Cancer Nanomedicines: Opportunities and Challenges


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ABSTRACT

The inherent leakiness of the tumor neovasculature allows for improved biodistribution of systemically administered nanomedicines. However, the high interstitial fluid pressure (IFP) created by these leaky tumor blood vessels may also prevent such constructs, as well as systemically administered chemotherapy or immunotherapy, from being maximally effective. One way to improve the efficacy of anti-cancer agents would be through the elimination of the high tumor IFP. CYT-6091, a nanomedicine comprised of tumor necrosis factor alpha (TNF) bound to PEGylated gold nanoparticles, has been shown to cause vascular leak in two preclinical tumor models. Associated with that vascular leak was a significant reduction in the high tumor IFP in implanted tumors, and a 6-fold increase in paclitaxel accumulation inside naturally occurring tumors in genetically engineered mice. CYT-6091 has been successfully tested as a single agent in a phase I clinical trial in advanced-stage cancer patients. Success in a CYT-6091 plus chemotherapy phase II study will demonstrate that cancer patients should be treated with CYT-6091 prior to chemotherapy in order to reduce tumor size in situ, potentially leading to less complicated follow-up surgery, less time in hospital, improved patient outcomes, and ultimately reduced healthcare cost associated with treating patients with cancer.

Keywords: tumor interstitial fluid pressure, tumor vascular disrupting agent, tumor necrosis factor alpha, cancer, nanomedicine

1 THE OPPORTUNITY

The promise of cancer nanomedicines, constructs between 10-100 nm in diameter, has for decades been that these engineered drugs would enable the systemic administration of toxic, but potent chemotherapies, vastly reducing or eliminating their debilitating side effects [1]. The reasoning was that by design, cancer patients could be dosed with higher levels of chemotherapy because these constructs would deliver their payloads to tumors and avoid healthy tissues and organs. This expectation was based on a myriad of anatomical descriptions of the unique biology of the newly formed blood vessels that support tumor growth, be it a primary or a metastatic tumor [2]. Unique in the body, the tumor neovasculature has fenestrations, actual pores, ranging in size from 0.2 to 1.2 µm, which would allow nanomedicines to exit the circulation and remain in the tumor. This process has been termed the “enhanced permeability and retention” effect (the EPR effect) [3].

Is the EPR effect limited only to administered nanomedicines?

2 THE CHALLENGE

Since many blood factors are smaller than 100 nm, they too can exit the circulation through the tumor neovascular fenestrations. [As a point of reference, red blood cells are 8 µm in size.] And, since the blood vessels on the periphery of tumors do not have such fenestrations, tumors develop a microenvironment wherein the osmotic pressure inside the tumor is greater than its surroundings [4]. This high interstitial fluid pressure (IFP) presents a physical barrier that inhibits systemically administered cancer therapies, whether small molecule therapeutic, nanomedicines or biologics, from penetrating the tumor microenvironment to reach their respective targets, the cancer cells, or immune regulatory factors. Consequently, chemotherapies, for example, are significantly less effective than would be predicted from in vitro and in vivo preclinical studies.

The solution to this problem is to eliminate the high tumor IFP created by the leaky tumor neovasculature, opening up the tumor microenvironment to subsequently administered therapeutics. The challenge has been that agents that alter or destroy blood vessels do not discriminate between healthy and tumor blood vessels. The potentially life-threatening side effects of such systemically administered agents have thus far prevented their systemic use. However, in patients with in-transit tumors on their limbs, surgical oncologists in Europe are routinely isolating that limb, hooking its major blood supply to a heart-lung machine, and infusing an otherwise toxic dose of the cytokine, tumor necrosis factor alpha (TNF) followed by chemotherapy [5]. In a variety of tumor indications, one treatment has been shown to result in dramatic local tumor shrinkage in approximately 85% of cancer patients [5]. This dramatic success has been attributed to the biologic
action of TNF, which causes apoptosis of vascular endothelial cells, resulting in a significant drop in tumor IFP, allowing more follow-on chemotherapy to sequester in the tumor, which in turn results in better tumor regression [5]. The challenge has been to deliver TNF systemically at a therapeutic dose, approximately 1 mg that avoids its life-threatening side effects.

Numerous clinical trials conducted with human recombinant TNF have shown that the maximum tolerated dose of systemically administered TNF is approximately 0.4 mg per dose [6]. With higher doses, patients experience vascular leak that requires medical treatment, with some patients experiencing the constellation of clinical symptoms consistent with septic shock.

Could a nanoparticle formulation solve this problem?

3 A NANOMEDICINE SOLUTION

We have designed a nanomedicine platform (Figure 1), whose core is a 27 nm particle of gold that is decorated with TNF and a linear form of polyethylene glycol (PEG) with a distal thiol group (PEG-Thiol) [7]. Both molecules bind independently to the gold nanoparticles through available thiols forming a dative covalent bond. The resultant TNF-bound, PEGylated gold nanoparticle drug has been assigned the name, CYT-6091.

![Figure 1. Blueprint of CYT-6091. CYT-6091 contains TNF and PEG-Thiol independently bound to 27 nm gold nanoparticles (NP).](image)

Each element of this construct serves a specific purpose. Gold nanoparticles were chosen as the platform to carry TNF because of their long clinical history of being used to safely treat patients with rheumatoid arthritis and on the well-known chemistry of gold nanoparticles binding thiol-containing molecules almost instantaneously, forming a dative covalent bond. Consequently, the gold nanoparticles are an ideal scaffold to bind TNF onto the particles’ surface. Rather than being packaged inside a liposome or a biodegradable polymer, each molecule of TNF bound to the gold nanoparticles’ surface is biologically active. However, we have also shown that simply binding TNF to 27 nm gold nanoparticles is not sufficient for targeting tumors, since this construct was rapidly taken up by the liver and spleen, the major organs of the monocyte phagocyte system (MPS) [7].

As shown in Figure 1, since PEG-Thiol is hydrophilic, these linear pieces of PEG-Thiol absorb water, forming a “water shield” around the gold nanoparticle, and in so doing prevent opsonization and immune recognition. Also, as drawn in Figure 1, the size of the PEG-Thiol polymer was chosen so that the active binding site of the gold-bound TNF is still exposed, allowing TNF to be active while bound to the gold nanoparticles [7].

The gold-bound TNF serves two functions on CYT-6091. First, with the passive extravasation of CYT-6091 through the tumor neovasculature (the EPR effect), the gold-bound TNF binds to TNFR1 receptors found on the endothelial cells that comprise the tumor neovasculature. Once TNF binds to its receptor, TNF then exerts its biologic action; apoptosis of these endothelial cells, destroying these blood vessels. Thus, TNF on CYT-6091 serves as an active tumor targeting ligand and as a vascular disrupting agent (a VDA).

We hypothesize that to be effective in treatment of patients with cancer, systemically administered CYT-6091 needs to achieve a dose of 1 mg of TNF. CYT-6091 was first tested as a single agent in a phase I dose escalation clinical trial in advanced-stage cancer patients [8]. The first patient treated in this study, given 0.1 mg of TNF formulated as CYT-6091, developed a fever of 40°C within 30 minutes. However, prior to this patient’s second treatment, the patient was pretreated with antipyretics that completely eliminated this post-treatment fever. Patients treated with doses as high as 1.2 mg of TNF formulated as CYT-6091, exceeding the 1 mg target dose, did not develop hypotension, the dose-limiting toxic effect of TNF. In addition, in core biopsies of tumors and adjacent healthy tissues from these patients, gold nanoparticles were seen 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 hypertensive 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.
A CYT-6091 phase Ib/II clinical trial that mimics the ILP protocol, pre-treating with CYT-6091 followed by standard-of-care chemotherapy is the next step in clinical development. Choosing the cancer indication has been guided by studies using murine tumor models and two genetically engineered mouse models (GEMMs) of pancreatic cancer, the MEN1 knockout (KO) for pancreatic neuroendocrine tumor (PNET) and Kras/p53 for pancreatic ductal adenocarcinoma (PDAC). Each murine model system provided specific data to support our hypothesized mechanism of action of CYT-6091 as a VDA.

For example, in the 4T1 murine mammary tumor model, a significant drop in tumor IFP was seen following CYT-6091 treatment [9], while in MC-38 murine colon carcinoma tumors, tumor specific vascular leak was observed [10]. However, we were concerned that the tumor vasculature supporting these implanted murine cancer cell tumors might not reflect the tumor vascular network found in naturally occurring tumors. We reasoned that GEMMs are the ideal models to evaluate the effect of CYT-6091 on the tumor vasculature.

As shown in Figure 2, using dynamic contrast enhanced magnetic imaging resonance, CYT-6091 treatment caused significant vascular leak in PNET tumors, not seen in muscle or in PBS-treated control animals.

**Figure 2. Imaging vascular effect of CYT-6091 by dynamic contrast enhanced magnetic resonance imaging (DCE-MRI) in PNET GEMMs.** The signal enhancement curve was used for quantifying the tumor vasculature and control muscle vasculature by DCE-MRI. The first 6 time-points are pre-injection of Gd-DTPA; the next 80 are post-injection of Gd-DTPA. The data are from the pancreatic and control muscle regions of Men1 KO mice at 24 h of post-injection (Red curve) and baseline (Green curve). The DCE-MRI images show that the tumor vascular leakage is significantly increased after CYT-6091 treatment (0.25 mg/kg) compared to baseline in Men1 KO mice, but no effect is seen in the mice after PBS treatment and there is minimal effect seen in the muscle tissues.

Further, we hypothesized that this increase in vascular leakiness would enable more follow-on chemotherapy to accumulate in the tumor. As seen in Figure 3, there was a 6-fold increase in the tumor levels of paclitaxel in PNET tumors after administration of CYT-6091 22 hr before paclitaxel compared with paclitaxel alone. Based on these preclinical data, a phase II study will be conducted in patients with pancreatic cancer, combining standard-of-care chemotherapy ± CYT-6091.

**Figure 3. Accumulation of paclitaxel in PNET GEMMs by LC-MS/MS.** Mean concentrations of paclitaxel in tumors after administration of PBS control (n=2), CYT-6091 alone at 0.25 mg/kg IV x1 (n=2), paclitaxel alone at 9 mg/kg IV x 1 (n=2), and paclitaxel at 9 mg/kg IV x 1 22h after CYT-6091 at 0.25 mg/kg IV x 1 (n=2). Studies were performed in Men1 KO mice and tumor samples were obtained 6 hours after paclitaxel treatment. Mean concentrations of paclitaxel in tumors were approximately 6-fold higher with CYT-6091 pretreatment compared with paclitaxel alone.

The ideal cancer nanomedicine would increase the delivery of TNF and chemotherapy to tumors, eliminating potential deleterious side effects from the systemic administration of TNF and the chemotherapy. Recently, the construct CYT-21625 has been developed that delivers both...
TNF and paclitaxel directly to tumors, with little to no free paclitaxel found in blood [11]. Since native paclitaxel does not bind to gold nanoparticles, a thiolated analog was created that releases native paclitaxel in the tumor. So, CYT-21625 further advances the ideal characteristics of nanomedicines in cancer treatment, using the tumor targeting and tumor vascular disrupting action of TNF coupled with the cytotoxic effect of paclitaxel on the cancer cells residing in the same areas of the tumor microenvironment to provide a “one-two punch” against cancer.

4 IN CLOSING

Using the biology of the tumor neovasculature to safely deliver TNF bound to PEGylated gold nanoparticles (CYT-6091) will lead to a change in strategy for the treatment of solid tumors. We envision that 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 eliminating the high tumor IFP, the protective tumor microenvironment will be destroyed, exposing more cancer cells to potent anti-proliferative agents. For the cancer patient, such a strategy may lead to less complicated surgeries and less time in hospital. In addition, since cancer often times recurs, treating cancer as a chronic disease, periodically treating with CYT-6091 to destroy the tumor neovascularization and suppress tumor growth may enable cancer patients to live with their disease. Overall, improved patient outcomes and survival will result in a dramatic reduction in healthcare costs associated with treating cancer.

5 REFERENCES