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New Opportunities and Old Challenges in the Clinical translation of Nanotheranostics

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Abstract

Nanoparticle-based systems imbued with both diagnostic and therapeutic functions, known as nanotheranostics, have enabled remarkable progress in guiding focal therapy, inducing active responses to endogenous and exogenous biophysical stimuli, and stratifying patients for optimal treatment. However, although in recent years more nanotechnological platforms and techniques have been implemented in the clinic, several important challenges remain that are specific to nanotheranostics. In this Review, we first discuss some of the many ways of 'constructing' nanotheranostics, focusing on the different imaging modalities and therapeutic strategies. We then outline nanotheranostics that are currently used in humans at different stages of clinical development, identifying specific advantages and opportunities. Finally, we define critical steps along the winding road of preclinical and clinical development and suggest actions to overcome technical, manufacturing, regulatory and economical challenges for the safe and effective clinical translation of nanotheranostics.

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Introduction

The first known intentional use of synthetic nanoparticles is found in the ornamented Roman 'Lycurgus cup' from the 4th century AD, which appears green in reflected light but turns ruby red when light is transmitted through it[1]. This cup is made of dichroic glass in which a colloidal dispersion of gold and silver nanoparticles causes the optical effect, which was ingeniously exploited some 2,000 years before similar nanoparticles were used for optical imaging in biomedical research. Today, nanotechnology is defined foremost by the scale of the involved structures – typically ranging between 1 and 100 nm – and is used in a vast array of applications. Materials at this scale differ vastly in their physical, chemical and biological properties from their bulk counterparts. Innovation in nanotechnology relies on the manipulation of materials and the exploration of their interactions with other systems, including living ones[2]. The juncture of nanotechnology and medicine gave rise to the field of 'nanomedicine' in the archetypal form of a nanoparticle carrying drug molecules, which quickly became one of the most intriguing, but also controversial, branches of a new science[3].

A first unique attribute of nanomedicines is the ability to modulate the distribution of a payload, resulting in improved bioavailability with increased deposition at the biological target and diminished systemic toxicity. A nanomedicine can improve the balance between desired efficacy and undesired toxicity (therapeutic index), thereby enabling the delivery of drug amounts that would not be possible if the same drug were administered freely. Another unique attribute of nanomedicines is their ability to create a 'nanoenvironment' providing the necessary solubility, stability and protection to the selected payload. This nanoenvironment provides stealthiness to the payload and allows it to reach the target unperturbed avoiding enroute degradation[4]. Besides their use as drug carriers, nanoparticles themselves interact with the surrounding environment and respond to multiple endogenous and exogenous stimuli[5]. This aspect motivated the use of nanoparticles for various diagnostic applications and, eventually, for the integration of therapeutic and diagnostic functions and the rise of theranostics[6]. Notably, the notion of theranostics has its origin in a single atom: iodine. The radioactive isotope of iodine ^{131}I has been used since 1941 for the treatment of thyroid diseases as it emits gamma rays for imaging and highly energetic electrons for killing iodide-accumulating thyroid cancer cells[7], therefore allowing both imaging and therapy. More recently, the clinical success and approval by the Food and Drug Administration (FDA) and European Medicines Agency (EMA) of the radiopharmaceuticals ^{177}Lu-DOTATATE (Lutathera®) for the treatment of neuroendocrine tumors, and ^{68}Ga-PSMA-11 (Locametz®) together with ^{177}Lu-PSMA-617 (Pluvicto™) for the positron emission tomography (PET) imaging-guided treatment of prostate cancer are establishing the potential of theranostics in the clinic.[8]

In the past decade, the concept of theranostics has been extensively applied to nanoparticles leading to nanotheranostics, which in its archetypal form is the combination of imaging agents and therapeutic substances in the same nanostructure (Figure 1). In addition to sharing the attributes listed above, nanotheranostics provide clear advantages over nanomedicines. First, they can be used without drugs to probe the accumulation of the nanoscale carrier at the diseased site and thus stratify patients that would benefit from the

nanotherapy. Second, they allow monitoring and predicting outcomes already at the time of administration, or shortly thereafter. Third, nanotheranostics can carry a high-density load of therapeutic and imaging agents to the biological target, thus increasing the signal-to-noise ratio for imaging while improving therapeutic efficacy. Fourth, nanotheranostics can be activated 'on command' by endogenous biological stimuli or exogenous energy sources, whereby a signal is produced or drug molecules are released upon interacting with the correct milieu (such as low pH or enzymatic activity). Finally, nanotheranostics can be engineered to report the release of a drug from the system itself[9].

Design and Formulation of Nanotheranostics

An incredible variety of therapeutic and imaging modalities has been integrated into nanoparticles (Table 1) to improve disease detection, boost therapeutic efficacy and monitor the outcome of medical interventions[6, 10, 11]. Based on the mode of integration, three basic strategies emerge in the design and formulation of nanotheranostics: first, nanocarriers may be formed with materials that themselves have imaging and therapeutic capabilities (innate); second, imaging and therapeutic components may be chemically modified and conjugated to the structure of the nanocarrier (modification); and, finally, imaging and therapeutic components may be dispersed and loaded within the structure of the nanocarrier without any chemical modification (encapsulation). Nanotheranostics can also be targeted to specific cell receptors by encapsulating or modifying the carriers with targeting moieties, or even by using materials with innate affinity for a particular molecule. The literature is filled with examples of innate, encapsulation, modification and targeted nanotheranostics as well as nanotheranostics that combine several of these design strategies, as extensively detailed in the Supplementary Information. A few examples are discussed in this section, highlighting their unique features and mechanisms of action (Figure 2).

Metallic nanoparticles are arguably the primary source of nanotheranostics with innate properties. Their magnetic, radioactive or plasmonic properties turn them into natural candidates for applications such as diagnostic, imaging and photothermal therapies. Super-paramagnetic iron oxide nanocubes and nanospheres provide a classic example of innate nanotheranostics (Figure 2A)[12–14]. They can be either used individually or clustered together to boost both the generated signal in magnetic resonance (MR) imaging and the efficacy of magnetic-induced ablation therapy. Combined MR imaging and delivery of chemotherapeutic agents has been also demonstrated by several authors, using gadolinium or manganese as the imaging reporting agent.[15, 16] Heavy-metal oxide nanoparticles have also been used as MR imaging agents and enhancers of external beam radiation therapy (Figure 2B)[17, 18].

Polymeric nanoparticle and lipid-based nanotheranostics are strongly associated with modification as a method to integrate imaging and therapeutic components. Functional groups present on the surface of the nanocarriers, or introduced during their assembly, can be used to label the surface with different agents. For example, two different radionuclides – one for imaging and one for internal radiotherapy – can be combined within the same nanoparticle (Figure 2C)[19, 20]. Also, radiolabeled nanoparticles can carry photosensitizers that induce reactive oxygen species via the Cerenkov effect, which enables the nanoparticles

to act as an internal light source, locally deploying a potent therapy[21–25]. A broad variety of nanoparticles has also been used to guide surgeons in the resection of malignant tissues and lymph nodes (Figure 2D)[26–29].

Although the surface of polymeric nanoparticles or liposomes can be modified, the encapsulation of the imaging and therapeutic agents has been the method most frequently chosen for the preparation of new theranostic nanoparticles. Typically, these agents are mixed together with the components of the nanocarrier during the assembly process. This preparation method has led to lipid nanocarriers that can be stimulated by external light sources, for instance using persistent luminescence nanoparticles incorporated with chemotherapeutic molecules into liposomes. Upon external irradiation, these systems serve both as anti-cancer and optical imaging agents (Figure 2E)[30–36]. Also, nanomedicines carrying different drug molecules can be triggered by high-intensity focused ultrasound under MRI guidance (Figure 2F)[37, 38]. The photoacoustic effect has also been exploited in conjunction with nanomedicines to enhance imaging depth and quantify drug release in vivo[39, 40]. Finally, focused ultrasound has been used to permeabilize the blood vasculature and facilitate the extravascular deposition of nanomedicines[41–44], as well as to burst vesicles loaded with therapeutic and imaging agents[45–48].

It is important to highlight that these strategies to incorporate theranostic components are often mixed together to obtain nanocarriers with different physico-chemical properties. For example, the loading of iron oxide nanoparticles for MR imaging into pH-responsive polymeric micelles combines innate nanotheranostic agents and encapsulation nanotheranostic[49]. Additionally, modifying the surface of nanomedicines with radionuclei for PET and single-photon emission computed tomography (SPECT) enables imaging the biodistribution of the nanomedicines and – in the case of PET – the quantification of the tissue-specific accumulation of nanomedicines in vivo[50–54]. Besides these examples, an extensive description of preclinical nanotheranostics is provided in the Supplementary Information.

Clinical Nanotheranostics

As scientists, we are not bound to one particle or imaging molecule or drug molecule, and we can devise new sophisticated nanotheranostic agents capable of combining multiple therapeutic and imaging modalities. However, caution must be taken with the huge ‘sandbox’ of imaging and therapeutic nanoparticles. The integration of multiple functions (imaging, therapies, targeting) in a single nanostructure must address a precise unmet clinical need – rather than being inspired by the ‘because we can’ credo – in order to maximize the odds for clinical translation.

Indeed, the variety of configurations shown in Figure 2 clashes against the modest number of theranostics studied in active and completed clinical studies (Table 2, 3). As of today, clinically relevant nanotheranostics are mostly limited to iron oxide nanoparticles, gadolinium-based and hafnium-based nanocomplexes, fluorescent nanoprobes, thermoresponsive liposomes and radiolabeled nanomedicines, which are mostly still undergoing clinical investigation at different levels of development. In this section, these

clinically relevant nanotheranostics are described, highlighting their technical features and advantages.

Nanoparticles with 'innate' imaging capabilities for thermal therapies

As mentioned, several metallic nanoparticles are well suited for theranostic applications as they have the innate ability to serve as imaging contrast enhancers and locally deploy heat upon stimulation by external energy sources. Already in the early 2000s, gold nanoparticles with a core diameter of a few nanometers were proposed as computed tomography (CT) contrast nanoparticles due to their high X-ray attenuation profile[55, 56]. Gold nanoparticles are also known to generate heat upon stimulation with an external light source via surface plasmon resonance.[57] An example is gold-silica nanoshells, which are currently being tested in humans for the photothermal ablation of prostate and head and neck cancer cells (Auroshell – Table 2 and 3)[58]. The nanoshells have a silica core of ~ 150 nm coated by a ~ 10 nm gold shell designed to optimally resonate with a near-infrared light stimulation (~ 800 nm) and produce sufficient heat already at 10 μg/ml local concentration of gold[59, 60]. However, nanoparticles for CT imaging typically require concentrations higher than 100 μg/ml to generate sufficient contrast in human applications, and they are ~ 10 nm spheres of solid gold.[61] It is then not surprising that, in the clinic, the gold nanoshells are detectable via MR and ultrasound imaging but not CT. The mismatch between the optimal conditions for heat generation and imaging, as well as the cost of the native material, are limiting the clinical development of gold-based true nanotheranostics. However, newer CT scanners able to gather spectral information might be able to change this situation, as the amount of gold required for imaging would be lower and compatible with that of thin nanoshells[62, 63].

Iron oxide nanoparticles (IONPs) have been developed preclinically in a myriad of different configurations (Table 1 and Supplementary Information)[64], and they have been approved for the treatment of anemia (20 nm ferumoxytol nanoparticles[65, 66]) and for magnetic hyperthermia of brain tumors (NanoTherm® particles[67], Table 2 and 3). In the treatment of high-grade gliomas, NanoTherm® are injected directly in the tumor to overcome the blood-brain barrier and then heated up via external alternating magnetic fields at a frequency of 100 kHz with a field strength of 2.5 – 18 kA/m[68]. Not only does magnetic hyperthermia with IONPs require dedicated equipment, but also the local high density of iron induces susceptibility artifacts in MR imaging, hence, disease progression is typically monitored using CT scans. As for gold nanoparticles, a truly nanotheranostic use of IONPs is limited by the mismatch between the high specific absorption rate needed for efficient heat generation and the requirements for optimal imaging. Extensive efforts are being dedicated to developing IONPs that overcome this mismatch.[13, 14]

IONPs have also been tested clinically to target cancer metastases in the lymph nodes and track immune and stem cells in a variety of conditions, including cardiovascular and neurological disorders, in cancer immunotherapy and in regenerative medicine (Table 2 and 3)[65, 69–71]. However, these studies have mostly focused on developing new methods for stem cell deposition and elucidating the dynamics of immune cells leveraging the good safety profile of IONPs in humans rather than designing novel theranostic approaches.

Nanoparticles with ‘innate’ imaging capabilities for radiosensitization

Gadolinium-based contrast agents are extensively used in MR imaging.[72] They are typically preferred to IONPs for their positive contrast, but they are also associated with the risk of transmetallation, which can lead to severe side effects in patients with poor renal clearance[73]. More recently, complexes of Gd are being actively investigated in the clinic as possible nanotheranostic agents. This is the case of the sub-5 nm AGuIX® particles[74–76], comprising a polysiloxane matrix with multiple 1,4,7,10-tetraazacyclododecane,1-glutaric acid-4,7,10-triacetic acid (DOTAGA) ligands carrying gadolinium atoms. The resulting nanocomplexes function both as MR contrast agents (longitudinal relaxivity of ~ 10 $mM^{-1}s^{-1}$ at 1.5T) and local enhancers for radiation therapy (~ 2-fold increase in DNA damage following irradiation). Notably, in clinical studies, AGuIX nanoparticles have been shown to detect cancer metastasis in the brain under MR imaging and modulate their progression upon whole brain irradiation. In particular, it has been documented that the rate of growth of brain metastasis diminishes with increasing AGuIX concentration within the lesions (Figure 3A)[77]. Indeed, the larger is the accumulation of gadolinium in the metastasis, the larger is the local enhancement in MRI signal and irradiation-induced damage. This demonstrates the truly theranostic nature of AGuIX, which could not be achieved with molecular-based Gd contrast agents due to insufficient metal concentration and retention at the target.

In addition to gadolinium, hafnium is another element with high atomic number and electron density that could be efficiently shaped into nanoparticles and used as a radiation enhancer. Hafnium oxide nanoparticles (NBTXR3) with an average diameter of 50 nm have been shown to locally deposit high energy upon exposure to external ionizing radiation[78]. In patients affected by advanced sarcoma, an intratumor injection under CT guidance of NBTXR3 before radiotherapy doubled the number of responding patients compared to radiotherapy alone.[79] This preliminary success is fostering the launch of multiple clinical studies on other cancers too (Table 2 and 3).

These are two elegant examples of how the innate properties of a chemical element can be harnessed to result in clinically relevant theranostic properties, but only after the element is reshaped into nanoscale objects providing the necessary material density.

Multimodal imaging nanoparticles for guiding surgical therapy

In the operating room, nanoparticles labeling specifically the malignant tissue provide information on its local extension and on the status of the resection margin. Typically, in this application, nanoparticles do not carry therapeutic agents, but rather recognize the diseased cells based on specific receptors and generate a local signal for more accurate detection. Strictly speaking these particles would be considered nanodiagnostics, but the association with surgical intervention (therapy) justifies their listing as nanotheranostics.

Optical imaging is by far the simplest, most cost- and time-effective detection modality in an operating room setting. Nanotheranostics for guiding surgery include silica-based nanoagents (C-Dots, also known as Cornell dots), carbon-based nanoparticles (CNPs, also known as carbon dots) and fluorophore-lipid complexes (Table 2). C-Dots are ~ 10 nm silica

spheres containing near-infrared (NIR) fluorophores, typically cyanine 5.5. The surface of C-Dots can be also modified to expose targeting moieties as well as radioisotopes (^{64}Cu, ^{124}I) to enhance specificity and sensitivity with PET imaging. Clinically relevant examples are the cRGDY-PEG-Cy5.5 – C-Dot targeting $\alpha_v\beta_3$ integrins for the optical detection of lymph nodes[80] and the multimodal ^{64}Cu-NOTA-PSMA-PEG-Cy5.5, which are C-Dots targeted against the prostate-specific membrane antigen. C-Dots are systemically injected, reach and accumulate within the diseased tissue, and, upon intraoperative optical or PET imaging, help visualize tumor margins and inspect lymph nodes to assess the stage of the disease and guide surgery (Figure 3B). A similar clinical application is proposed for CNPs, which are ~ 10 nm carbon spheres designed to fluoresce upon UV exposure. As for C-Dots, the CNP surface can be readily derivatized to expose multiple chemical groups and biologically relevant molecules[81, 82]. CNPs have been tested on a huge variety of tumors, including colon rectal, gastric, rectal, breast, cervical and thyroid cancer (Table 2)[83, 84]. Another potentially relevant agent for guiding the resection of malignant masses is LipImage 815. This is a ~ 50 nm complex resulting from mixing various lipids with NIR IR780 iodide molecules[85]. After multiple tests in rodents, LipImage 815 was validated in larger animals to assess toxicity and identify the effective dose[86, 87]. However, these preliminary feasibility studies have not yet been followed by human trials.

The advantage of nanoparticles over single molecules in guiding surgical intervention mostly resides in the straightforward surface modification that supports the attachment of multiple targeting moieties and different imaging agents to increase specificity and signal-to-noise ratio. However, clinical studies have yet to demonstrate the full advantage of using multimodal, targeted imaging nanoparticles for image-guided surgery in terms of disease-free survival, overall survival, post-operative complications and rate of recurrence, as it has been already shown for indocyanine green and other fluorescent molecules[88, 89] (Figure 3B). Furthermore, in the future, newer particles capable to fluoresce in the short-wave infrared region will play an increasingly important role, as tissue absorption, scattering and autofluorescence in this portion of the spectrum (900 – 1700 nm) are negligible compared to visible wavelengths[90]. Preclinical short-wave infrared imaging with specialized fluorochromes enables high-resolution in-vivo imaging at depths not possible with conventional fluorochromes[91].

MRI-guided thermoresponsive liposomes

Because a variety of lipid-based nanoparticles have been already translated into the clinic as nanomedicines, thermosensitive liposomes are promising for nanotheranostics. For example, ThermoDox® is a liposomal formulation of doxorubicin whose lipid bilayer, resulting from mixing in a specific molar ratio three phospholipids, becomes unstable at temperatures higher than ~40°C[92]. Within the circulatory system, the thermoresponsive liposomes firmly encapsulate doxorubicin, limiting undesired drug leakage and off-site effects but, at the diseased site, the bilayer can be destabilized by localized heating, which can be obtained via a high-intensity focused ultrasound source or via radiofrequency ablation, triggering the release of the therapeutic cargo. Clinical data have shown that the overall survival of patients with hepatocellular carcinomas undergoing conventional radiofrequency ablation can be improved by 40% or even 100% upon exposing thermoresponsive doxorubicin liposomes to

2 or 3 min of ablation per unit tumor volume, respectively[93] (Figure 3C). This theranostic approach could only be achieved by integrating a drug into a nanostructure designed to respond to external energy sources.

Companion nanodiagnostics for patient stratification

To lodge within the malignant tissue, most nanomedicines rely on the enhanced permeability and retention effect, a phenomenon that occurs in tumors with leaky vasculature that allows nanoparticles in the bloodstream to selectively accumulate within the malignant tissue. Indeed, due to its rapid and chaotic growth, the tumor vasculature is not continuous but has openings, named fenestrations, with sizes of hundreds of nanometers[94]. Therefore, most nanomedicines with a diameter smaller than ~ 200 nm could passively accumulate within the malignant tissue by crossing these fenestrations. However, the clinical relevance of the enhanced permeability and retention effect is still debated[94]. Agents with a high safety profile, like IONPs, could be administered before nanotherapy to probe the vascular permeability of tumors and inform oncologists on the most adequate intervention. This approach has been recently tested in the clinic by characterizing the accumulation of systemically administered ferumoxytol in primary solid tumors[95] and metastatic lesions[96] via MR imaging. These studies found a positive correlation between the MR contrast enhancement induced by IONPs and the therapeutic response to irinotecan-loaded liposomes (Onivyde®2, Table 3). Despite a major difference in size between the ~20 nm ferumoxytol and the ~100 nm Onyvide®, these studies provide clinical evidence of the usefulness of IONPs as a nanoscale companion diagnostics to select patients who are likely to respond to nanotherapy[95,96].

A similar, but potentially more quantitative approach, consists in labeling nanomedicines with a sufficiently long-lived radionuclide. The clinical imaging of radiolabeled nanomedicines was likely first performed in 1976, when Anthony Segal and colleagues labelled liposomes containing bleomycin with ^{111}In and detected them using scintigraphy in patients with liver cancer[97]. Since then, planar gamma scintigraphy has been used to quantitatively assess in patients the biodistribution and tissue deposition of various liposomal nanomedicines[98–103], Ca-Na_2EDTA nanoparticles[104] and a copolymer-doxorubicin conjugate[105]. Following this, 3D SPECT imaging was used to detect ^{99m}Tc-radiolabeled DOXIL/Caelyx in patients with non–small-cell lung cancer and head and neck cancer to probe the particles' biodistribution[106]. More recently, the first clinical PET imaging study of nanomedicines was performed in patients with primary and metastatic breast cancer, who received HER_2-targeted liposomal doxorubicin (MM-302) radiolabeled with ^{64}Cu[107]. PET and CT imaging showed both inter- and intra-patient heterogeneity in uptake of the liposomes in primary tumors and metastases. Despite this, a correlation was observed between the intratumor deposition of ^{64}Cu and doxorubicin, and patients' disease progression-free survival[107]. In another important clinical example, a docetaxel-entrapping polymeric nanoparticle (CPC634) was labeled with ^{89}Zr by modifying the carrier's surface and was imaged in cancer patients[108]. Again, high inter- and intra-patient heterogeneity in tumor uptake was documented. In the same study, no correlation was found between the tumor deposition of ^{89}Zr-CPC634 nanoparticles and the response to chemotherapy on 7 late-stage, heavily pretreated patients with various forms of cancer. Importantly, however,

individual lesions with the highest nanoparticle uptake showed up to 50% reduction in size at 96 hours post treatment (Figure 3D).

The nuclear imaging of nanomedicines is historically the first example of clinical nanotheranostics. All these studies also highlight the advantages of using radiolabeled nanomedicines as a surrogate to test the actual, unlabeled nanomedicine. First, the radiolabeled nanomedicine is comparable in size and surface physico-chemical properties to the native therapeutic nanoparticle, implying that the former should more accurately match the biodistribution and pharmacokinetics of the latter. Second, the tissue-specific deposition of the radiolabeled nanomedicines can be accurately quantified, providing important information on dosing and drug accumulation during disease evolution. Third, accurate quantification enables the objective and precise characterization of inter- and intra-patient heterogeneity in nanomedicine deposition and nanotherapy response, building a database of fundamental information that could help develop more effective nanotheranostics for the future. However, radiolabeling requires extra manufacturing steps and characterization efforts that unavoidably result in higher costs.

Nanoradiotheranostics

Alongside the nuclear imaging of nanomedicines, the use of nanoradiotheranostics in the clinic was also reported. A key exploratory study in patients with metastatic cancer documented the administration of PEGylated liposomes radiolabeled with the β^- -emitter ^{188}Re[109]. A partial therapeutic effect was observed in some metastatic lesions, which also showed uptake of the radiolabeled liposomes by SPECT and CT imaging. However, dosimetry studies showed that the liver and spleen of patients received the highest dose, which caused the termination of the clinical work. Nonetheless, these preliminary studies, together with the fact that regulatory approval for clinical use has been obtained for a number of molecular radiotheranostics[110], highlight the potential of nanocarriers in radiotherapy for oncological applications.

Challenges and opportunities in Clinical Translation

Two decades from the launch of the US Cancer Nanotechnology initiative, almost 40 nanoparticle formulations have been clinically approved, and several more are advancing towards clinical translation[111–113]. Most are used as therapeutic nanomedicines for cancer, iron deficiency and, more recently, rare and infectious diseases. The latter include Onpattro®, the first approved siRNA-encapsulating liposome for the treatment of the rare condition known as hereditary transthyretin-mediated amyloidosis[114], and Comirnaty® and Spikevax®, the nanoparticle-based mRNA-vaccines against the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)[115]. Whereas some nanoparticle-based medicines have carved a market space worth multiple billions of US$, the same has not yet happened for the many nanotheranostics available on the laboratory benches.

The composition, architecture, imaging modality and therapeutic strategy affect the clinical translatability of nanotheranostics and together contribute to shaping the depth and width of the so called ‘valley of the death’ between laboratory benches and clinical beds (Figure 4). However, it is important to recall that the rate of translation into the clinic continues

to be moderate (< 10%) even for more conventional therapeutic agents, such as small molecules, that have been developed by the pharmaceutical industry and reviewed by international regulatory bodies for decades[116, 117]. The imbalance between the number of preclinical studies and the number of clinical products certainly remains a contentious point, but it should not be taken, just yet, as a measure of the impact of nanomedicines or nanotheranostics. A few companies developing nano-health products have failed to improve outcomes over the current standard treatments, with BIND Therapeutics being the most notable example[114], but most nanotheranostics are simply getting lost along the winding road of clinical translation because of the lack of information, technical resources and funding and owing to improper design of clinical trials rather than for their potential or actual performance. In this section, technical, economical and regulatory challenges are critically discussed.

The fate of systemically administered nanotheranostics

The goal of systemically injected nanoparticles for therapy and imaging is to reach the diseased tissue, deploy the therapeutic cargo, generate a signal for imaging and eventually clear out of the body without inducing any undesired damage. When it comes to nanoparticle accumulation within diseased tissue, nano skeptics often refer to a meta-analysis by Warren Chan and colleagues supporting the notion that tumor uptake of systemically injected nanoparticles is modest and, on average, equal to 0.7% of the injected dose (%ID)[118]. Although tumor accumulation is certainly important, a proper assessment of nanoparticle performance should also include traditional pharmacokinetic and toxicological parameters[119]. Indeed, a more recent meta-analysis by William Zamboni and colleagues compared the tumor (AUC_{tumor}) and systemic (AUC_{blood}) exposure (concentration and duration) of nanomedicines using the same dataset of Chan and coworkers. They found that the AUC_{tumor}/AUC_{blood} ratio was ~100 times higher than the tumor ID%[120]. Incidentally, with the advent of more sophisticated data mining tools and artificial intelligence algorithms, similar meta-analyses could be efficiently conducted on a larger cohort of studies stratifying the results in terms of nanoparticle attributes such as size, shape, surface properties, deformability and composition, as well as disease type and stage, and animal species. This represents an immense opportunity to objectively assess the performance of nanohealth products as well as identify subsets of conditions for which nanomedicines could be more effective than molecular or cellular therapies[121–123].

Those nanoparticles that do not accumulate in the diseased tissue can be either directly excreted through the glomerular pores in the kidneys or deposited over time into organs of the reticulo-endothelial system, mainly the liver and spleen. The glomerular pore size in healthy kidneys is ~10 nm, as determined via direct morphometric analysis of electron microscopy images[124], which correlates nicely with a cut-off size for kidneys' filtration of 6 to 8 nanometers, documented for a variety of nanoparticles[125, 126]. By contrast, in the liver most nanocarriers would degrade, if biodegradable, and be eventually excreted through the hepatobiliary circulation and the gut, or accumulate, if not biodegradable. In this context, it should be highlighted that the mechanisms of excretion and the biodegradation products for nanoparticles are of high concern to regulatory bodies and could impact the preclinical and clinical development of nanotheranostics. Focusing on rapid elimination

rather than biodegradation could be a safer and less expensive approach, as the dwelling time of nanocarriers in the body and amount of byproducts would be minimized. This is an important consideration for the 5 nm AGuIX nanocomplexes, for which longer residence times in the body could lead to transmetallation. Different nanoparticles, such as IONPs and liposomes, whose hydrodynamic diameter is significantly larger than 10 nm, are designed to circulate for longer, accumulate within the diseased tissue, and eventually degrade. Interestingly, looking at clinically relevant nanotheranostics, rapid excretion does not seem to be a necessary condition to access and succeed in clinical studies (Table 4). Rather, clinically relevant nanotheranostics are mostly made of well-known and extensively characterized materials (iron oxide, gold, lipids, silica) and are realized following simple manufacturing steps involving mixing, chemical conjugation and filtration. Therefore, investigators should keep in mind that efforts to translate nanotheranostics with augmented complexity and made with ‘exotic’ materials can only be justified by a significant improvement over the current clinical standards.

Certified preclinical development

In the preclinical development of any health product, the definition of good manufacturing practices (GMPs) and the collection of preclinical toxicological data are crucial steps, and represent a large portion of the ‘valley of death’. GMPs are fundamental to comply with the requirements of regulatory bodies and ensure that nanotheranostics are fabricated with the same properties over multiple production batches (reproducibility), while toxicological studies must be conducted under good laboratory practices and be fully certified by regulating bodies. These two steps cannot be undertaken by research laboratories within academic settings, as they require specific expertise, certifications and, most importantly, financial support well beyond the figures commonly granted by governmental agencies for industrial research. Just to put things in perspective, the launch of Onpattro took 15 years of preclinical and clinical development and $2.5B total investments.[127, 128] Currently, the medication is sold at ~$400k per patient per year with global net product revenues of $150 millions, as estimated using the Electronic Data Gathering, Analysis, and Retrieval (EDGAR) system.

Defining proper manufacturing protocols for nanotheranostics generally costs hundreds of thousand US$, typically requiring a first phase to realize the so called ‘GMP-like’ product followed by a second phase to obtain the actual GMP product for in-human use. The precise cost and duration of the two phases depend on technical, geographical and economic factors. The complexity of the technology, the number and type of fabrication steps and the too often neglected sterilization process all affect manufacturing costs. For instance, if the resulting product cannot be sterilized following standard procedures because these would impact the material properties and the final performance of the product, then the entire fabrication process should be conducted under sterile conditions, inevitably boosting the cost. Also, there is a lot of variation in costs and expertise among countries as well as within different areas in the same country. Not to mention that there are countries that lack any capacity in GMP manufacturing of nano-products. Finally, the cost of raw materials and the currently ongoing supply chain issues also affect development costs. Whereas small molecules and monoclonal antibodies have reached a level of development that enables most

contract research organizations and contract development and manufacturing organizations to deal with GMP manufacturing and toxicological studies, this is not yet the case for nanotheranostics. If a contract development and manufacturing organization with specific expertise does not exist or cannot be involved without compromising the protection of intellectual rights, then a dedicated GMP facility may be needed, resulting in much longer development times, larger investments, much higher risks, and an inevitable widening of the 'valley of death'. A large portion of nanotheranostics described in the literature falls under this less fortunate scenario. Therefore, in several cases, the lack of specific expertise for manufacturing becomes an insurmountable obstacle that explains the imbalance between the number of nanotheranostic agents described in the scientific literature and the number of clinically approved products. Consequently, it is not surprising that most nanotheranostics currently used in the clinic are based on consolidated technologies – iron oxide and gold nanoparticles, silica nanoparticles and liposomes – or molecular complexes resulting from the mixture of relatively inexpensive and well-characterized materials (Table 4). Fortunately, also thanks to the efforts dedicated to developing the lipid nanoparticles used for the SARS-CoV-2 vaccination campaign, a larger portion of contract development and manufacturing organizations are becoming familiar with a variety of nano- and micro-manufacturing and characterization techniques, which should facilitate the access of nanotheranostics to the clinic.

Once at least a GMP-like product is available, a contract research organization can conduct toxicological analyses, typically on rodent and non-rodent species, taking up to a few million US$. Even in this case, it is difficult to give precise figures, as costs depend on multiple factors. It is however important to remark that the cost of GLP toxicological studies tends to increase quasi-linearly with the number of therapeutic and imaging components associated with the nanotheranostic. For instance, the addition of an imaging component via different appendices (metallic or non-metallic ligands) and in different locations (surface modification or core encapsulation) to a nanomedicine might affect the overall biodistribution of the carrier and therefore the actual imaging accuracy, safety and therapeutic efficacy[108, 129, 130]. For example, liposomes containing ^{111}In within the lipid bilayer have significantly higher liver trapping than those encapsulating the same molecule in the aqueous core[129].

Another important point is that changes often occur in dosage, materials and manufacturing steps during the preclinical development phase. These changes can dramatically impact the performance of the agent and therefore require new toxicological studies, as well as the possible addition of new steps in the GMP workflow. Furthermore, especially in the case of highly innovative products, new ad-hoc analytical techniques may have to be developed, which can be far from current standards and challenging to validate[131]. Within this context, new opportunities are provided by the convergence of biology with physical sciences, mathematical modelling and organ-on-chip platforms, which could be used to efficiently and accurately fill the gap between clinical validation and animal testing. The development and validation of computational approaches, such as the Quantitative Nanostructure-Toxicity Relationship;[132] advances in in-vitro techniques, such as microfluidics and organs-on-chip[133]; and new approaches to data processing with machine learning and artificial intelligence tools[121 134, 135] may facilitate the accurate reproduction of in-vivo conditions.

As costs grow with complexity, nanotheranostics are expected to be more expensive than their therapeutic counterparts[136]. Selecting innate nanotheranostics could possibly reduce the complexity and contain costs, but it is difficult to generalize. This consideration suggests once again that including multiple imaging modalities, therapeutic molecules and targeting moieties without a clear medical need only widens and deepens the ‘valley of death’. At the same time, it is important to note that adding a nanotheranostic agent upfront could reduce cost further down the road by stratifying patients that will truly benefit from a nanomedicine rather than just applying it and then learning through follow-up imaging studies that the uptake of the agent was too low to achieve an effect. Indeed, this is something that a companion nanotheranostic could have revealed upfront.

Regulatory process for nanotheranostics

It is accepted that the existing criteria based on quality, safety, efficacy, performance and benefit/risk balance, and the two-framework structure of ‘medicinal product’ and ‘medical device’ are adequate for regulating medicines, devices and more complex health products, like nanotheranostics. In the US, the FDA Office of Combination Products selects the most competent experts for the evaluation of nano-based products. In the EU, the centralized procedure of EMA is used for evaluating and supervising nanomedicines, but several nano-based products, especially those intended for the treatment of iron deficiency, are approved at the national level[137].

The regulatory classification of all the products intended for use on humans follows the fundamental principle of the ‘main’ mechanism of action (MOA). As nanotheranostics possess both therapeutic and diagnostic capabilities and embody different MOAs, they may fall in different regulatory classifications and, therefore, different nanotheranostics may follow different pathways for regulatory oversight and approval.[138] According to current legislation, any product intended for prevention, in-vivo diagnosis or treatment and acting mainly via a metabolic, immunological or pharmacological mode is defined as a medicinal product, and is governed by the pharmaceuticals legislation. In addition, products containing more than one active agent are defined either as ‘fixed combination medicinal products’, if they involve two or more substances acting as medicinal products, or as ‘combined medicinal products’, if one of the components is a medical device integral to the product but the main MOA is exerted by the medicinal substance. By contrast, any product intended for medical purposes and acting mainly via a physical, mechanical or chemical mode is defined as a medical device and is governed by the medical devices regulation. A regulatory classification becomes more complex when the intended action of the product is physical but also pharmacological, which identifies so-called ‘borderline products’, and when new materials, or combinations of materials, are to be introduced into the human body. However, it is important to point out that the legislation foresees well-defined interactions between the medicinal product and medical device frameworks in these borderline cases, so that all the needed expertise is made available.

The decision on the proper classification of a product is crucial, as the regulatory path as well as duration and costs of product development and testing are different. In the US, the decision is taken by the FDA centralized offices, including the Office for Combination

Products together with the Center for Drug Evaluation and Research, Center for Biologics Evaluation and Research and Center for Devices and Radiological Health. In the EU, the responsibility resides within the authority of each Member State. However, the new EU regulation on medical devices has reinforced the harmonisation process among the EU Member States, establishing a central body where divergences might be resolved.

The regulations and guidelines cannot address all possible variables that could emerge in the development of innovative and complex health products. When performing studies with nanotechnology-based products, it is recognized that they are closer in complexity to biological structures (such as proteins and viral particles) than to small synthetic molecules, because beyond the identified pharmacological, immunological and metabolic interactions there are multiple additional layers of physical and spatial dynamics between the product and the host. The inherent physico-chemical properties – such as size, surface charge, shape and imaging detectability – and biological activity of nanomaterials, as well as their interaction with plasma or serum proteins (the corona) and blood cells, the stable incorporation of imaging agents and the release profile of the drug molecules should all be ascertained during preclinical studies.

In spite of the regulatory complexity and challenges posed by the convergence of different regulatory frameworks, it is important to recall that investigators have the opportunity to establish an open discussion with regulators early in the product development phase. This interaction can happen with the FDA Office of Combined Products, where the classification of the product takes place and the coordination of the relevant competences for the regulatory review can be identified in a timely manner in the different FDA Centers. In the EU, although the decision on the classification as medicinal product or medical device is officially given at a national level, there is a centralised regulatory framework coordinated by EMA that can provide scientific orientation at a very early stage – the EMA Innovation Task Force. This early interaction with regulatory scientists and officers could mitigate risks and costs and facilitate dialogue during a formal investigational new drug application, reducing the depth and width of the ‘valley of death’.

Harmonisation of expertise and knowledge

Nanotheranostics lie at the intersection of multiple disciplines. Far more than in medicinal chemistry and pharmacology, research laboratories focusing on the design, engineering and validation of nanotheranostics must build and accumulate a critical mass of knowledge and expertise to be effective. Whereas medicinal chemists and pharmaceutical scientists can enjoy spending long hours perfecting the potency and water solubility of their molecules before handling them to a contract research organization for GMP manufacturing and GLP preclinical toxicological analyses and efficacy studies, nanotheranostic laboratories typically have to address internally all the steps in the development process from the synthesis to the in-vitro physico-chemical and biological characterizations and the preclinical validation in small rodents. Therefore, the development of nanotheranostics requires a research team integrating expertise in physics, chemistry, materials science, engineering, biotechnology, biology, pharmacology, medicine and biomedical imaging.

Once the academic laboratory has produced a prototype nanotheranostic, its clinical translation requires a gamut of expertise typically not found within the wet benches of universities and research institutions, revolving around standardized manufacturing practices, accurate analytical and toxicological analyses, regulatory guidelines, clinical trial design, financing and healthcare reimbursement. The number of contract development and manufacturing organizations and contract research organizations with a documented track record in handling the manufacturing and toxicological characterization of nanotheranostics is very limited. The communication among academic laboratories and external contract organizations has to be developed for the specific product at hand. It also commands for reciprocal education and willingness to learn, while protecting intellectual property and know-how.

As mentioned earlier, another crucial connection is that between investigators and regulators. In particular, an early investigator-regulator dialogue helps exchange knowledge on novel developments in nanotechnology, materials science, drug delivery and related fields. This is fundamental to provide all the tools required to critically evaluate a nano-health product and more efficiently advance it towards the clinic. This early dialogue also serves to anticipate and address specific bottlenecks in the preclinical and clinical development of complex products, such as nanotheranostics. This approach offers the opportunity to shift the paradigm in drug and device development, seeing the regulator as an ally rather than an additional hurdle along the ‘valley of death’.

Finally, scientists should learn to resist the temptation to integrate multiple functions – multiple molecules and therapeutic modes, multiple imaging agents for different modalities, multiple targeting moieties – into nanocarriers simply ‘because they can’. This approach can perhaps facilitate the publication of manuscripts, even in high-impact journals. However, its utility to patients and to the scientific community is highly questionable. If the complexity of nanotheranostics is not justified by a clear unmet medical need or a dramatic (meaning orders of magnitude) improvement over the current standards, it is unlikely that the technology would reach the clinic and therefore benefit patients. Also, it is well recognized that true innovation does not derive from adding together known concepts and functions, but rather from encouraging diverse thinking at the interface among multiple disciplines.

Future Perspectives

The abundance of preclinical nanotheranostics demonstrates the tremendous potential of combining imaging, therapy and targeting in one nanostructure. However, strategies are needed to support the clinical integration of these technologies and address the challenges that widen the ‘valley of death’. In terms of technical challenges, more efforts are needed to rationalize and optimize the performance of ‘innate’ theranostic materials, where a significant mismatch still exists between imaging and therapeutic performances, while leveraging lipid and polymeric nanoparticles that are currently being tested, or even already used, in the clinic. More systematic studies on the therapeutic and imaging performances as well as on pharmacokinetics and pharmacodynamics could facilitate the selection of the most appropriate materials and nanostructure configurations. This approach would require a change in research funding schemes, balancing the quest for absolute novelty with that for

rational design and optimization, which is often considered as a mere incremental activity. New opportunities are provided by the implementation of funding schemes focusing on different technology readiness levels and following the development of a product from the laboratory bench to clinical testing. Although this is already happening in some countries, more resources should be allocated to unconventional technologies – like nanotheranostics – that are less likely than small molecules to attract the interest of large pharmaceutical companies at an early developmental stage.

Regarding manufacturing, a ‘low hanging’ but promising fruit is the conversion of existing nanomedicines into nanotheranostics. This strategy would not only build on consolidated knowledge and minimize fabrication costs, but it would also help conduct more efficiently, and possibly more successfully, clinical trials by properly stratifying patients. Moreover, the combination of nanotheranostics with artificial intelligence tools would help extract precious information on the disease biology as a function of the specific patient, stage and used technology. Importantly, the impact that the SARS-CoV-2 pandemic has had on the pharmaceutical manufacturing community, favoring the rapid conversion and even the launch of new contract research organizations and contract development and manufacturing organizations for the realization and testing of nanocarriers and unconventional therapeutics, cannot be neglected.

In terms of regulatory approval, most agencies have established protocols to facilitate early engagement, already during the preclinical development phase, with regulatory scientists, although there is no dedicated path for nanotheranostics. This represents a unique opportunity, as early interactions could help set requirements and expectations, instruct the scientists in the regulatory bodies on new materials and technologies, and, more importantly, help shape the proper path (medicinal product vs medical device) for the proposed nanotheranostics. As communication and cross-fertilization between fields are crucial, a new opportunity is provided by the unprecedented proliferation of biotechnological accelerators, launched by academic institutions as well as by private entities. These initiatives have the objective to connect scientists with experts in regulatory sciences, patent attorneys, manufacturers and venture capitalists and help scientists familiarize with communities that are very different from academia. These accelerators serve to build common knowledge and trust around new technologies and should become a new model for funding and overseeing the development of novel medical technologies.

In conclusion, scientific soundness and medical relevance are necessary conditions for the clinical translation of nanotheranostics, but this translation can only occur if academia establishes a symbiotic interaction with other stakeholders involved in the process.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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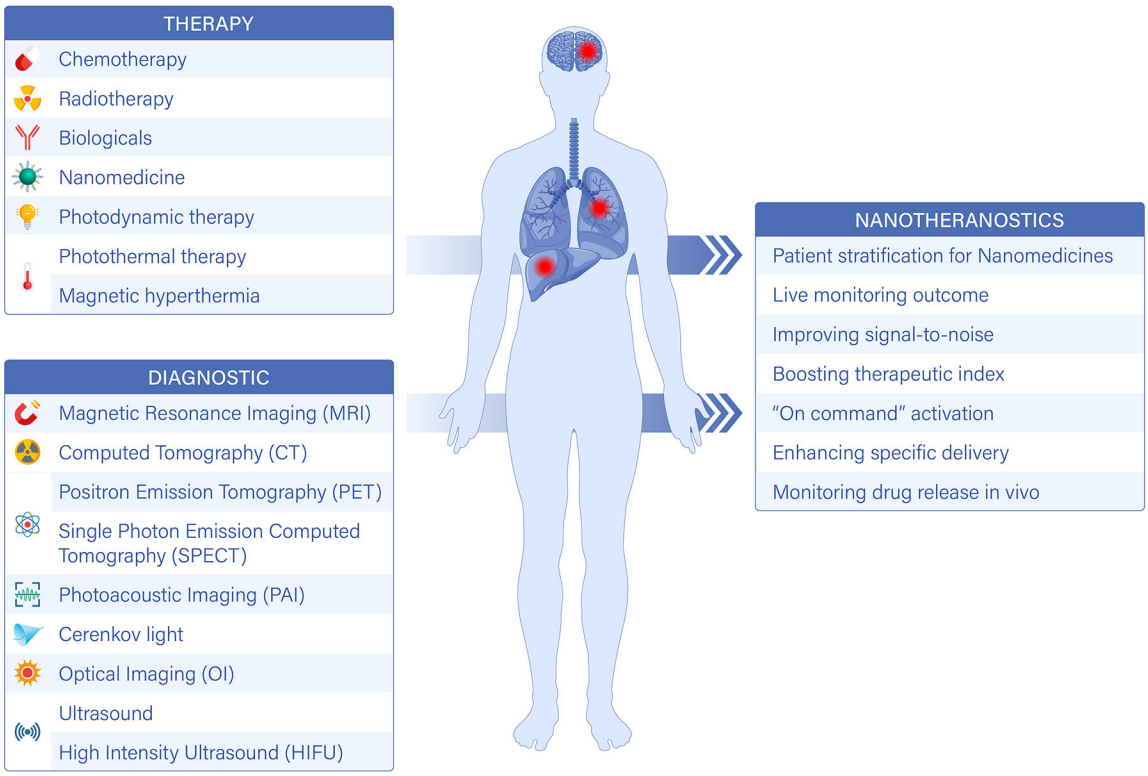

Figure 1 |. Theranostic nanoparticles.

Nanotheranostics combine therapeutic and imaging functions in a nanocarrier. This can be achieved by the incorporation of specific therapeutic and imaging agents into a nanoparticle, or can be a result of the innate properties of the material, or a combination of the two. Targeting molecules (such as small ligands, peptides, aptamers, antibodies and fragments of antibodies) can be incorporated into a nanoparticle to enhance the recognition of specific cellular and subcellular targets. It is also possible to use materials with the ability to specifically recognize the diseased tissue. Moreover, nanotheranostics can be designed to be activated by endogenous and exogenous stimuli. Compared to molecular agents and nanomedicines, nanotheranostics offer several advantages, including patient stratification, 'on command' activation and enhanced therapeutic efficacy.

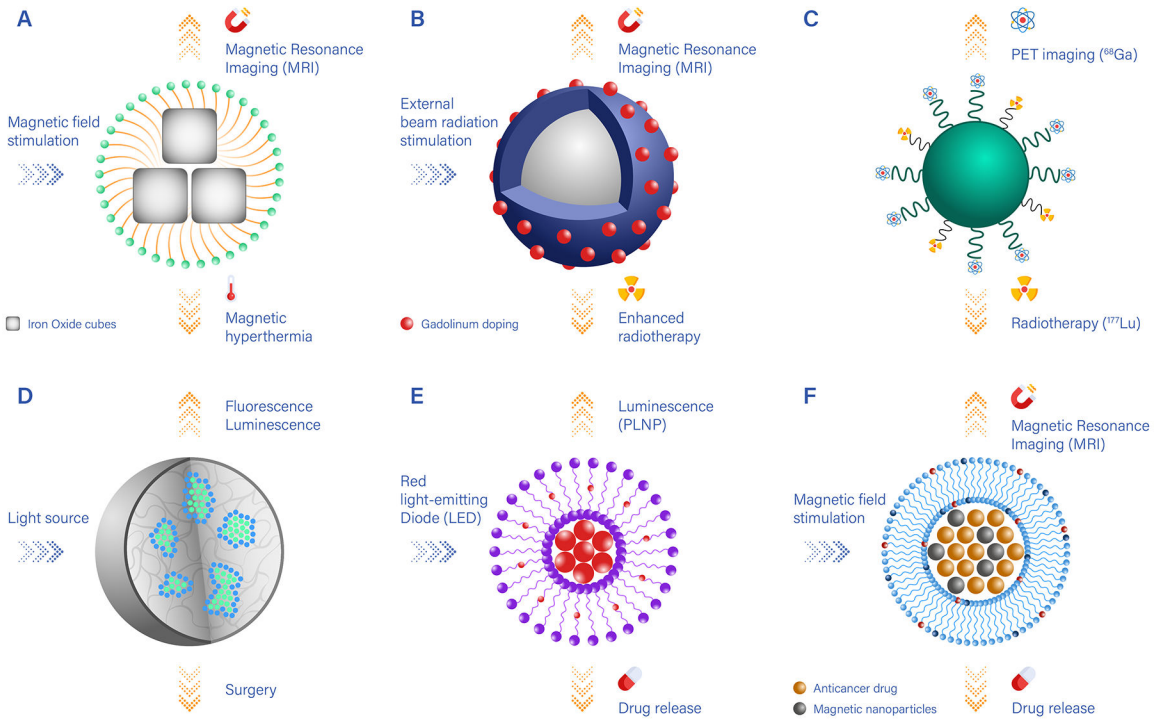

Figure 2 |. Examples of nanotheranostics with different imaging, therapeutic and targeting components.

A. Clusters of iron oxide nanocubes coated by polymeric or lipid chains (yellow) and presenting surface-targeting moieties (blue). Upon magnetic stimulation, iron oxide nanocubes generate thermal energy, which can be used for magnetic hyperthermia, and can be visualized via magnetic resonance imaging (MRI); **B.** Nanocarriers carrying heavy metals (such as Gd) locally enhance the therapeutic efficacy of external beam radiation and can be imaged via MRI; **C.** Nanocarriers conjugated to two different radionuclides can be used for positron emission tomography (PET) imaging (using ^{89}Zr) and radiotherapy (using ^{177}Lu); **D.** Nanoparticles encapsulating luminescent/fluorescent molecules, which under external light stimulation can be visualised and guide surgical resection; **E.** Nanocarriers loaded with persistent luminescence nanoparticles (PLNP) and chemotherapeutic drugs that are released by passive diffusion over time; **F**. Nanocarriers loaded with iron oxide particles for MR imaging guidance, which under high-intensity focused ultrasound stimulation trigger the release of anti-cancer molecules.

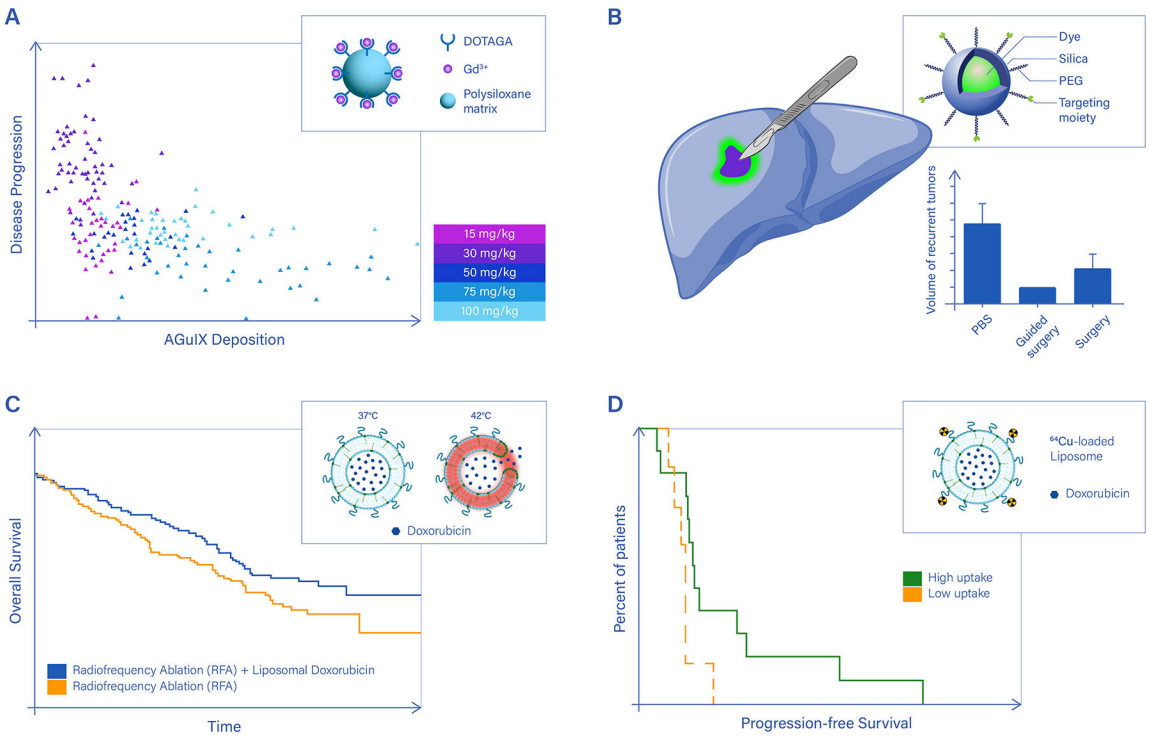

Figure 3 |. Clinical demonstrations of nanotheranostics.

A. Enhancing the efficacy of external beam radiation therapy using Gd-based nanoparticles (AGuIX) under magnetic resonance imaging (MRI). Clinical data on cancer metastases to the brain show a positive correlation between AGuIX deposition within the lesions, as detected via MRI, and disease regression. Upon external beam radiation therapy, brain metastases receiving higher amounts of AGuIX grow less or even reduce in diameter (relative lesion size < 1) over a 28-day observation period. DOTAGA: 1,4,7,10-tetraazacyclododecane,1-glutaric acid-4,7,10-triacetic acid ligands. The legend shows the dose of AGuIX injected in the patients. Each symbol in the plot corresponds to a different injected dose and patient.[77] **B.** Multimodal imaging nanoparticles carrying targeting moieities to specifically recognize cancer cells help visualize tumor margins, inspect lymph nodes, and accurately guide surgical resection of the bulk malignancy. However, to fully unveil the advantage of this approach, future clinical studies will have to address patient survival and disease recurrence, as was done for indocyanine. The Kaplan-Meier plot compares the overall survival for patients with rectal cancer following a laparoscopic lateral pelvic lymph node dissection performed with or without indocyanine green fluorescence imaging[89]. Image-guided surgery with indocyanine positively correlates with improved survival and reduced risk of local disease relapse. **C.** Boosting the spatio-temporal specificity of conventional therapies using thermoresponsive nanomedicines. The Kaplan-Meier plot shows a significant improvement in overall survival for hepatocellular carcinoma patients treated with radiofrequency ablation plus thermosensitive doxorubicin liposomes[93]. The chemotherapeutic agent is released preferentially at the tumor site, following the localized temperature increase and consequent destabilization of the liposomes. Drug release from thermosensitive nanomedicines has been achieved in clinical settings using

radiofrequency ablation and high-intensity focused ultrasound; **D.** ^{89}Zr-labeled, docetaxel loaded polymeric nanoparticles have been used to treat patients with various cancers (primary and metastatic) and image particle deposition in each single lesion for 7 patients. Although at the overall patient level no statistically significant correlation was found between nanoparticle accumulation in the cancer tissue and response to therapy, individual lesions with higher nanoparticle deposition (red lesion in Patient 1 and yellow lesion in Patient 6) underwent a reduction in size of up to 50% within a 96-hour observation period[108].

Panel a adapted from Ref.[77], panel b from Ref.[89], panel c from Ref.[93], panel d from Ref.[108].

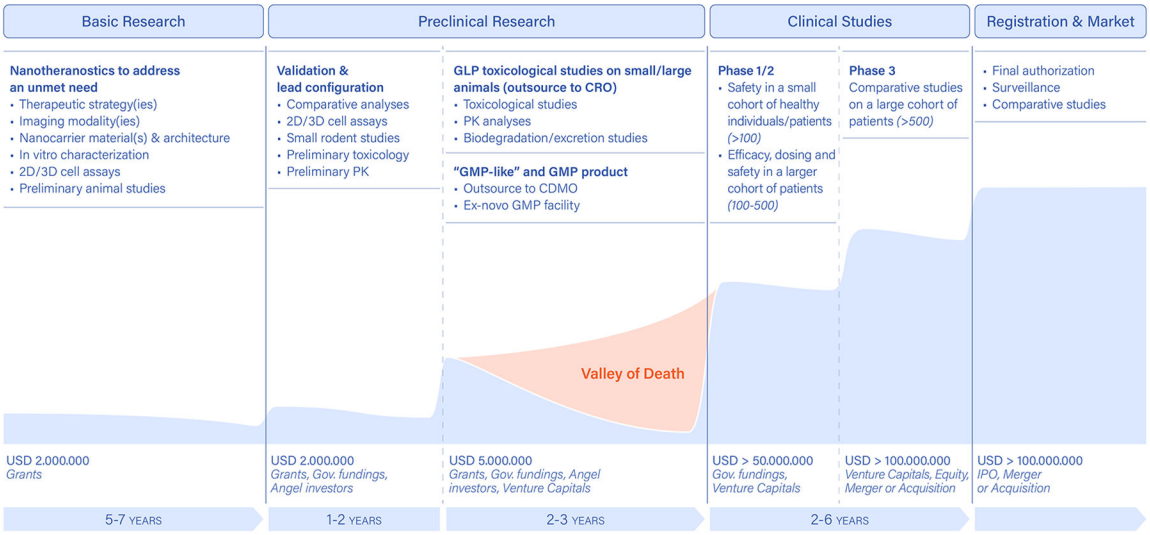

Figure 4. Milestones in the clinical translation of nanotheranostics.

Early design, development and characterization of novel nanotheranostics entirely relies on research grants, which help demonstrating a new idea and technology all the wat up to small-rodent models. This basic research phase is almost exclusively funded by governmental research grants. More systematic analyses and in-vitro/in-vivo characterizations of the new technology are conducted in the preclinical research phase. This aims at identifying a lead product, with a specific imaging/therapeutic agent combination as well as material and architecture configurations. This phase is typically funded by industrial-like governmental grants, such as small business innovation research (SBIR) and small business technology transfer (STTR) programs in the US; European Innovation Council (EIC) Transition and Accelerator programmes in the EU, and angel investors and foundations. A second portion of the preclinical research phase deals with the manufacturing and toxicological testing of the proposed product, following well-coded procedures (good manufacturing practice, GMP, and good laboratory practice, GLP) in agreement with the relevant regulatory bodies (FDA, EMA and others). This phase can be funded by governmental agencies, angel donors and foundations, as well as venture capital firms investing in early-stage products. Finally, the clinical studies aim to assess the safety, efficacy and economic convenience of the proposed product over different phases, depending on the type of product and disease. These activities typically require significant capitals involving multiple venture capital firms as well as pharmaceutical companies. PK: pharmacokinetics; CRO: contract research organization; CDMO: contract development and manufacturing organization).

Table 1 |

Most relevant preclinical and clinical nanotheranostics.

Imaging modality	Therapeutic modality	Imaging agent	Therapeutic agent	Stage	Ref.
Magnetic Resonance Imaging	nanomedicine	Gd^{3+}, Mn^{2+}, IONP	small molecule	pre-Clinical	14–16
	thermal ablation	IONP	IONP (magnetic) PTA (photothermal)	pre-Clinical & Clinical	12–14, 59, 60, 67
	radiation therapy	Gd^{3+}, Gd_2O_3, IONP	Au, Bi, Gd-complexes	pre-Clinical &Clinical	17, 18, 74, 78
	Image-guided cell therapies	IONP (intracellular)	cellular therapies	Clinical	69, 70
Nuclear Imaging	'companion' nanoparticle	^{64}Cu, ^{89}Zr, … (PET imaging)	nanomedicine	pre-Clinical &Clinical	51, 107, 129
	radiation therapy	^{64}Cu, ^{89}Zr, … (PET imaging)	^{177}Lu, ^{90}Y, $^{186/188}Re$	pre-Clinical	50, 109
	CRIT	^{64}Cu, ^{89}Zr, … (PET imaging)	porphyrins, chlorins	pre-Clinical	21, 23–25
Optical Imaging	photodynamic therapy	Cy5.5, Cy7; PLNP	porphyrins, chlorins, nanomedicine	pre-Clinical	30–33
	image-guided surgery	Cy5.5, Cy7; PLNP; DCNP	surgery	pre-Clinical &Clinical	26, 80
	nanomedicine	endogenous, AuNP, CNT	small/macro-molecules	pre-Clinical	82, 85
Ultrasound	enhanced delivery	vesicles	small/macro-molecules, nanomedicine	pre-Clinical	41–44
	triggered drug release	vesicles	drug-loaded vesicles	pre-Clinical &Clinical	37, 38, 40

HIFU: high-intensity focused ultrasound; CRIT: Cerenkov radiation-induced therapy; IONP: iron oxide nanoparticle; PET: positron emission tomography; PLNP: persistent luminescence nanoparticle; DCNP: down-conversion nanoparticle; NP: nanoparticle; CNT: carbon nanotube; MRI: magnetic resonance imaging; AuNP: Gold nanoparticle; PTA: photothermal ablation.

Table 2 |
Active and Recruiting Clinical Trials for Nanotheranostics.

Information from clinicaltrial.gov.

Imaging modality	Therapy	nano-particle	clinical application	Phase	Start Date	Ref.
Magnetic Resonance Imaging	surgery	SPIO	sentinel node	NA	07/2018 05/2020 07/2021	NCT05288686 NCT05161507 NCT04803331
				Phase1/2	11/2021	NCT05359783
	small molecule	USPIO	neuro-inflammation	Phase 3	03/2020 05/2020	NCT04722692 NCT04261777
				Phase 1	06/2022	NCT05357833
		USPIO	vascular inflammation		09/2021	NCT03948555
	nutritional supplement	SPIO	iron deficiency	Phase 3	08/2018 02/2020	NCT03619850 NCT04268849
				Phase 4	02/2020 01/2020 09/2021	NCT04205266 NCT04080908 NCT04278651
	radiation therapy	SPIO	hepatocellular carcinomas and liver metastasis	NA	11/2020	NCT04682847
	magnetic hyperthermia	NanoTherm	prostate cancer	NA	11/2021	NCT05010759
	radiation therapy	AGuIX	brain tumor and metastasis	Phase 1/2	03/2022	NCT04881032
				Phase 2	03/2019 09/2021	NCT03818386 NCT04899908
			lung & pancreatic cancer	Phase 1/2	05/2021	NCT04789486
			gynecologic cancer	Phase 1	05/2018	NCT03308604
	photothermal therapy	AuroShell	prostate cancer	NA	01/2020	NCT04240639
Computed Tomography	radiation therapy	NBTXR3	NSCL	Phase 1	02/2021	NCT04505267
			head & neck squamous cell	Phase 2 Phase 3	04/2021 01/2022	NCT04862455 NCT04892173
			pancreatic cancer	Phase 1	07/2020	NCT04484909
			esophageal cancer	Phase 1	11/2020	NCT04615013
Optical Imaging	surgery	Carbon NP	sentinel node	NA	01/2018 02/2022	NCT03550001NCT05167149
				Phase 2	01/2022	NCT05229874
				Phase 2/3	01/2021	NCT04759820
	surgery	C-dots	prostate cancer	Phase 1	02/2021	NCT04167969
			sentinel node	Phase 1/2	04/2014	NCT02106598
	surgery	ONM-100	Peritoneal Carcinomatosis	Phase 2	11/2021	NCT04950166

NA means that the trial didn't have FDA-defined phases. SPIO: super-paramagnetic iron oxide nanoparticles; USPIO: ultra-small SPIO; carbon NP: carbon nanoparticle; C-dots: carbon dots. NanoTherm are iron oxide nanoparticle, AGuIX are polymeric gadolinium complexes, AuroShell are silica gold nanoshells, NBTXR3 are hafnium oxide nanoparticles; ONM-100 are fluorescent imaging nanoparticles.

Table 3 |
Completed Clinical Trials for Nanotheranostics.

Information from clinicaltrial.gov.

Imaging modality	Therapy	Nano-particle	clinical application	Phase	Last Update	Reference	Results	Comments
Magnetic Resonance Imaging	surgery	SPIO	sentinel node	NA	06/2017 06/2019 10/2020 10/2020 09/2022	NCT01927887 NCT02739425 NCT01815333 NCT03898687 NCT05054062	Y N Y N P	SPIO for lymph node visualization in melanoma patients
	small molecule	USPIO	neuro-inflammation	NA	07/2022	NCT02549898	P	no correlation between neuroinflammation and USPIO signal enhancement
				Phase 1	03/2023	NCT02511028	N	-
	cell therapy	SPIO	cell mobility & retention	NA	06/2021	NCT00972946	P	safety and feasibility of cell labeling with SPIO for MRI tracking
				Phase 1	08/2018	NCT03651791	P	
				Phase 1/2	03/2021	NCT00781872	N	-
	Cell tracking	USPIO	inflammation in cardiovascular disorders	NA	02/2013 12/2014 04/2015	NCT01169935 NCT01323296 NCT00368589	P P P	safety and feasibility of cell labeling with SPIO for MRI tracking
				Phase 2/3	05/2017	NCT02319278	P	
	nutritional supplement	SPIO	iron deficiency	NA	03/2014	NCT01374919	P	safety of Ferumoxytol
				Phase 2	05/2018	NCT01052779	P	efficacy of Ferumoxytol vs iron sucrose
				Phase 3	01/2015 01/2015 06/2018 04/2022 04/2022 04/2022	NCT00255437 NCT00255424 NCT02694978 NCT01114204 NCT01114139 NCT01114217	P P P Y Y Y	safety of i.v. iron therapy in kidney disease patients
				Phase 4	03/2012 04/2022	NCT01148745 NCT01227616	Y P	safety of Ferumoxytol in chronic kidney disease patients
	magnetic hyperthermia	SPIO	prostate cancer	Phase 0	05/2017	NCT02033447	N	-
	radiation therapy	AGuIX	brain tumor and metastasis	Phase 1	06/2019	NCT02820454	P[77]	AGuIX phase I study protocol in brain metastasis patients
	photothermal therapy	AuroShell	prostate cancer head & neck	NA	02/2017 03/2021	NCT00848042 NCT02680535	Y P	demonstration of a software for mapping tissue ablation and preoperative planning
	irinotecan liposomes	SPIO	breast cancer and solid tumors	Phase 1	11/2019	NCT01770353	P	efficacy of liposomal irinotecan in metastatic breast cancer patients

Imaging modality	Therapy	Nano-particle	clinical application	Phase	Last Update	Reference	Results	Comments
Nuclear Imaging	Nanomedicine	^{89}Zr-CPC634	multiple solid tumors	Phase 1	10/2020	NCT03712423	P[108]	localization of ^{89}Zr-CPC634 in malignant tissue
		^{64}Cu-MM-302	Breast Cancer Brain Tumours	Phase 1	01/2017	NCT01304797	P[107]	localization of ^{64}Cu-MM-302 in malignant tissue
					n/a	NCT02735798	N	product withdrawn (trial never started)
		^{188}Re-liposome	solid tumors	Phase 1	10/2014	NCT02271516	N	study terminated for safety concerns
Computed Tomography	radiation therapy	NBTXR3	sarcoma	Phase 1	10/2020	NCT01433068	N	-
				Phase 2/3	04/2021	NCT02379845	P	demonstration of NBTXR3 as theramostic nanoparticles
Optical Imaging	surgery	carbon NP	sentinel node	NA	03/2016	NCT02724176	P	demonstration of carbon nanoparticles for patient stratification
		ONM-100	Cancer (breast, lungs, prostate, ovarian, lungs…)	Phase 2	11/2022	NCT03735680	N	-
			Lung cancer	Phase 2	03/2023	NCT05048082	N	-

NA means that the trial didn't have FDA-defined phases. Y indicates results posted at clinicaltrials.gov, N results not posted, and P results published in a scientific manuscript. SPIO: super-paramagnetic iron oxide nanoparticles; USPIO: ultra-small SPIO; Carbon NP: carbon nanoparticle; C-dots: carbon dots; NanoTherm: iron oxide nanoparticle, AGuIX: polymeric gadolinium complexes, AuroShell: silica gold nanoshell, NBTXR3: hafnium oxide nanoparticles; ONM-100: fluorescent imaging nanoparticles; 89Zr-CPC634: 89Zr-labeled polymeric nanoparticles carrying docetaxel; 64Cu-MM-302: 64Cu- labeled liposomes carrying doxorubicin; 188Re-liposome: 188Re-labeled liposomes.

Table 4 |

Main attributes of clinically relevant nanotheranostics.

Nanotheranostic	size (nm)	composition	blood longeivty	route of Administration	First Trial	Major Application
ferumoxides[64]	50 – 100	dextran-coated IONP	< 1 h	iv/local	09/1999	liver imaging
ferumoxytol[66]	17 – 31	polyglucose sorbitol carboxymethylether - coated IONP	~ 15 h	iv/local	05/2004	anemia treatment
AuroShell[58]	150	Au nanoshell on a silica core	3 – 6 h	iv	04/2008	cancer ablation
^{64}Cu-MM-302[107]	~ 100	^{64}Cu-labeled HER_2-targeted PEGylated liposomal doxorubicin	~ 33 h	iv	03/2011	cancer chemotherapy
Carbon NP[83]	~ 10	carbon pellet (after surface modification ~ 150)	-	local	01/2012	lymph node dissection
NBTXR3 [78]	50	functionalized hafnium oxide nanoparticles	-	local	01/2014	head & neck cancer
Cornell Dots [80]	~ 10	radiolabeled molecular targeted PEGylated silica matrix	-	iv/local	04/2014	lymph node dissection
^{188}Re-Liposome[109]	~ 80	^{188}Re-labeled PEGylated liposome	~ 40 h	iv	10/2014	terminated
AGuIX [77]	~ 5	Gd chelated-polysiloxane	> 6h	iv	03/2016	cancer radiotherapy
^{89}Zr-CPC634[108]	65	radiolabeled docetaxel-loaded nanoparticles	~ 60 h	iv	04/2018	cancer chemotherapy

IONP: iron oxide nanoparticle; AGuIX: polymeric gadolinium complexes; AuroShell: silica gold nanoshells; NBTXR3: hafnium oxide nanoparticles; Carbon NP: carbon nanoparticle; ^{89}Zr-CPC634: ^{89}Zr-labeled polymeric nanoparticles carrying docetaxel; ^{64}Cu-MM-302: ^{64}Cu-labeled liposomes carrying doxorubicin; ^{188}Re-liposome: ^{188}Re-labeled liposome; iv: intravenous administration.