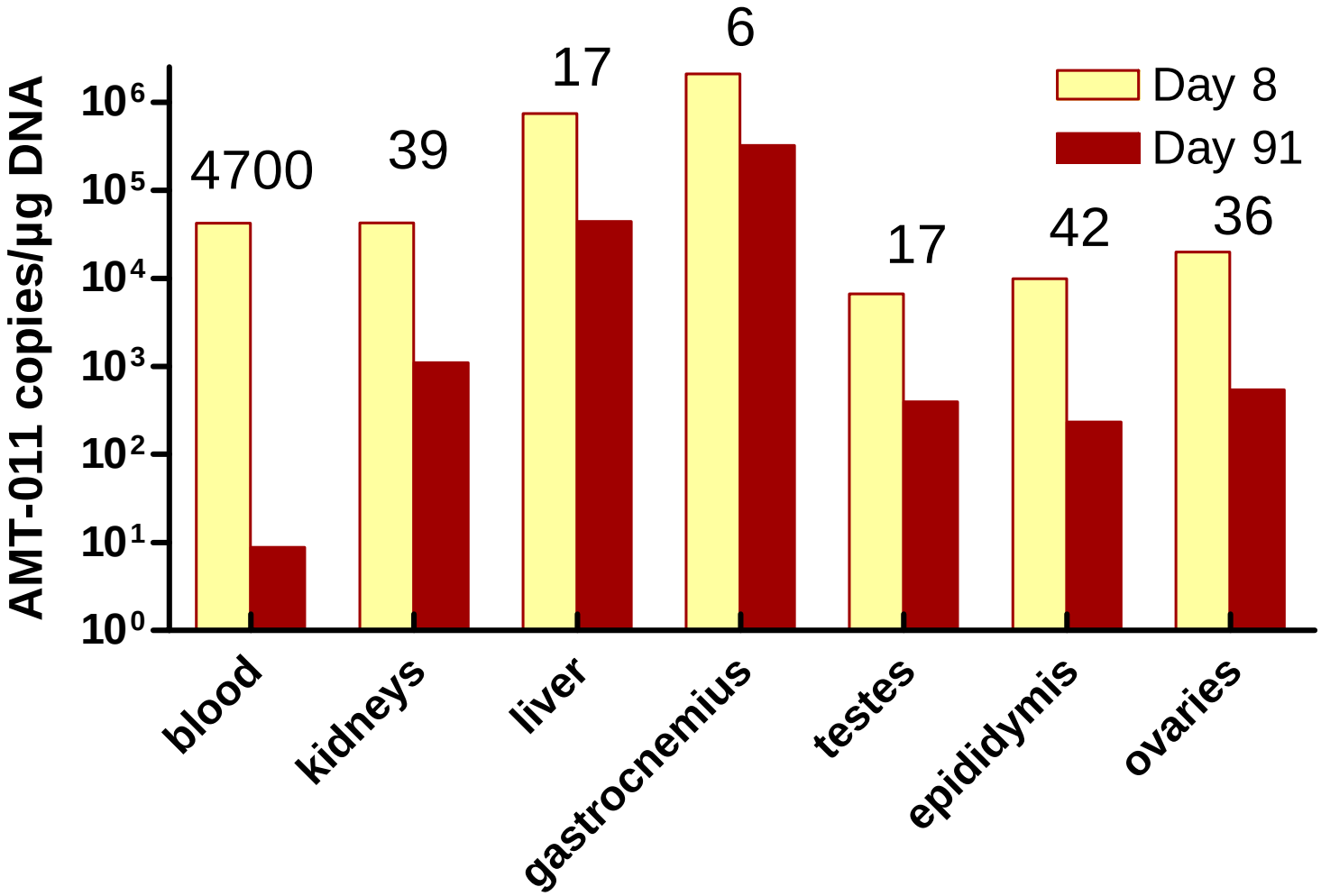
Biodistribution

- Tropism:
  - Tropism affects shedding
  - Difference between targeted and non-targeted tissues in wash-out
  - Extracellular vector probably rapidly degraded (depending on tissue)

Biodistribution



Biodistribution



Animal species

- Permissive species to vector
- Tropism preferably similar to humans
- Technical feasibility – depending on relevant tissue/excreta to be studied

- Choice for ‘routine’ safety studies (e.g. toxicity) and shedding may be different – target tissue not always most relevant
- Normal animals, or disease model?

Animal species

- Mice:
  - Little injectable required – more extensive studies possible in early phase of development
  - Numbers!
  - Technical limitations: access to body fluids very restricted, blood volume limited
- Rats
  - Less frequently used for preclinical research
  - Use of metabolic cages well established (CROs!)

Animal species

- Rabbits
  - Smallest commonly used non-rodent species
  - Established methods for obtaining semen
- Larger animals: easy access to body fluids, urine and faeces
  - Suitable animals include primates (cynomolgus, rhesus,marmoset monkeys), dogs, (mini-)pigs, etc.

Questions

- How predictable are animal experiments with respect to duration, extent and route of shedding?
  - More than with chemical entities, tropism and biodistribution are species-specific.
  - Animal species for shedding studies should be permissive, exhibit a relevant biodistribution profile, and meet the technical demands!
- Clinical route or worst case approach?
  - How important is shedding for gene therapy in brain?

Questions

- Do we need to test every GTP for shedding?
  - Same vector, different transgene
  - Use of marker genes (QPCR versus localisation)
- On which type of data should decisions for shedding studies be based?
  - Preclinical pharmacology or GLP-compliant data from biodistribution studies?
  - Should the eventual data be derived from GLP-compliant studies?
  - Additional animal studies to be performed (i.e. outside the standard safety package)?

Questions

- Are *in vivo* tests always required?
  - Even if – as is known for AAV – shed virus is probably inactive
- Could *in vitro* assays be employed?
  - Standard set of in vitro studies?
  - Can they replace/reduce *in vivo* studies?

Questions

- Analytical methods:
  - Guidance as to ‘how’ and ‘which’ desired.
  - Scientific evaluation of effect of matrices.
    - Urine and faeces probably hostile to live virus.
    - Effect of matrix on assay? Part of validation?