Exposing the tumor microenvironment: how gold nanoparticles enhance and refine drug delivery

*“Cancer will be treated as a medical disease first, treating tumors *in situ*, and then using surgery to remove any residual cancer.”*

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In recent years, there has been a surge of interest in nanoparticle-based drug delivery, with a particular focus on cancer chemotherapy. Among the many nanoparticle substrates that have been developed, gold nanoparticles have several advantages: they are chemically stable and biocompatible, they can be prepared in a range of sizes, and they can easily be functionalized by taking advantage of the stability of the gold–sulfur bond [1,2]. The key question however is “do gold nanoparticles provide a therapeutic benefit over other nanoparticle constructs to enhance tumor drug delivery?” Although gold and other nanoparticle-based cancer medicines (nanomedicines) carrying potent chemotherapies may improve the biodistribution of these drugs, the fundamental question is whether these new nanomedicines are more effective in killing the cancer cells within the tumor microenvironment. Sadly, the answer has often been no.

In a number of high profile clinical trials using biodegradable polymers, micelles or liposomes, the clinical benefit to patients has been marginal, at best, and in some studies the responses have been less effective than the native chemotherapy itself [3,4]. Such disappointing results have led some to question whether there is a real benefit of nanotechnology-based medicines in the treatment of

cancer patients. This commentary offers the insight that the key to effective nanoparticle drug delivery is exposing the tumor microenvironment using a vascular disrupting agent (VDA) in combination with a potent chemotherapy, and that gold nanoparticles are uniquely suited to deliver this one-two punch to the tumor.

The challenge & opportunity: intratumor fluid pressure & VDAs

A primary challenge in treating cancer is often the collateral damage that occurs to healthy organs and tissues when treated with potent cytotoxic drugs. Targeting tumors with nanoparticle-based therapies has been touted as a way of eliminating these deleterious side effects [5]. The primary reason for this expectation is that, unique in the body, the new blood vessels that support tumor growth have fenestrations anywhere from 200 to 400 nm in size. Consequently, the enhanced vascular permeability caused by these fenestrations has been thought to enhance retention of nanoparticle-based therapies at the site of tumors, resulting in improved safety of potent, but toxic cancer therapies; this is the so-called enhanced permeability retention (EPR) effect [6,7]. However, the benefits of the EPR effect may not be as large as had been thought, and are now estimated to account for a less than twofold increase in delivery of anticancer drugs to tumors [8]. The overall benefits of nanoparticle delivery have been questioned in a recent paper

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that reviewed the nanoparticle literature over the last 10 years and concluded that the median delivery efficiency of nanoparticles is low, with only 0.7% of the injected dose ending up in the tumor [9]. Although this study has been criticized, and drug delivery efficiencies may be “*closer to 10% than 0.7% for nanomedicines that have been approved or are in clinical trial*” [10], the fact remains that nanomedicines that rely on the EPR effect alone have not been notably successful.

A consequence of this enhanced tumor vascular permeability is a significant increase in osmotic pressure in solid tumors [11,12]. This resultant high tumor interstitial fluid pressure (IFP) creates a physical barrier that limits the ability of systemically administered cancer therapies from reaching their target, whether it is a check point inhibitor or the cancer cells themselves. This problem is even greater for nanomedicines, since nanoparticle-based drugs are much larger than a typical active pharmaceutical agent alone.

To address this problem, VDAs have been developed that are designed to disrupt the tumor neovasculature, resulting in a reduction of tumor IFP. By reducing IFP, the penetration of nanomedicines, traditional chemotherapies and even the newer immunotherapies into the tumor microenvironment would dramatically improve, which should lead to increased treatment efficacy for all classes of cancer drugs.

These VDAs fall into two classes: those that rely upon local cytokine activity, primarily that of TNF α , and those that bind to the colchicine-binding site of β -tubulin to destroy tumor blood vessels [13]. However, to date none of these agents has been approved for cancer treatment, due in part to side effects that may be related to the agent's activity on healthy blood vessels. For maximal safety and efficacy, such agents need to be targeted to tumors. It is here that gold nanoparticles play a vital role, as exemplified by the gold nanoparticle-based cancer nanomedicine, CYT-6091.

A gold nanoparticle platform

The core of CYT-6091 is a 27 nm gold nanoparticle to which TNF and thiolated PEG are individually bound [14]. Because TNF is decorated on the surface of the gold nanoparticles, this construct takes advantage of both the EPR effect and TNF's biological action on blood vessels by limiting its biodistribution solely to the tumor microenvironment. In a Phase I clinical study of CYT-6091, the first patient treated with the lowest dose of CYT-6091 developed a high fever that was completely eliminated by antipyretic pretreatment. In contrast, patients treated with doses of TNF formulated as CYT-6091, even tenfold higher than the first dose administered, experienced no hypotension, the dose-limiting toxic effect of TNF [15]. In addition,

core biopsies of tumors and adjacent healthy tissue from these patients showed the gold nanoparticles in the tumor, but not in adjacent healthy tissue. These observations suggest that in the circulation CYT-6091 separates the clinically manageable pyrogenic effect of TNF, a blood effect, from its dose-limiting toxic hypotensive effect on blood vessels (its VDA activity) and that CYT-6091 traffics to solid tumors, avoiding healthy tissue and organs. In this way, CYT-6091 harnesses the anti-tumor therapeutic potential of TNF without inducing its severe life-threatening side effects.

Combinational cancer therapy

Similar to other blood vessel cancer therapies, such as bevacizumab (Avastin®), simply destroying or inhibiting the vascular blood supply to a tumor is not sufficient to kill tumors. The ideal cancer therapy would destroy the high tumor IFP microenvironment, while delivering a potent chemotherapy such as paclitaxel (Taxol®) directly to tumors, avoiding healthy organs and tissues. The construct CYT-21625 has been developed to meet these criteria. CYT-21625 is comprised of TNF and an analog of paclitaxel independently bound to a pegylated gold nanoparticle [16].

With the larger, hydrophilic thiolated PEG absorbing water, while the smaller, hydrophobic paclitaxel analog excluding it, a multilayered nanoparticle is created. With the exclusion of water, the release of paclitaxel from the gold nanoparticles is dramatically limited, with little to no free paclitaxel being measured in blood following intravenous administration of CYT-21625 [16]. Preclinical *in vivo* studies showed that the paclitaxel carried on CYT-21625 is slowly released in the tumor, so this formulation acts as a slow release depot of paclitaxel [16]. CYT-21625 is thus the ideal nanomedicine construct, relying upon the tumor targeting of TNF to cause vascular disruption, while limiting the biodistribution of paclitaxel to tumors, sparing healthy organs and tissues from its effects and enabling the intracellular action of paclitaxel to kill cancer cells in the tumor.

Ideal preclinical cancer model

Demonstrating the potential clinical benefit of CYT-21625 in a preclinical model is most clearly seen in a genetically engineered mouse model of neuroendocrine pancreatic cancer, *MEN1* KO (PNET). By developing pancreatic cancer over a 6–12 month period of time, these animals also develop the tumor microenvironment that most closely resembles that seen in pancreatic cancer patients. PNET mice treated with CYT-21625 exhibited a significant increase in tumor-specific vascular leak as measured *in vivo* by dynamic contrast enhanced magnetic resonance imaging (DCE-MRI),

corresponding to a significant drop in the elevated insulin levels that is a consequence and a hallmark of these tumors. These effects were not seen with equivalent doses of either paclitaxel or TNF treatment alone [NILUBOL N, ZIQUANG Y, PACIOTTI GF *ET AL.* UNPUBLISHED DATA, 2016].

Similarly, PNET mice treated with CYT-6091 exhibited a significant increase in tumor-specific vascular leak as measured *in vivo* by DCE-MRI, which corresponded to a sixfold increase in tumor paclitaxel levels, when paclitaxel was administered hours after CYT-6091 pretreatment, compared with the same dose given alone [ZIQUANG Y, LIBUTTI SK, UNPUBLISHED DATA, 2016]. Taken together the data for CYT-6091 and CYT-21625 demonstrate the clear benefit of coupling chemotherapy treatment with prior VDA treatment. In other words, these data suggest that the vascular leakiness attributable to TNF improves the response to chemotherapy.

Future perspective

Using the biology of the tumor neovasculature to safely deliver TNF bound to immune avoiding pegylated gold nanoparticles will lead to a change in strategy for

the treatment of solid tumors. Cancer will be treated as a medical disease first, treating tumors *in situ*, and then using surgery to remove any residual cancer. In effect, by destroying the high tumor IFP, the protective tumor microenvironment will be destroyed, exposing more cancer cells to potent antiproliferative agents. For the cancer patient, such a strategy may lead to less complicated surgeries, less time in hospital and improved outcomes, resulting in a dramatic reduction in healthcare costs associated with treating cancer.

Financial & competing interests disclosure

L Tamarkin is the President and CEO of CytImmune Sciences Inc., a company with a gold nanoparticle-based medicine in development. D Kingston owns stock options in CytImmune Sciences, Inc. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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