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Nanoparticles: Take Only Pictures, Leave Only Footprints

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Abstract

A phase I study of a tumor-targeted nanoshell in five patients documents an important milestone in the development of nanoparticles for molecular imaging in humans.

The hype behind nanomedicine has generated intense hope in the media and scientific literature for new cures and diagnostic tests. Nanoparticles (NPs) offer seemingly ideal characteristics for imaging and therapy, including multivalency—which enables the attachment of targeting, multimodal diagnostic and therapeutic payloads—and enhanced bioavailability, owing to longer plasma residence times than a free drug or imaging agent. Thus, a plethora of NPs for medical use have been developed and tested in animals in laboratories around the world. However, for many NPs animal models are the end of the road. In this issue of *Science Translational Medicine*, Phillips and colleagues make the move from animals to humans with an ultrasmall NP for cancer imaging—a first of its kind in translation (1).

PHARMACOKINETIC FOOTPRINTS

The attrition in translation of nano to humans relates to the poorly understood or defined safety and regulatory issues surrounding the prolonged pharmacokinetics of NPs. Indeed, although the main focus for NP clinical feasibility has been on particle diameter, equally important issues include charge, shape, hardness, and surface chemistry (2, 3). The longer the half-life of the NP in the body, the longer—and more expensive—are the toxicity studies that must be performed before an agent can be considered for clinical translation. Moreover, the more complex a NP becomes, with multiple attachments, such as targeting moieties and therapeutic payloads, the more difficult is the approval process because the fate of each component must be individually understood and the toxicity related to breakdown products must be demonstrated.

Similarly, because humans are not evolved to metabolize most of these synthetic NPs, they are viewed with some skepticism by regulators regarding how the body will actually metabolize them. This is often poorly understood for familiar, low-molecular weight and structurally defined drugs, so understanding the fate of NPs is even more challenging. Similarly, the longer plasma residence times that improve NP bioavailability also raise concerns over excretion, exposure, immunogenicity, and long-term toxicity. In the specific case of quantum dots (Q dots), there is added concern over the long-term retention of heavy metals found in their core. As a consequence, progress toward the clinic for all NPs has been slow.

Now, two biomedical imaging studies in *Science Translational Medicine* raise hope that we may soon see progress in the use of NPs in humans. Both examples are instructive because the design of the NPs is carefully crafted to account for their use in patients and to overcome some of the more obvious disadvantages of NPs. At the same time, both reveal considerable challenges ahead.

FIRST-IN-HUMAN PICTURES

Phillips *et al.* developed a nanoparticle hybrid PET-optical imaging agent based on the Cornell dot (C dot) technology (4). The first aim of their clinical study was safety and pharmacokinetics when administered intravenously to five terminal patients with melanoma; the second was imaging efficacy (1). Because the NP agent was systemically administered, it had to be carefully designed to avoid issues of slow clearance and biodistribution so that it would be rapidly and completely cleared by the kidneys. At the center of the NP construct was a silica C dot nanoshell, designed and selected to avoid the heavy-metal issues with conventional Q dots, encapsulating the fluorescent dye, Cy5. The encapsulation of the Cy5 dye, and presumably other fluorophores of the same class, resulted in a multifold increase in fluorescence compared with that of unencapsulated dyes. This is a happy coincidence that could not be tested in this small study but has been previously evaluated in pre-clinical models (4).

In order for this agent to be eliminated through glomerular filtration rather than slower hepatic excretion, it was important that the diameter of the NP be less than 10 nm (Fig. 1). However, even at this size the unadorned C-dot (6 to 7 nm) could have ended up primarily in the liver when injected intravenously. The authors added poly(ethylene glycol) (PEG) to help the particles evade the mononuclear phagocytic system so that 90% of the particle was removed by the kidney without substantial catabolism. This created a more favorable pharmacokinetic profile for human use that eliminated ~97.5% of the nanoparticle, mostly intact, from the bladder within 72 hours, thus reducing concerns of residual inorganic particles in the body and resulting toxicity.

It should be noted that the 10-nm guide-post depends to some extent on the hardness of the molecule. More flexible agents can be renally excreted at larger diameters; however, this threshold is a good rule of thumb (2, 5). In their study, Phillips *et al.* targeted the C-dot (Cy5)-PEG to the $\alpha\beta3$ integrin using the cyclic peptide cRGDY. cRGDY was also radioiodinated with iodine-124 (a positron emitter) via tyrosine residues on the peptide, for positron emission tomography (PET) imaging. When injected into the five patients at microdoses sufficient for imaging (average effective dose of 185 megabecquerels), the agent produced no adverse events or biochemical abnormalities, and only minor free iodine uptake was noted in the thyroid (Fig. 1), as is expected with any peptide iodination.

This phase I study was strictly a safety study, so there are still unknowns regarding the practical utility of the agent as a PET agent and as an intraoperative optical agent, but the preliminary results affirm the idea that smaller intravenous NPs can receive investigational new drug (IND) status and be safe for human use. Moreover, the study provides hints about the utility of such an agent, such as the ability to detect a hepatic metastasis in one patient

and an incidental pituitary microadenoma; however, larger studies will be necessary to determine efficacy. Of course, the C dot will not have all the advantages of larger NPs, such as multivalency or bioavailability, but it has major practical advantages that may allow it to move forward to the clinic.

RIGHT ON THE DOT

As mentioned, nanomedicine offers several advantages over traditional imaging approaches, including targeting, longer circulation times and bioavailability, and multimodality. Phillips *et al.* showed for the first time that inorganic, bright PET-optical hybrid imaging agents might translate to use in the clinic for cancer imaging (1). This opens the door to the translation of another class of imaging agents, quantum dots, which have to date been deemed too risky (as heavy metal-based) for use in people.

With the hope that the C dots pave the way for Q dots, Pan *et al.*, also in this issue of *Science Translational Medicine*, demonstrated a different approach to targeted cancer imaging with NPs (6). In 26 intact human bladder specimens, a Q-dot₆₅₀ conjugated to a CD47 antibody was instilled intravesically so as to detect cancer. The authors used blue light cystoscopy *ex vivo*, but this form of imaging endoscopy could easily translate to bladders *in vivo*. The anti-CD47 Q-dot₆₅₀ performed well, with a sensitivity of 83% and a specificity of 90.5%. The Q dot used in this application was larger than the 10-nm renal excretion cutoff; however, because the agent is administered intravesically and then washed out, it bypasses this limitation. Previous experience with intravesical instillation followed by washing shows that systemic absorption from this type of administration is very low. Thus, once again the NP is eliminated intact from the body.

The topical application of this antibody–Q-dot conjugate makes full use of the exceptionally bright fluorescence of Q dots while avoiding potential toxicities associated with prolonged systemic exposure. This agent is fine for such topical applications but would likely not be successful as an intravenous agent for the reasons outlined above that have to date hindered translation. As a means of detecting bladder cancers not visible with conventional white light cystoscopy, this approach by Pan *et al.* (6) holds promise. Moreover, other potential clinical applications of Q dots for oncology include enhancing endoscopy for colon cancer and imaging the lymph nodes, draining tumors to permit their selective resection (7, 8). In both instances, it would be assumed that there is minimal systemic absorption and near complete recovery of the Q dot.

Both studies point to the challenges ahead for nanotechnology in cancer imaging and therapy. The cost of bringing such agents to market means that they must be useful in a broad range of conditions and not just in highly targeted populations, such as patients with metastatic melanoma or localized bladder cancer—although these patient populations are the most obvious cohorts for first-in-human testing. Moreover, to achieve near-complete utilization of these agents in these small populations, the agents must be particularly effective with few, if any, competitors.

In the case of the C dots, the targeting moiety cRGDY probably does not meet these criteria. A positive integrin scan in melanoma has no current practical meaning in terms of directing therapy because no integrin-targeted therapies are currently available. This is because integrins are expressed both on tumor vessels and tumor cells, leading to ambiguity about whether the agent identifies angiogenesis or tumor. Slight accumulation of the ^{124}I -cRGDY-PEG-C dot in the bone marrow and muscle observed by Phillips *et al.* (1) raises some concerns regarding mechanism and longer-term toxicity. For diagnostic purposes alone, 2-[^{18}F]-fluorodeoxyglucose (FDG) already provides an excellent and widely available means of diagnosing melanoma in patients. Recognizing that this C dot-based NP is strictly a model for future agents with other targeting moieties, the limited value of cRGDY must be acknowledged nonetheless.

Likewise, CD47 is not an established target for bladder cancer, although preclinical and clinical studies suggest that this “don’t eat me” signal is widely expressed on the surface of solid tumors (9). Like all cell-surface targets, bladder cancer expression will be highly heterogeneous, with variable cell surface expression. Only 80% of bladder cancers express it, and not all those that express it do so in sufficient amounts to be diagnostic or therapeutic. This coupled with inadvertent washout may explain the sensitivity of only 83% in the ex vivo human bladder study by Pan *et al.* (6). Moreover, CD47 is also found on macrophages and dendritic cells. Thus, a single target, such as CD47, might falsely identify a considerable number of lesions as cancers, owing to target expression on non-tumor cells.

Nevertheless, the authors of both studies in *Science Translational Medicine* describe platform imaging technologies that should allow many different targeting moieties to be conjugated to the respective NP. Therefore, although progress has been made in NP platforms, the targeting moieties will need optimization for the specificity desired in the clinic. Topics worthy of consideration for future work should include pairing diagnostic agents with therapeutic agents and making use of the multivalency of NPs to attach multiple targeting agents to each NP.

Phillips (1) and Pan (6) provide reasons to be optimistic about a future involving the clinical translation of NPs and should go a long way toward appeasing critics who say that such progress is impossible. Convincing regulatory agencies to approve NPs for testing in humans, even in the limited study by Phillips *et al.*, is a major accomplishment because it sets a precedent and enables regulators to become familiar with this new class of agents (10). The more safety data that can be obtained from these kinds of studies, the more established and routine will become the rules for approving NPs. However, a consistent theme is that NPs should be designed to leave the body quickly and intact, leaving only footprints in the form of images.

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Fig. 1. Nano's footprints.

The nanoparticle created by Phillips *et al.* (1) is composed of a silica shell encapsulating a Cy5 fluorophore (the "C dot"). PEG is bound to the nanoshell on one end and ¹²⁴I-cRGDY on the other. After injection, most of the hybrid optical-PET imaging agent was excreted intact by the kidneys and bladder. The liver catches larger or uncoated nanoparticles, so this was not a major tissue for ¹²⁴I-cRGDY-PEG-C dot accumulation. Small amounts of the tracer were detected in the thyroid (free iodine) and GI tract. The biodistribution [adapted from (1)] demonstrates that the urinary tract is the dominant route of excretion, which is favorable for translation.