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Biodistribution Studies Demystified: Informing the Efficacy of Cutting Edge Therapeutics

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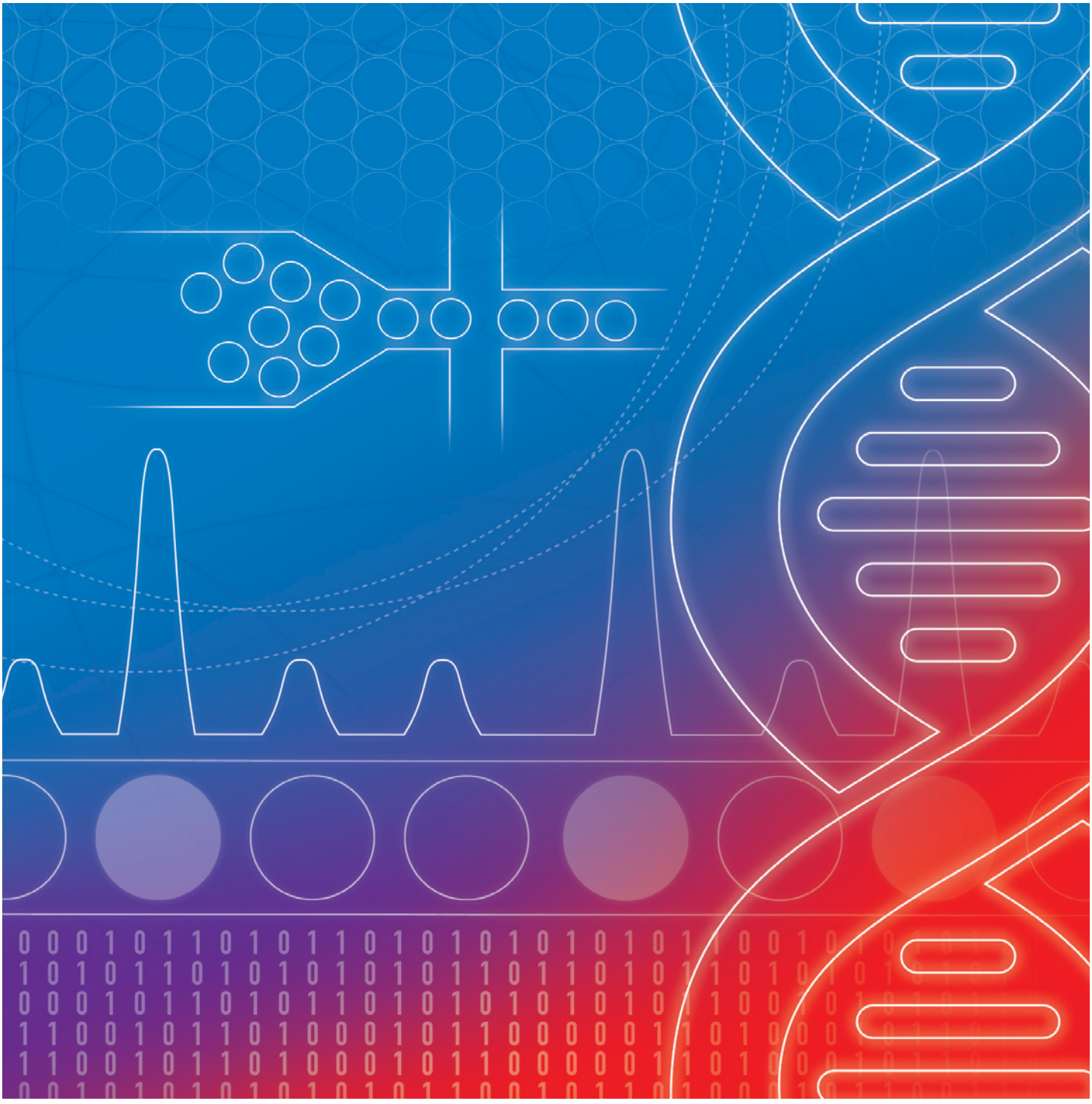

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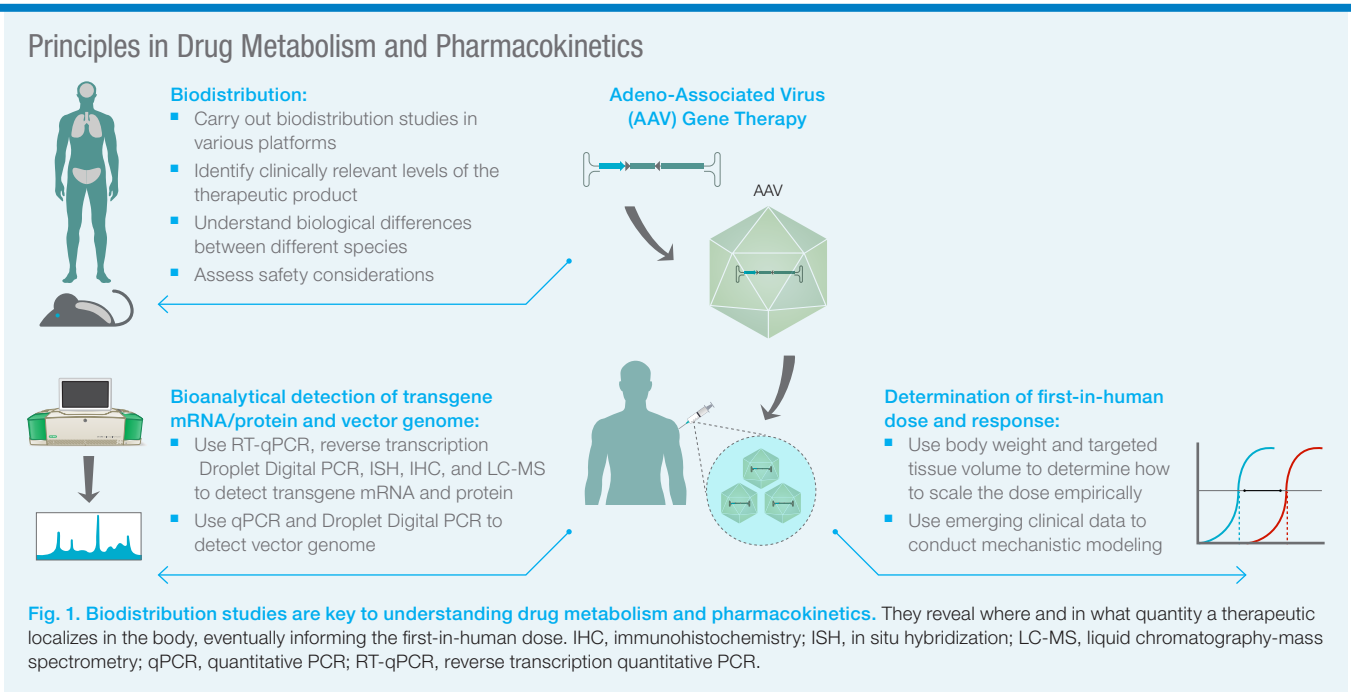

Fig. 1. Biodistribution studies are key to understanding drug metabolism and pharmacokinetics. They reveal where and in what quantity a therapeutic localizes in the body, eventually informing the first-in-human dose. IHC, immunohistochemistry; ISH, in situ hybridization; LC-MS, liquid chromatography-mass spectrometry; qPCR, quantitative PCR; RT-qPCR, reverse transcription quantitative PCR.

Cutting Edge Therapeutics

The recent progression of novel cell and gene therapy modalities has been extraordinary. For viral vectors, the rapid expansion of clinical pipelines and the number of potential candidates moving toward commercial approval have skyrocketed since the milestone approval of Glybera (uniQure N.V.), a recombinant AAV therapy for hereditary lipoprotein lipase deficiency, by the U.S. Food and Drug Administration (FDA) in 2012 (Mendell et al. 2021). A similar pace and progressive trend for genetically modified autologous cells is evident. Less than a decade ago, two lentiviral-transduced autologous T-cell products for the treatment of pre–B-cell acute lymphoblastic leukemia (ALL) or B-cell lymphomas were approved by regulatory agencies (Shah and Fry 2019). Since then, developmental pipelines for gene-modified cellular therapeutics have significantly expanded in the number of unique assets and their targeted disease indications.

Collectively, these novel medicinal cell and gene modalities represent cutting edge therapeutics that have the potential to significantly improve or cure complex and devastating conditions that would otherwise have few or no therapeutic options…

Close behind the clinical promise of autologous cell therapies, the inception of cellular reprogramming to generate induced pluripotent stem (iPS) cells has fueled several developmental milestones and propelled this potential therapeutic modality to clinical evaluations. These cells have offered an ideal starting point for differentiation into multiple therapeutic cell types to treat numerous conditions, including circulatory system disorders and neurodegenerative conditions (Kim et al. 2022). Finally, the rise of RNA-based therapeutics, fueled partly by the coronavirus disease 2019 pandemic, has also demonstrated promise in the space of in vivo gene modification for targets previously thought to be undruggable (Liu and Wang 2022). Collectively, these novel medicinal cell and gene modalities represent cutting edge therapeutics that have the potential to significantly improve or cure complex and devastating conditions that would otherwise have few or no therapeutic options.

Biodistribution: A Critical Tool in Cell and Gene-Based Therapeutics

Elucidating these novel therapeutic modalities' complete mechanisms of action will allow them to fulfill their true potential. Mechanistic studies include both pharmacokinetic and toxicological characteristic assessments and are typically performed in both nonclinical and early clinical studies of potential therapeutic candidates (Figure 1). These studies effectively aim to determine the presence, persistence, and clearance of the drug at the molecular level in target tissues and an array of nontarget tissues (Vervaeke et al. 2022). In other words, they establish where the therapeutic goes when it is administered, how long it lasts, and the functional consequences of the therapeutic's localization.

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These analyses, known as biodistribution studies, are essential for predicting and assessing therapeutic efficacy and toxicity that occurs from intended or unintended localization of the therapeutic (Kamiyama et al. 2021). Further, findings about biodistribution can inform necessary details required for future clinical studies, such as dose selection, administration route, and dosing schedule (Sugimoto et al. 2021).

Biodistribution studies are mandated by multiple regulatory agencies, including the U.S. FDA and the European Medicines Agency (EMA). However, each agency has a slightly different interpretation of the meaning and methods of the study, and there is currently no international consensus or synchronization (Kamiyama et al. 2021). Further, the overall testing objectives are highly dependent on the identity of the therapeutic modality, as each has a unique mechanism of action and specific associated risks. Viral vectors, for instance, deliver genes to target tissues and cells through tissue tropism derived from the vector capsid design. However, there is ample opportunity for unintended transgene uptake and subsequent expression when off-target tissues process the vector (Zheng et al. 2011). Thus, there is a potential risk for organ toxicity (Gonin and Gaillard 2004). In stark contrast to viral vectors, the clinical efficacy of chimeric antigen receptor (CAR) T cells has been shown to correlate positively with the in vivo expansion once administered (Sugimoto et al. 2021). However, this clinical efficacy and underlying in vivo cell expansion are often paired with cytokine release syndrome and, in severe cases, the potential for multiple organ failure (Sugimoto et al. 2021). Yet another novel therapeutic type with a unique therapeutic mechanism, undifferentiated iPS cells, always poses a risk of tumorigenicity. Thus, determining iPS-derived–cell-product fate once administered is crucial in assessing the risk of malignant transformation (Kamiyama et al. 2021).

Because of the diversity of therapeutic modalities and their mechanisms of action, preclinical biodistribution studies utilize both in vitro cell systems as well as in vivo models (Silva Lima and Videira 2018). Cell systems offer more simplistic and straightforward platforms to elucidate basic therapeutic mechanisms. However, these in vitro systems have significant limitations in extrapolating results to more complex living systems, necessitating the use of animal models. For in vivo biodistribution studies, the consensus from regulators specifies that animal models need to be representative of the human situation and responsive to the therapeutic modality (Silva Lima and Videira 2018). Typical animals utilized in biodistribution studies include mice, rats, rabbits, monkeys, and dogs (Gonin and Gaillard 2004). At a minimum, nine key tissues should be evaluated: the administration site, blood, brain, gonads, heart, kidneys, liver, lungs, and spleen (Kamiyama et al. 2021). Depending on the therapeutic, evaluation of other bodily fluids and excrement may also be required or recommended (Kamiyama et al. 2021).

Early-phase clinical studies are often utilized as further elucidation of biodistribution and functional consequences of the therapeutic modality (Buscail et al 2015). More importantly, early-phase clinical biodistribution studies present the opportunity for long-term follow-up of individuals who have received cell and gene therapy products. These studies are designed not only to assess the long-term persistence of the therapeutic but are also intended to capture delayed adverse events that may occur. These long-term follow-up studies are vital for therapeutic modalities that utilize permanently integrating vectors or long-term stable transgene expression, modalities that exhibit latency, or modalities with other key characteristics that may trigger delayed malignant transformation or other undesirable side effects in recipients (U.S. FDA 2020).

Methods Utilized in Biodistribution

Given the significance of biodistribution in therapeutic development, it is critical to utilize sensitive methods in these studies. Regulatory agencies mandate that methods used must demonstrate the highest sensitivity currently available (Kamiyama et al. 2021). Current methods are based on either imaging technologies or molecular-based methods for nonclinical and clinical studies.

Commonly used imaging technologies include in vivo imaging systems (IVIS) with fluorescence labeling, positron emission tomography (PET), single photon emission computed tomography (SPECT) with radioisotope labeling and magnetic resonance imaging (MRI). Alternatively, qPCR, immunohistochemistry, and in situ hybridization are commonly used molecular-based methods (Kamiyama et al. 2021) (Figure 2).

Imaging-based techniques can facilitate real-time and noninvasive data acquisition by selective imaging of the therapeutic within tissues. However, these technologies are limited to cellular-based therapeutics, as they lack the resolution to detect viral vectors or mRNAs. Further, the imaging methods ideal here are not high throughput and are relatively expensive per test (Kamiyama et al. 2021). Finally, successful imaging depends on labeling the cells for subsequent visualization. Thus, there is a general concern that labeling may influence the cells' behavior and cause erroneous results (Kamiyama et al. 2021).

Among molecular-based techniques for biodistribution studies, PCR-based methods are the most common (Gonin and Gaillard 2004). Primarily, qPCR and RT-qPCR exhibit the highest sensitivity and specificity of target molecules over a wide dynamic range and thus are recommended by regulatory agencies (Ma et al. 2021). However, qPCR and RT-qPCR–based methods can have significant drawbacks in the context of biodistribution studies. The quantitative power of qPCR and RT-qPCR–based methods depends on the utilization of a standard curve. However, target DNA/RNA recovery can be variable among different sample matrices, thus a matrix-matched calibration curve is required (Nakayama et al. 2023). Utilization of additional calibration curves can increase the overall cost and complicate the interpretation of the obtained results. Further, the susceptibility of qPCR and RT-qPCR–based methods to amplification inhibitors is well known (Nakayama et al. 2023). Even with matrix-matched calibration curves, variability can be demonstrated across different tissue types, indicating matrix effect or variability in DNA recovery (Nakayama et al. 2023).

Droplet Digital PCR (ddPCR™) technology is a viable alternative to qPCR-based methods. By partitioning the sample into tens of thousands of droplets of known volume and subsequently performing PCR amplification to the endpoint, ddPCR technology can provide absolute quantification without the use of a standard curve. Further, running the sample to the endpoint minimizes the matrix effect and reduces overall sensitivity to PCR inhibitors that may be present in the sample.

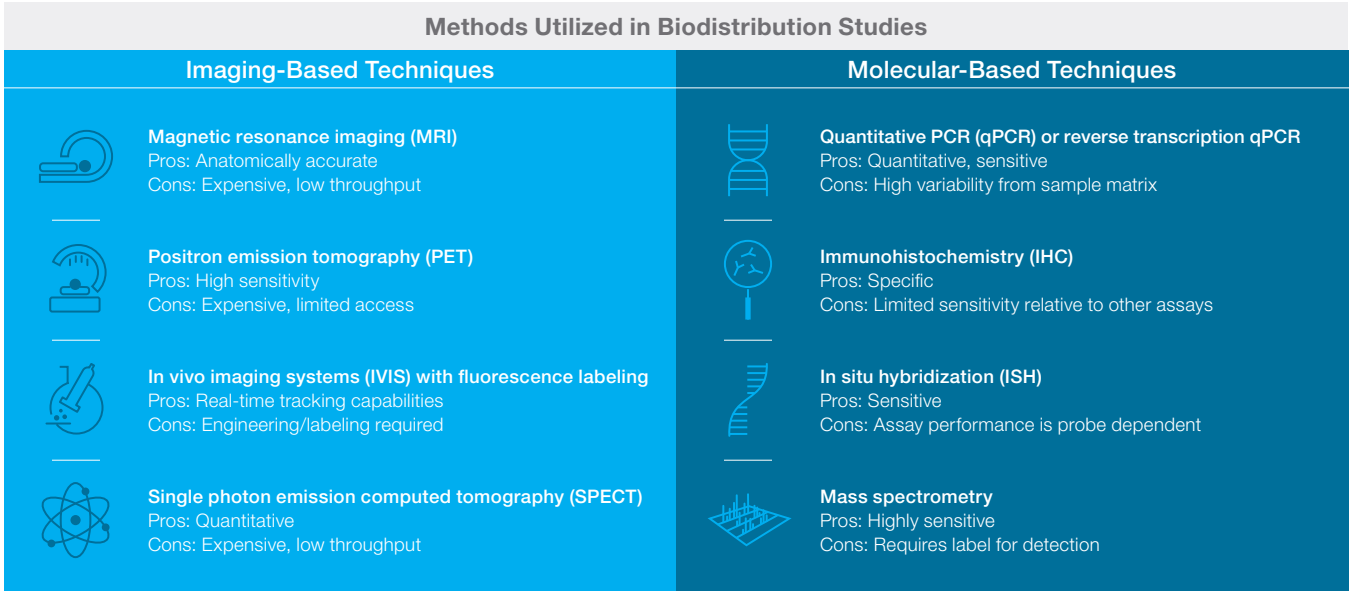

Fig. 2. Imaging and molecular-based techniques commonly utilized in biodistribution studies. Biodistribution studies combine real-time and molecular data to get an accurate understanding of how a new therapeutic behaves in human and animal models.

The inherent benefits of ddPCR technology are gaining traction in biodistribution studies. In one study, a robust ddPCR method for quantifying $CD8^+$ T-cell biodistribution across various tissue samples in immunodeficient mice was established. There it was found that ddPCR technology demonstrated superior accuracy to qPCR technology when DNA recovery was normalized (Nakayama et al. 2023). In another study, ddPCR technology was used to perform high-throughput quantification of human neural stem cells in frozen and paraffin tissue sections or whole blood with a sensitivity of 100—200 human neural stem cells (Wang et al. 2020). This sensitivity is significantly higher than the gold standard methods available today.

Wrapping It Up

Cell and gene therapy has made remarkable strides with recent approvals and an even more impressive clinical and preclinical pipeline. Determination of biodistribution in both the preclinical and early clinical phases is an essential aspect of developing these novel therapies. Essentially, the presence, persistence, and clearance of the drug at the molecular level, both in target tissues and an array of nontarget tissues, must be assessed. This will inform developers of potential side effects that will need to be managed and will provide crucial details about the drug's mechanism of action. A robust and sensitive set of analytics is vital to biodistribution studies because enhanced resolution, sensitivity, and specificity can provide developers with a more precise and complete picture of the distribution tendencies of the therapeutic. Using PCR-based methods, including qPCR and/or ddPCR assays, developers can study biodistribution with high precision and sensitivity to inform subsequent clinical studies, ultimately maximizing the chances of efficacy, safety, and therapeutic success.

References

Buscail L et al. (2015). First-in-man phase 1 clinical trial of gene therapy for advanced pancreatic cancer: Safety, biodistribution, and preliminary clinical findings. Mol Ther 23, 779–789.

Gonin P and Gaillard C (2004). Gene transfer vector biodistribution: Pivotal safety studies in clinical gene therapy development. Gene Ther 11, S98–S108.

Kamiyama Y et al. (2021). Biodistribution studies for cell therapy products: Current status and issues. Regen Ther 18, 202–216.

Kim JY et al. (2022). Review of the current trends in clinical trials involving induced pluripotent stem cells. Stem Cell Rev Rep 18, 142–154.

Liu A and Wang X (2022). The pivotal role of chemical modifications in mRNA therapeutics. Front Cell Dev Biol 10, 901510.

Ma H et al. (2021). qPCR and qRT-PCR analysis: Regulatory points to consider when conducting biodistribution and vector shedding studies. Mol Ther Methods Clin Dev 20, 152–168.

Mendell JR et al. (2021). Current clinical applications of in vivo gene therapy with AAVs. Mol Ther 29, 464–488.

Nakayama M et al. (2023). Novel cell quantification method using a single surrogate calibration curve across various biological samples. AAPS J 25, 26.

Shah NN and Fry TJ (2019). Mechanisms of resistance to CAR T cell therapy. Nat Rev Clin Oncol 16, 372–385.

Silva Lima B and Videira MA (2018). Toxicology and biodistribution: The clinical value of animal biodistribution studies. Mol Ther Methods Clin Dev 8, 183–197.

Sugimoto H et al. (2021). Insights on Droplet Digital PCR–based cellular kinetics and biodistribution assay support for CAR-T cell therapy. AAPS J 23, 36.

U.S. Department of Health and Human Services, Food and Drug Administration, Center for Biologics Evaluation and Research (2020). Long Term Follow-Up After Administration of Human Gene Therapy Products — Guidance for Industry. https://www.fda.gov/media/113768/download, accessed July 19, 2023.

Vervaeke P et al. (2022). Regulatory guidelines and preclinical tools to study the biodistribution of RNA therapeutics. Adv Drug Deliv Rev 184, 114236.

Wang F et al. (2020). Comparative strategies for stem cell biodistribution in a preclinical study. Acta Pharmacol Sin 41, 572–580.

Zheng C et al. (2011). Assessment of the safety and biodistribution of a regulated AAV2 gene transfer vector after delivery to murine submandibular glands. Toxicol Sci 123, 247–255.

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