T.J. Nakchedi[1,2], C.A. Herberts[1], T. van den Hoorn[1], A.M.G. Pasmooij[1], M.L. Manson[2]
[1] College ter Beoordeling van Geneesmiddelen (CBG)/Medicines Evaluation Board (MEB)
[2] Pharmacy, Leiden University Medical Center

Regulatory experience with non-clinical studies of cell-based therapies

An analysis of studies on the biodistribution and tumorigenicity

Introduction

Cell-based therapies, which are classified as Advanced Therapy Medicinal Products (ATMPs), are at the forefront of drug innovation and highly science driven. Compared to traditional small molecules they require a more tailored approach when assessing the safety aspects biodistribution and tumorigenicity. [1,2]

With respect to biodistribution there are various methods, however, there is a need for clarity on which methods are most suitable for the product at hand. Regarding tumorigenicity there is a debate whether *in vitro* studies are sufficient for risk assessment and thereby deeming *in vivo* studies irrelevant.

Aim: *Investigating the **need** and **nature** of studies on the biodistribution profile and tumorigenic potential of cell-based therapies in order to provide consistency amongst regulators as well as developers.*

Methods

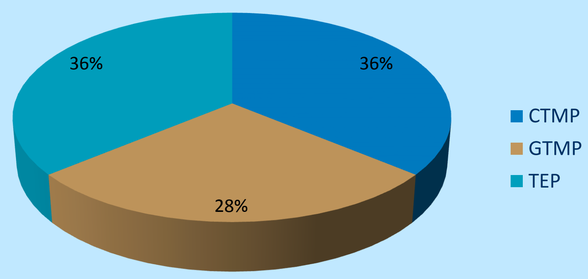
- Collection of scientific advice reports from EMA database (January 2013-June 2018) and sorting products into CTMP, GTMP (with cells), TEP.

->Exclusion: Products discussed in scientific advice reports lacking information on the non-clinical package.

- Analysis of biodistribution and tumorigenicity data collected from scientific advice reports using developed score table.

- Systematic literature search to create an overview on biodistribution methods.

Distribution of products



CTMP: Cell therapy medicinal product
GTMP: Gene therapy medicinal product (with cells)
TEP: Tissue engineered product

Results

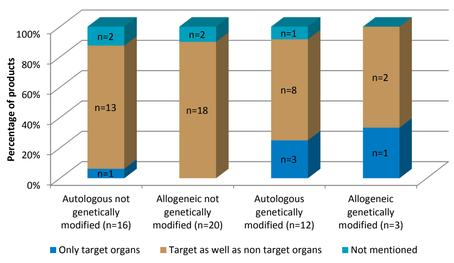
Biodistribution

Total (n=89 products):
Biodistribution studies performed and/or planned: 57 % (n=51), not necessary 28% (n=25), not mentioned 9% (n=8)

Migration

Total (n=51 products):
Migration assessment in target organs only:10 % (n=5)
Migration assessment in target as well as non target organs: 80% (n=41)
Not mentioned: 10% (n=5)

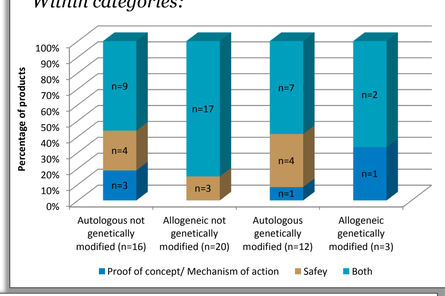
Within categories:



Type of information acquired

Total (n=51 products):
Information on proof of concept/mechanism of action:10% (n=5)
Information on safety: 21% (n=11)
Information on both proof of concept/mechanism of action and safety: 69% (n=35)

Within categories:



Total (n=51 products):
Quantifiable measurements were possible for 84 % (n=43). Not mentioned for 16% (n=8).

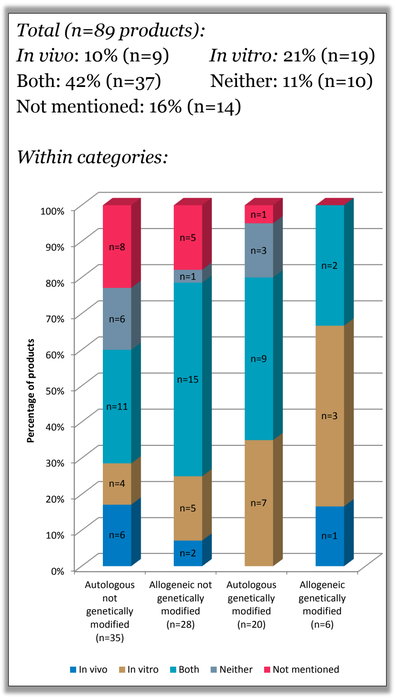
Figure 1. Analysis of biodistribution data

Tumorigenicity

Types of studies

Total (n=89 products):
In vivo: 10% (n=9) *In vitro:* 21% (n=19)
Both: 42% (n=37) Neither: 11% (n=10)
Not mentioned: 16% (n=14)

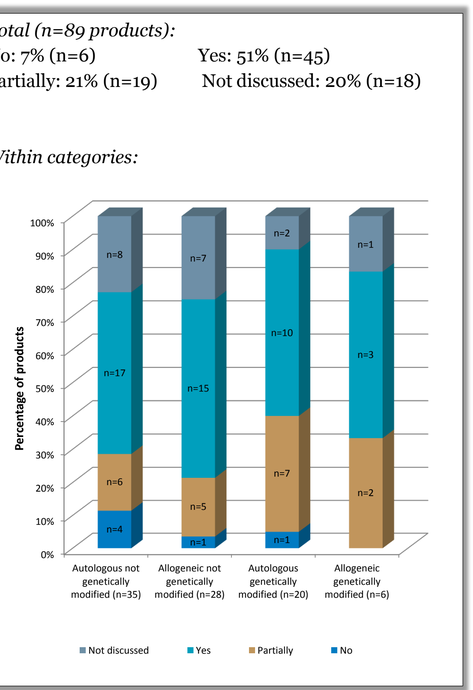
Within categories:



Informativeness tumorigenicity package

Total (n=89 products):
No: 7% (n=6) Yes: 51% (n=45)
Partially: 21% (n=19) Not discussed: 20% (n=18)

Within categories:



Relevance *in vivo* studies

Total (n=46 products)
Yes: 17% (n=8)
No: 83% (n=38)

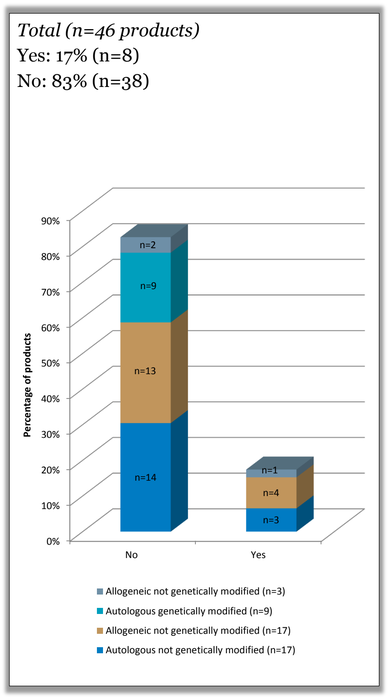


Figure 2. Analysis of tumorigenicity data

Table 1. Overview of selected biodistribution methods that can be used for *in vivo* and/or *ex vivo* measurements

Method	Description	Label Internal External	Serial measurements	Imaging speed	Imaging time frame	Sensitivity, Detection limit	Resolution (depth, 2D/ 3D)	Anatomical information	Whole body imaging	Live cell imaging	Clinical utility	Advantages/ Disadvantages	Examples
((q)RT)PCR *Ex vivo*	Method for detecting cells by measuring target DNA amplification	Internal label: primers, GAPDH, GFP, Alu sequences, probes	-	-	Single time point/ sample	1/600,000 cells (~0.0002%), Highly sensitive Alu based: 10 cells/mouse organ, 0.1 human cells in 1.5×10^6 heterogeneous cells.	Single cell	-	-	-	-	Advantages: Low cost, simple, high sensitivity and specificity Disadvantages: Invasive, dependent on acquired DNA sample	MSC's, chondrocytes, bone marrow cells, Human Umbilical Cord Blood Cells
Histology *Ex vivo*	Method for visualizing stained or labelled cells	Internal: H&E staining, DAPI, Prussian blue, rhodamine B fluorescence, PKH26, immunostaining, (DIG)-labeled DNA probes External: Antibodies	-	-	Single time point/ sample	Single cell	Resolution dependent on microscope	-	-	-	-	Advantages: Low cost, image acquisition can be done any time and slides can be reassessed Disadvantages: Time consuming	MSC's, immune cells, muscle precursor cells, NK cells, DC's, bone marrow cells, human neural stem cells
PET (Immuno-PET) *In vivo/ Ex vivo*	Highly sensitive, non invasive method for (quantitatively) tracking cells	Internal tracers: ^{64}Cu-(169cDb), ^{52}Mn, [^{89}Zr]Zr-oxine, [^{111}In]In-oxine, In(iii) and Zr(iv)), ([^{18}F]F-AraG, External label: Antibodies	Yes	Minutes-hours	Imaging during 30-60 minutes, imaging up to 7-14 days (depending on tracer/ label)	High sensitivity (10^{-9} to 10^{-12} M, single cell), 100-25.000/ 1x10^4 cells cells (depending on instrumentation and tracer),	Resolution: 1-2mm, ~3-5 mm^3 Limitless depth, 2D images (3D in combination with CT)	No	Yes	Yes	Yes	Advantages: High sensitivity, multiple labels possible, quantifiable Disadvantages: Low spatial resolution, radioisotopes have a short half-life, low-resolution imaging at the cellular or sub-cellular level, expertise required, potential false positives after cell death	Liver stem/progenitor cells, CD8+/CD4+ T cells, T cells (CAR/TCR), B-cells, muscle precursor cells, dendritic cells, stem cells, human induced pluripotent stem cells, hepatocytes
MRI *In vivo/ Ex vivo*	Non invasive method, with high spatial resolution for (real time) tracking of cells	Internal tracers: SPIO, ^{19}F	Yes	Minutes-hours	Up to 24 weeks (SPIO)	Sensitivity: Cells (not single cells)	Resolution: ~<1-3mm^3, 10-100µm Limitless depth, 3D images	Yes	Yes	Yes	Yes	Advantages: High spatial resolution, stable label(s) Disadvantages: Low sensitivity, low-resolution imaging at the cellular or sub-cellular level, not quantifiable	Kidney cells, stem cells, PBMC's, glial restricted precursor cells, MSC's, immune cells, breast cancer cells, muscle precursor cells

Conclusions

Biodistribution studies
Need: Performed and/or planned for majority of the products
Nature:
- **Migration assessments mostly in target as well as non target organs**
- **Quantifiable measurements for 84% of products**
- **Choice of method dependent on: need for *in vivo/ex vivo* measurements, costs, feasibility and accessibility**

Tumorigenicity studies
Need: For half of the products *in vivo* studies are performed and/or planned, for 83% of these products the studies were not relevant
Nature:
- **For 21% only *in vitro* studies, for 10% only *in vivo*, for 42% both**
- **For 51% of the products the tumorigenicity package was deemed fully informative**

References:
1Flory E, Reinhardt J. European Regulatory Tools for Advanced Therapy Medicinal Products. Transfusion Medicine and Hemotherapy. 2013;40(6):5-5.
2 Herberts C, Kwa M, Hermsen H. Risk factors in the development of stem cell therapy. Journal of Translational Medicine. 2011;9(1):29.
Table 1: contact t.j.nakchedi@umail.leidenuniv.nl

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