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Imaging-guided nanomedicine development

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Abstract

Nanomedicine research is an active field that produces thousands of studies every year. However, translation of nanotherapeutics to the clinic has yet to catch up with such a vast output. In recent years, the need to better understand nanomedicines' *in vivo* behavior has been identified as one of the major challenges for efficient clinical translation. In this context, non-invasive imaging offers attractive solutions to provide valuable information about nanomedicine biodistribution, pharmacokinetics, stability or therapeutic efficacy. Here, we review the latest imaging approaches employed in the development of therapeutic nanomedicines, discuss why these strategies bring added value along the translational pipeline, and give a perspective on future advances in the field.

1. Introduction

Nanomedicine, the application of nanotechnology to disease prevention and treatment, initially sparked great excitement as a promising avenue to improve drug delivery. Indeed, preclinical nanomedicine research is still a blooming field that produces thousands of studies every year. To date, however, the number of nanoformulations approved for clinical use is comparatively low [1,2]. Clinical nanomedicine applications are so far mostly limited to cancer, but promising preclinical studies indicate that its use in other scenarios like cardiovascular disease [3,4], organ transplantation [5] or autoimmune disorders [6] could be beneficial. These applications, which exploit nanoparticle formulations to modulate the immune system in a so-called nanoimmunotherapeutic fashion, are producing exciting results and instilling new vigor to the field.

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Declaration of interests

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The paucity in nanomedicine translation can be attributed to a range of challenges, which include production scalability and regulatory issues, but mainly relate to deficient understanding of nanoformulations' *in vivo* behavior and a lack of patient selection methods [1,7]. In this setting, the value of imaging is increasingly recognized at different steps along the nanomedicine development pathway as it can provide critical information in a non-invasive fashion. In this review, we discuss recent advances in the use of imaging strategies applied to therapeutic nanomedicine development over the past two years, and provide an outlook on the field's future directions.

2. Imaging techniques

Imaging techniques like X-ray computed tomography (CT), magnetic resonance imaging (MRI) or positron emission tomography (PET) have revolutionized medical care. These techniques allow non-invasive *in vivo* visualization of anatomical structures and biological processes to diagnose and prognose disease. However, no standalone imaging technique is perfect, as all have their own strengths and weaknesses. While a detailed discussion about their limitations is out of the scope of this article, a brief summary of their features is included in Table 1. In the context of nanomedicine development, nuclear (PET and single-photon emission computed tomography [SPECT]), anatomical (MRI and CT), as well as optical imaging techniques (such as fluorescence molecular tomography [FMT], fluorescence imaging, or intravital microscopy) are the most widely used to study nanoformulations *in vivo* (and *ex vivo*). Given the limitations of standalone imaging techniques, a combination of two or more of them is increasingly preferred in order to attain optimal characterization. Typically, multimodal imaging combines two techniques that bring together complementary information, such as functional and anatomical data as in PET/CT or PET/MRI. Using hybrid scanners both acquisitions can be performed in a single session. However, multimodal imaging can also be approached asynchronously, by acquiring images at different times in different scanners.

3. Imaging applications in nanomedicine development

Therapeutic nanomedicine development can greatly benefit from the use of different imaging modalities. These nanomedicines may intrinsically contain contrast-generating or imaging agents, or may be modified to do so, which allows their *in vivo* tracking by the corresponding imaging technique. In this section, we summarize the most recent, innovative and relevant examples of imaging-based strategies applied to different aspects and stages of a nanoformulation's development.

3.1 Evaluation of *in vivo* behavior.

One of the greatest challenges facing nanomedicine translation is the lack of understanding of their *in vivo* performance in terms of stability, pharmacokinetics, as well as tissue and cellular distribution. These features can be investigated using anatomical, nuclear and optical imaging methods. However, not all techniques are equally suited for the purpose. While CT, MRI and optical imaging have been used to track nanoparticles *in vivo*, only PET and SPECT can afford truly quantitative information [8]. Moreover, due to their high sensitivity, nuclear imaging techniques allow minimal modification of the nanomaterials to introduce

the required radioactive tag. On the other hand, nuclear imaging's low spatial resolution limits its use to whole-body and tissue level distribution, leaving cellular (and subcellular) evaluation to optical imaging techniques.

3.1.1. Biodistribution and pharmacokinetics.—Nanotherapeutics with inorganic cores, such as iron oxide or gold, can be tracked *in vivo* by MRI [9-11] and CT [10,12,13], respectively. The generated contrast can be quantified to derive information about tissue distribution and clearance kinetics. On the other hand, nanomedicines labeled with fluorophores or carrying a fluorescent nanocrystal core can be visualized using fluorescence imaging methods [14,15]. While these optical methods have issues related to tissue absorption or penetration *in vivo*, this is an affordable semi-quantitative approach that can be further complemented by *ex vivo* microscopy. For example, a recent work used X-ray- and fluorescence-based imaging methods to study nanoparticle dynamics associated with different pulmonary delivery methods [16].

In recent years, however, the multimodal combination of highly sensitive and quantitative PET or SPECT with anatomical CT imaging is being increasingly favored for the assessment of nanomedicines' biodistribution and pharmacokinetics [8]. With the advent of hybrid PET/MRI scanners, anatomical reference with high soft-tissue contrast is available for a more accurate localization of PET hot spots. Nuclear imaging approaches allow dynamic and longitudinal evaluation of a radiolabeled nanomedicine and *in vivo* comparison of its biodistribution in different species (Figure 1A) [3,4,17]. Importantly, the imaging results can be validated *ex vivo* by radioactivity counting in tissues of interest, and by autoradiography, which provides information about regional distribution in tissues with sub-millimeter resolution.

3.1.2. Targeting.—Non-invasive imaging can be used to assess the ability of nanotherapeutics to reach their targets, *e.g.* a tumor or inflammatory lesions, such as atherosclerotic plaques. Again, while MRI and CT can do this if a nanoformulation contains appropriate contrast-generating agents, PET and SPECT yield more accurate information due to their high sensitivity and truly quantitative nature. However, these techniques require auxiliary anatomical reference, typically in combination with CT. A more precise localization of accumulation spots can be achieved by combination with MRI due to this technique's excellent soft tissue contrast. This is particularly critical for nanomedicines targeting small regions of interest such as the atherosclerotic vessel wall [18].

Imaging-based strategies additionally enable evaluation of the efficiency of delivery-enhancing interventions. This is of great value in the development of nanodrugs that need to cross impassable biological barriers such as the blood-brain barrier (BBB). Two recent studies made use of FMT/CT [19] or MRI [20] in combination with other optical imaging methods to evaluate the effect of ultrasound-mediated permeation of the BBB on the accumulation of polymeric and liposomal nanoparticles. In both studies, the use of fluorophores allowed the authors to validate the *in vivo* imaging results using fluorescence microscopy *ex vivo* (Figure 1B). A similar approach was implemented to assess magnetic targeting of an iron oxide-gold core-shell photothermal nanotherapy formulation by MRI [9].

Targeting evaluation at the cellular level, however, requires the use of optical imaging methods. Sofias *et al.* employed a combination of PET and intravital microscopy to investigate the fate of $\alpha_v\beta_3$-integrin targeted nanoformulations [21]. The information thus gathered revealed significant differences between targeted and untargeted nanoformulations at tissue level, and a new mechanism of nanoparticle accumulation via phagocyte "hitchhiking". Importantly, the use of fluorescent labels allows further investigation of a nanomedicine's fate at the cellular level by *ex vivo* fluorescence microscopy or fluorescence-activated cell sorting. This is particularly important in the development of nanoimmunotherapies that work by selectively targeting a given immune cell population [17].

3.1.3. Nanoformulation integrity.—Most nanomedicines are composite materials, typically made of a nanocarrier and a drug. Generally, their evaluation focuses on the nanocarrier, and it is assumed that the nanoconstruct remains stable over time after administration. However, early release of the cargo is an issue with most formulations, and this can be investigated by non-invasive imaging. SPECT, for instance, allows multiplexing by using two isotopes with different gamma photon emission energies, which can be used to label different components of a nanomedicine. This approach was adopted by Llop *et al.* to investigate the biodistribution and pharmacokinetics of a composite iron oxide-polymeric nanoformulation *in vivo* by SPECT [22]. A slightly different approach was followed by Lamichhane *et al.*, who used ^{111}In-labeled liposomes encapsulating a ^{18}F-labeled carboplatin derivative [23]. Analysis of the ^{111}In signal by SPECT and ^{18}F by PET using a multimodal preclinical PET/SPECT/CT system showed similar tissue distribution, suggesting a stable integration of both components. Analogous approaches can be implemented using optical methods, which also allow multiplexing , and more specifically exploiting Förster resonance energy transfer [24,25]. We anticipate these multiplexing strategies to gain traction in early nanomedicine evaluation as they can provide critical information about a nanoconstruct's *in vivo* stability.

3.2. Imaging-guided therapy.

The use of imaging to improve therapeutic outcome in a personalized manner has become a feasible goal. These imaging-guided approaches rely on labeled nanomaterials to generate a trackable *in vivo* signal to detect –and quantify– their accumulation in tissues in a so-called theranostic fashion. Down the line, these strategies could evolve to enable patient selection and dose adjustment protocols. In recent years, numerous studies have reported on inorganic core nanoparticles with intrinsic contrast-generating or imaging properties engineered for therapeutic purposes. Wang *et al.*, for instance, developed a polymeric nanocapsule containing iron-based magnetic nanocrystals, indocyanine green and doxorubicin for imaging-guided dual photodynamic and chemodynamic therapy [26]. The magnetic and fluorescent properties were exploited for thorough characterization of the nanocapsules' *in vivo* tumor accumulation by MRI and fluorescence imaging, respectively, whereas thermal infrared imaging was used to monitor photodynamic therapy [26]. Jing *et al.* pursued an analogous strategy using a composite nanomaterial containing Fe_3O_4 superparamagnetic nanoclusters, MnO_2 nanosheets, the anticancer drug curcumin and the

photosensitizer chlorin e6 for dual chemotherapy and photodynamic therapy guided by MRI and fluorescence imaging [11].

Indeed, nanoparticles for photothermal or photodynamic therapy have intrinsic imaging capabilities, and this feature is exploited for guiding treatment [12,27-30]. Moreover, these nanoformulations are frequently engineered to incorporate MRI [9,31-33] and/or CT [32-35] contrast agents in order to visualize their distribution at the whole-body level. For instance, Sharma *et al.* developed gold nanorods for imaging-guided photothermal therapy, reliant upon the MRI, X-ray, and optical imaging properties of the nanomaterial [36] Another such example is the combination of bismuth nanoparticles with up-converting nanophosphors in core-shell multimodal nanoparticles for CT and up-conversion luminescence imaging-guided photothermal therapy [37]. In a similar fashion, a photothermal nanotherapeutic that activates its imaging and therapeutic features in the presence of tumor-overexpressed β-gallactosidase was recently reported [38]. The combination of anatomical imaging contrast agents and fluorophores has another interesting oncological application. Firstly, the nanomaterials can be located at whole-body level using the anatomical technique and subsequently used for precise tumor resection using fluorescence guidance. Yang *et al.* report on an α-lactalbumin-stabilized ultra-small gold quantum cluster with intrinsic detectability by near-infrared fluorescence imaging, CT and MRI, and therapeutic activity through inhibition of the MAPK and PI3K–AKT pathways. The authors prove that the nanomaterial is renally cleared due to its small size and can be used for intraoperative surgical imaging (Figure 1C) [39].

3.3 Treatment monitoring.

Finally, non-invasive imaging can be used to evaluate the therapeutic efficacy of a nanomedicine. This approach allows direct investigation of the formulation's therapeutic effect as well as its mechanism of action. For instance, we implemented one such strategy to translate a statin-loaded nanobiologic for the treatment of atherosclerosis from mice to large animals. Using a multimodal imaging approach to longitudinally monitor treatment in rabbits and pigs, combining PET- and MRI-based readouts, we were able to assess the anti-inflammatory effect of the nanobiologic formulation using restricted group sizes [3]. Similarly, the effect of a trained-immunity promoting nanobiologic was imaged by both ^{18}F-fluorodeoxyglucose (^{18}F-FDG) PET, to measure metabolic activation in the bone marrow, and by immunoPET using a CD11b-targeted nanobody to quantify the expected increased myelopoiesis (Figure 1D) [17]. In clinical trials, imaging-based readouts could be included to directly assess treatment response [40], potentially eliminating the need for large patient cohorts and long follow-up periods, as well as the associated high costs.

4. Perspective and conclusion

Non-invasive imaging strategies like the ones summarized here can be integrated into nanoformulation screening procedures to speed up early-stage development. Formulation libraries can be generated and thoroughly characterized for biodistribution, pharmacokinetics, targeting or cell specificity using complementary imaging techniques as discussed above [17,41,42]. The wealth of imaging data generated in these screenings

demands high-throughput analyses, which will greatly benefit from integration of artificial intelligence (AI) into the development pipeline [43,44]. Furthermore, mathematical modelling of these data can greatly help to understand the nanomaterial's *in vivo* performance as well as biological aspects influencing its behavior [45,46]. Thus, those formulations with poor *in vivo* behavior can be easily identified and discarded before further evaluation and waste of resources.

On the other hand, the excessive reliance on mouse studies has been traditionally blamed for the limited translational success of nanomedicine. This is partly due to a comparatively low number of available large animal models of disease, although in recent years there has been increasing interest in the development of this type of valuable research tools [47,48]. In this context, we envision that large animal studies will be increasingly relevant. Choosing multiple robust non-invasive imaging readouts to longitudinally evaluate a nanoformulation's efficacy in these translational studies allows the use of a limited number of animals [3]. This approach generates large amounts of data on independent markers of therapeutic efficacy and exploits the statistical power of longitudinal assessments, thereby increasing the likelihood of finding a statistically significant and biologically relevant effect.

One key challenge in nanomedicine translation remains the identification of patients that would benefit most from a nanotherapeutic intervention. In most cases, inter- and intra-patient disease heterogeneity is difficult to assess by common testing and therefore non-invasive imaging can greatly aid in this process [49]. Development of imaging-based patient selection protocols to homogenize cohorts in clinical trials would de-risk nanomedicine translation to the clinic by increasing the likelihood of detecting a response in amenable subjects. While most theranostic approaches have seemingly very little translational potential, simpler surrogate "nanoreporter" PET imaging [50,51], or analogous strategies using MRI [52], have yielded promising results. Ultimately, these patient selection protocols would be performed not just in trials but also in the clinic before the start of treatment, where they could additionally help to tailor the therapeutic regimen in an intrinsically personalized manner.

In conclusion, non-invasive imaging is being increasingly integrated at different stages of the nanomedicine development pipeline. We believe that the discussed strategies provide extremely valuable *in vivo* data and could at last help to bridge the translational gap between bench and bedside.

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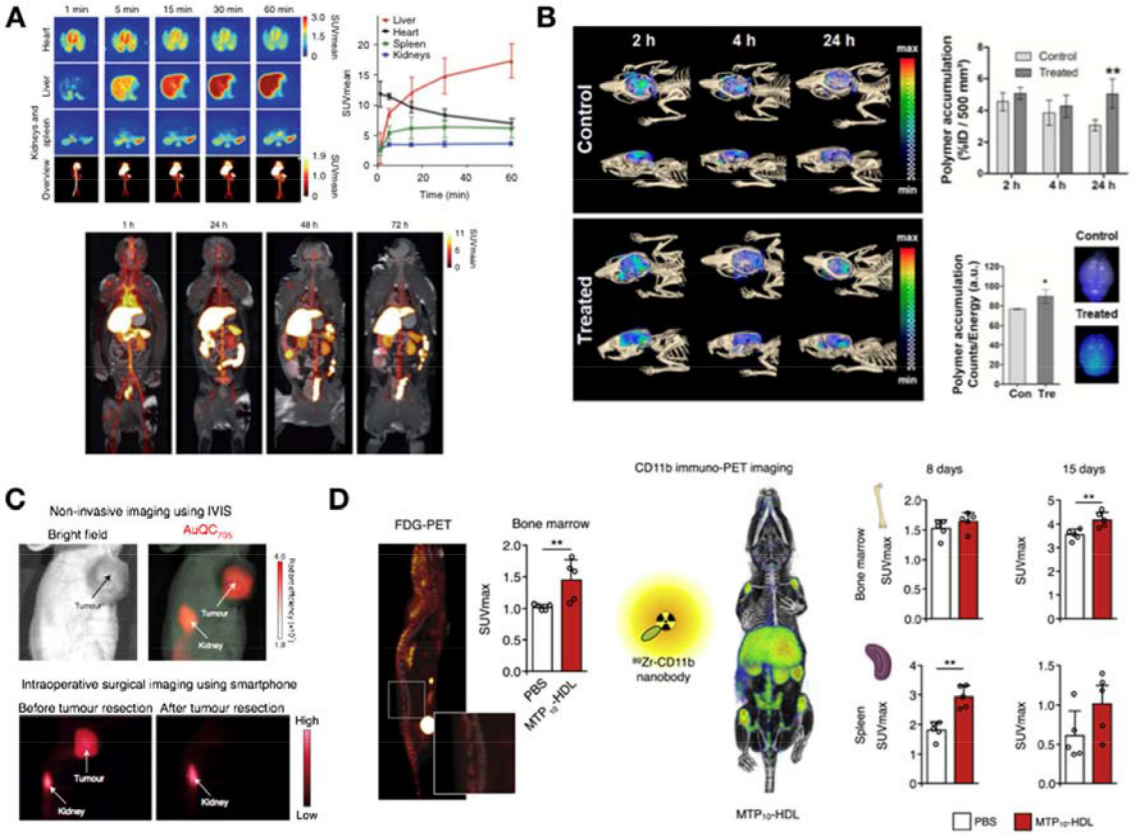

Figure 1. Non-invasive imaging applications in nanomedicine development.

A) Biodistribution and pharmacokinetics. The tissue distribution and clearance kinetics of a TRAF6 inhibitor-loaded nanobiologic was evaluated in non-human primates by PET/MRI. The formulation was radiolabeled with ^{89}Zr and monitored dynamically for the first hour after administration (top left). Quantitative data could be derived from selected tissues (top right). Subsequently, static scans were performed at 24, 48 and 72 hours post injection (bottom). Adapted from Lameijer *et al.* [4]. B) Targeting efficiency. A sonoporation treatment to enhance delivery across the BBB was evaluated by *in vivo* FMT/CT imaging using fluorophore-labeled nanoparticles. A significant increase in the florescent signal was measured 24 hours post-administration in treated animals compared to controls (top right). The results were corroborated *ex vivo* by fluorescence reflectance imaging of explanted brains (bottom right). Adapted from May *et al.* [19]. C) Imaging-guided therapy. An ultra-small gold quantum cluster nanoparticle ($AuQC_{705}$), detectable by near-infrared fluorescence, CT and MRI, was successfully employed to guide tumor resection by fluorescence imaging using a portable smartphone imaging system prototype. Adapted from Yang *et al.* [39]. D) Treatment monitoring. Non-invasive imaging can be used to monitor nanomedicine treatment efficacy and its underlying mechanisms of action. A trained-immunity promoting nanobiologic (MTP_{10}-HDL) was developed as a novel anticancer therapy. Its effects on immune response activation were monitored by PET imaging of metabolic activation in the bone marrow using ^{18}F-FDG (FDG-PET, left) and myelopoiesis in bone marrow and spleen using a radiolabeled nanobody (CD11b immuno-PET, right). Adapted from Priem *at al.* [17].

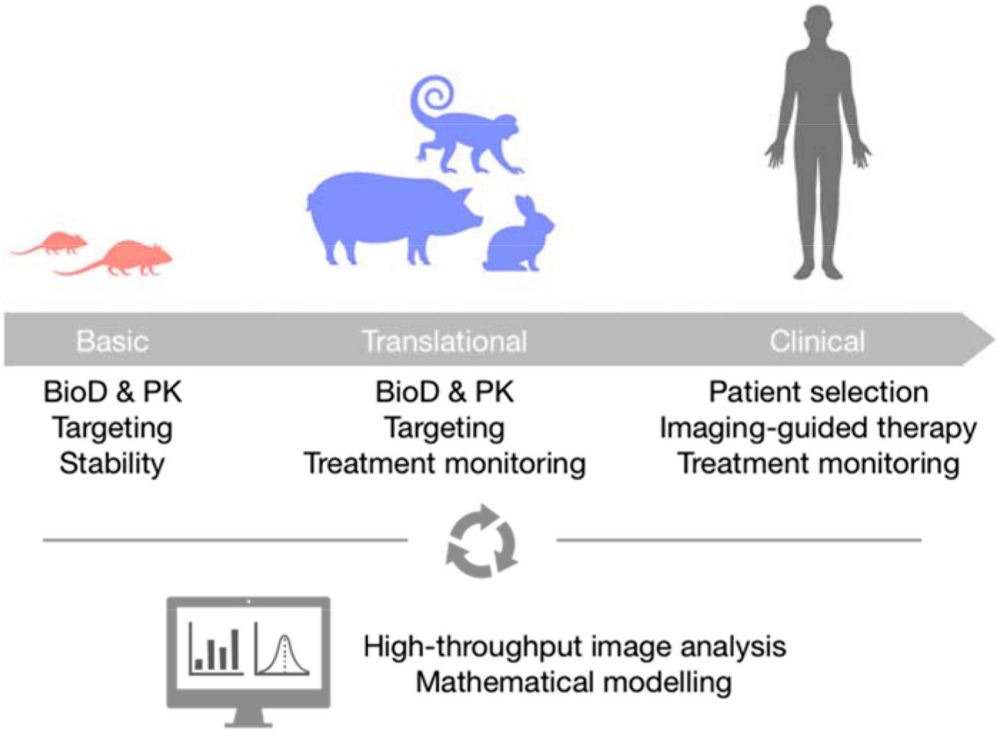

Figure 2. Integration of imaging along the translational pipeline.

Non-invasive imaging can be integrated at different stages of a nanoformulation's development. At the early stages, imaging-based screening of promising candidates can be performed to elucidate their *in vivo* behavior in terms of biodistribution (BioD), pharmacokinetics (PK), targeting or stability. In addition to assessing *in vivo* behavior, translational studies in large animal models can benefit from the integration of non-invasive imaging to longitudinally investigate treatment response using limited group sizes. Finally, in the clinic, imaging-based protocols can aid in selecting amenable patients, guiding therapy and monitoring response. At all stages, AI-based image analyses will be of paramount importance to generate quality data and facilitate mathematical modelling in order to understand and possibly predict nanomedicines' performance.

Table 1.

Features, advantages and disadvantages, and examples of imaging agents of techniques commonly used in the context of nanomedicine development [8,53,54].

Technique		Imaging agent	Spatial resolution	Sensitivity	Penetration in tissue	Advantages	Disadvantages
Anatomical	CT	Au, Iodine	<0.2 mm [P] 0.5–1 mm [C]	mM	No limit	Fast; high spatial resolution	Ionizing radiation; low contrast sensitivity
	MRI	Iron oxide, Gd, Mn	<0.1 mm [P] 1–2 mm [C]	μM-mM	No limit	High spatial resolution; soft tissue contrast	Low contrast sensitivity; time-consuming
Nuclear	PET	^{18}F, ^{64}Cu, ^{68}Ga, ^{89}Zr	1–2 mm [P] 6–10 mm [C]	fM	No limit	High sensitivity; quantitative	Ionizing radiation; expensive; limited spatial resolution
	SPECT	^{99m}Tc, ^{111}In, ^{123}I, ^{125}I	0.5–2 mm [P] 7–15 mm [C]	<pM	No limit		
Optical	FI	Fluorophores, quantum dots	1–5 mm [P]	nM	mm-cm	High sensitivity; multiplexing; inexpensive	Low penetration depth
	FMT	Fluorophores, quantum dots	<1 mm [P]	pM	cm	High sensitivity	Signal attenuation; limited penetration
	IVM	Fluorophores, quantum dots	<1 μm [P] 1 μm [C]	<nM	<mm	Cell-level resolution; real time imaging	Complex setup; limited field of view

[P]: preclinical; [C]: clinical; FI: fluorescence imaging; FMT: fluorescence molecular tomography; IVM: intravital microscopy.