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Synergy in cancer treatment between liposomal chemotherapeutics and thermal ablation

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Abstract

Minimally-invasive image-guided tumor ablation using short duration heating via needle-like applicators using energies such as radiofrequency or microwave has seen increasing clinical use to treat focal liver, renal, breast, bone, and lung tumors. Potential benefits of this thermal therapy include reduced morbidity and mortality compared to standard surgical resection and ability to treat non-surgical patients. However, improvements to this technique are required as achieving complete ablation in many cases can be challenging particularly at margins of tumors > 3 cm in diameter and adjacent to blood vessels. Thus, one very promising strategy has been to combine thermal tumor ablation with adjuvant nanoparticle-based chemotherapy agents to improve efficiency. Here, we will primarily review principles of thermal ablation to provide a framework for understanding the mechanisms of combination therapy, and review the studies on combination therapy, including presenting preliminary data on the role of such variables as nanoparticle size and thermal dose on improving combination therapy outcome. We will discuss how thermal ablation can also be used to improve overall intratumoral drug accumulation and nanoparticle content release. Finally, in this article we will further describe the appealing off-shoot approach of utilizing thermal ablation techniques not as the primary treatment, but rather, as a means to improve efficiency of intratumoral nanoparticle drug delivery.

Introduction

Our perspective and identification of a robust and exciting paradigm that incorporates liposomal nanotechnology for the treatment of cancer stems from our many years advancing and exploring minimally-invasive, image-guided thermal tumor ablation (1). This methodology is based upon coagulating tumor using short duration heating [< 15 min] by

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directly applying temperatures $> 50^{\circ}\text{C}$ via needle-like applicators using energies such as radiofrequency or microwave to treat focal liver, renal, breast, bone, and lung tumors (2). Potential benefits of this thermal therapy include reduced morbidity and mortality compared to standard surgical resection and ability to treat non-surgical patients (3). However, clinicians have been unable to achieve complete ablation in many cases, particularly at tumor margins and adjacent to blood vessels, presenting substantial barriers toward clinical efficacy (3). Thus, one very promising strategy has been to combine thermal tumor ablation with adjuvant nanoparticle-based chemotherapy agents to improve ablation efficiency (4). Here, we will primarily review principles of thermal ablation to provide a framework for understanding the mechanisms of combination therapy, review currently available studies on combination therapy, and include preliminary data on the role of such variables as nanoparticle size and thermal dose on improving combination therapy outcome. We will discuss how thermal ablation strategies can be used to improve overall intratumoral drug accumulation and nanoparticle content release. Additionally, we have further shown that this increased tumor destruction is in part due to intratumoral drug accumulation. Thus, in this article we will further describe the interesting off shoot approach of utilizing thermal ablation techniques not as the primary treatment, but rather, as a means to improve efficiency of intratumoral drug delivery.

Overview of focal tumor ablation thermal therapy as a treatment modality for focal malignancies

Traditionally, local tumor removal has required major surgery. Over the last decade, improvements in imaging technologies have enabled the development of minimally-invasive thermal tumor ablation that uses imaging guidance for the accurate percutaneous placement of needle-like applicators (1). Tumor destruction for these methods relies primarily upon subjecting the entire tumor volume to cytotoxic temperatures that induce tumor coagulation and necrosis from energy sources such as: radiofrequency (RF), microwave, ultrasound, and laser (1). Potential benefits of image-guided ablation of focal neoplasms compared to conventional surgical options include: 1) the ability to place needle electrodes using imaging guidance and without the need for large (or any) incision; 2) the ability to ablate and/or palliate tumors in non-surgical candidates; 3) substantially reduced morbidity, lower costs, and improved quality of life; and 4) performance of these procedures on an outpatient basis (1, 2, 5).

Historically, the greatest attention has been given to the clinical potential of image-guided ablation procedures for the treatment of colorectal metastases to the liver and to primary liver tumors due to the significant morbidity and mortality of standard surgical resection, combined with the large number of patients who cannot tolerate such radical surgery (6, 7). Indeed, over 100,000 RF ablation procedures for treating focal liver tumors have been performed over the last ten years. This fact alone (i.e., rapid adoption without complete validation and comparative outcome studies attests to both the scope of the clinical need and the less than ideal treatment options for these patients. More recently, the important clinical potential of these techniques has been reported for the treatment of neoplasms in other sites, including the kidney ($>1,000$ cases), breast, bone, lung, and retroperitoneum (2).

Regardless of the energy source used, the traditional (and current clinical) endpoint of thermal ablation is adequate tissue heating so as to induce coagulative necrosis throughout the defined target area. Relatively mild increases in tissue temperature above baseline (40°C) can be tolerated by normal cellular homeostatic mechanisms. Low temperature hyperthermia (42-45°C) results in reversible cellular injury, though this can increase cellular susceptibility to additional adjuvant therapies such as chemotherapy and radiation (8, 9) [FIG. 1A-B]. Irreversible cellular injury occurs when cells are heated to 46°C for 60 min, and occurs more rapidly as the temperature rises, so that most cell types die in a few minutes when heated at 50°C (10). Immediate cellular damage centers on protein coagulation of cytosolic and mitochondrial enzymes, and nucleic acid-histone protein complexes, which triggers cellular death over the course of several days (11). The exact temperature at which cell death occurs is multifactorial and tissue specific. Based upon prior studies demonstrating that tissue coagulation can be induced by focal tissue heating to 50°C for 4-6 min (12), this has become the standard surrogate endpoint for thermal ablation therapies in both experimental studies and in current clinical paradigms. However, studies have shown that depending on heating time, the rate of heat increase, and the tissue being heated, maximum temperatures at the edge of ablation are variable. For example, maximum temperatures at the edge of ablation zone, known as the “critical temperature,” have been shown to range from 30°C to 77°C for normal tissues and from 41°C to 64°C for tumor models (a 23°C difference) (13). Likewise, the total amount of heat administered for a given time, known as the thermal dose, varies significantly between different tissues (13). Thus, the threshold target temperature of 50°C should be used only as a general guideline.

The primary goal of most ablation procedures is to eradicate all viable malignant cells within a designated target volume. Based upon examinations of tumor progression in patients undergoing surgical resection and the demonstration of viable malignant cells beyond visible tumor boundaries, in most cases (or except when otherwise indicated) tumor ablation therapies attempt to include at least a 0.5-1.0 cm “ablative” margin of seemingly normal tissue for liver and lung, though less may be needed for some tumors in the kidney (14, 15). However, this cannot be reliably achieved for tumors > 2.5 cm in diameter with current technology using a single application of RF due to technologic and bio-physical limitations that retard uniform heating of the entire tumor volume to temperatures sufficient for inducing coagulation (2, 3). This necessitates multiple treatments to adequately treat the tumor. However, clinical strategies based upon multiple applications of RF have met with variable success, and are otherwise unattractive given that they can be extremely labor and time intensive, expensive, and prolong the duration of therapy (2, 5). Thus, further increases in thermal ablation efficiency are necessary to benefit greater numbers of patients and to treat larger tumors.

Overcoming existing limitations of RF tumor ablation – the rationale for combining thermal ablation with adjuvant chemotherapy

While substantial efforts have been made in modifying ablation systems and the biologic environment to improve the clinical utility of percutaneous ablation, limitations in clinical efficacy persist. Clinical studies using RF ablation for liver and kidney tumors report that

local tumor control can be achieved in 80-90% of cases where the tumors measure less than 2.5 cm in diameter (16). However, current thermal ablation techniques are proving less satisfactory for the treatment of larger tumors. For example, one study of RF ablation of hepatocellular carcinoma reports local recurrence in 65% of tumors greater than 3.5 cm in diameter, and in 75% of tumors larger than 5 cm (3). For the percutaneous RF ablative treatment of colorectal metastases, local recurrences of 35-89% have been reported for tumors greater than 2.5 cm (17-19). Additionally, with further long-term follow-up of ablation therapy, there has been an increased incidence in detection of progressive local tumor growth for all tumor types and sizes despite initial indications of adequate therapy (2, 3, 14, 17, 20). This suggests that there are residual patches of untreated disease in a substantial, but unknown number of cases, a result that falls far short of our goal of completely eradicating all tumor treated by RF ablation. Therefore, current strategies have evolved from this need to increase the completeness of RF tumor destruction, even for small lesions. More recent avenues of investigation have focused on the potential gains in tumor destruction that can be achieved by combining tumor ablation with adjuvant chemotherapy or radiation. The rationale for the potential synergy of these traditionally separate treatment paradigms is based upon several key concepts.

Firstly, conventional thermal ablation only takes advantage of temperatures that are sufficient by themselves to induce coagulation necrosis ($>50^{\circ}\text{C}$). Yet, based upon the exponential decrease in RF tissue heating there is a steep thermal gradient in tissues surrounding an RF electrode. Hence, there is substantial flattening of the curve below 50°C , with a much larger tissue volume encompassed by the 45°C isotherm. Modeling studies demonstrate that were the threshold for cell death to be decreased by as few as 5°C , tumor coagulation could be increased up to 1.5 cm (upto a 59% increase in spherical volume of the ablation zone) (21). Therefore, target tumors can be conceptually divided into three zones: 1) a central area, predominantly treated by thermal ablation, which undergoes heat-induced coagulation necrosis, 2) a peripheral rim which undergoes reversible changes from sub-lethal hyperthermia, and 3) surrounding tumor or normal tissue that is unaffected by focal ablation, though still exposed to adjuvant systemic therapies (1, 21) [FIG. 2].

Secondly, combination therapy likely increases tumor cell death through a 'two-hit' exposure of sub-lethal hyperthermia and chemotherapy or radiation-induced injury. Several studies have demonstrated that tumor death can be enhanced when combining RF thermal therapy with adjuvant chemo- or radiosensitizers (4, 22, 23). This combined approach targets the sizable peripheral zone of tissue exposed to sub-lethal, temperatures (i.e., largely reversible cell damage induced by mildly elevating tissue temperatures to $41-45^{\circ}\text{C}$) surrounding the heat-induced coagulation (4). Improved tumor cytotoxicity is also likely to reduce the local recurrence rate at the treatment site. Although there is high temperature heating throughout the zone of RF ablation, heterogeneity of thermal diffusion (especially in the presence of vascularity) retards uniform and complete ablation (24). Since local control requires complete tumor destruction, ablation may be inadequate even if large zones of ablation that encompass the entire tumor are created. By killing tumor cells at lower temperatures, this combined paradigm will not only increase necrosis volume, but may also create a more complete area of tumor destruction by filling in untreated gaps within the ablation zone (25). Combined treatment also has the potential to achieve equivalent tumor

destruction with a concomitant reduction of the duration or course of therapy (a process which currently takes hours to treat larger tumors, with many protocols requiring repeat sessions). A reduction in the time required to completely ablate a given tumor volume would permit patients with larger or greater numbers of tumors to be treated. Shorter heating time could also potentially improve their quality of life by reducing the number of patient visits and the substantial costs of prolonged procedures that require image guidance.

Thirdly, increased understanding of the effects of ablation at a cellular level has and will continue to lead to combination therapies with adjuvant agents that potentiate pro-apoptotic pathways and maximize tumor destruction. For example, several recent studies combining RF ablation with pro-apoptotic agents such as paclitaxel, or anti-heat shock protein agents such as quercetin have increased tumor coagulation and animal endpoint survival in small animal tumor models (26, 27). In particular, as newer agents, such as antiangiogenic drugs, are developed to target specific molecular pathways and become available, many of these may have specific uses when combined with ablative therapies (28, 29).

RF ablation and adjuvant doxorubicin – an early model for combination therapy

Given the potential synergy of combining therapies, an early preliminary study administered RF ablation in conjunction with percutaneous intratumoral injection of free doxorubicin in a rat breast adenocarcinoma model (2). Tumor destruction 48 hr following the last intervention measured 6.7 ± 0.6 mm for RF alone, and doxorubicin alone produced only 2-3 mm of necrosis ($p < 0.01$). However, increased coagulation was observed with combination doxorubicin and RF therapy (11.4 ± 1.1 mm; $p < 0.001$), confirming that combining thermal ablation with direct intratumoral drug injection can increase tumor coagulation. However, although initial image-guided tumor ablation strategies sought to increase local drug delivery by direct injection (30, 31), this approach has encountered many difficulties in clinical practice that have led to its disfavor. Many have reported difficulty in achieving uniform diffusion of percutaneously injected drugs such as ethanol and doxorubicin over larger tumor volumes (32). Intravascular direct catheter injection of highly concentrated chemotherapy has also been tried, but has had limited clinical adoption due to the substantially invasive nature of surgical or angiographic device implantation (33, 34). Hence, the use of a straightforward IV chemotherapeutic delivery route would be potentially clinically beneficial - were sufficient intratumoral drug delivery and/or accumulation possible without inducing systemic toxicity [FIG. 3].

These limitations, combined with the development and availability of preparations in which chemotherapeutic drugs are encapsulated in nanoparticle delivery vehicles, led to the incorporation of adjuvant liposomal doxorubicin (Doxil) into the combination therapy paradigm (4, 21, 35). Liposome particles are completely biocompatible, cause very little toxic or antigenic reaction and are biologically inert. Water-soluble drugs can be trapped in the inner aqueous compartment, whereas lipophilic compounds may be incorporated into the liposomal lipid membrane. Incorporation into liposomes protects the drug from the destructive environment in vivo. Some liposomes are capable of delivering their drug load inside the cell and even inside different cell compartments (36). The primary drawback of

initial liposomal preparations, (i.e., their elimination from the systemic circulation by the reticuloendothelial system (RES) due to rapid opsonization (37) has now been substantially overcome by surface-modification using flexible hydrophilic polymers such as polyethylene glycol (36-44) to prevent plasma protein absorption on liposome surfaces and subsequent recognition and uptake of liposomes by the RES. Hence, touted benefits for the use of these “stealth” liposomal carriers include reduced systemic phagocytosis and a resultant prolonged circulation time, selective agent delivery through the leaky tumor endothelium (an enhanced permeability and retention effect), as well as reduced toxicity profiles (42, 45). As a result, this doxorubicin-containing formulation is widely accepted for clinical practice (46-48).

Subsequent studies combined RF ablation with adjuvant liposomal doxorubicin. Experiments in a rat breast adenocarcinoma have demonstrated significant increases in mean tumor coagulation diameter from combination RF/Doxil therapy (13.1 mm) compared to RF alone (6.7 mm) (49). Confirmatory studies performed in different models demonstrated similar gains in overall ablation-induced tumor coagulation. For example, in a large animal canine subcutaneous sarcoma model, combination therapy, increased mean tumor coagulation diameter from 23 mm with RF alone to 37 mm with RF/Doxil, representing an increase of 212% in necrosis volume, with most of the gain occurring in the larger peripheral periablation zone (21) [FIG. 4].

Similarly, increases in intratumoral drug accumulation with combination therapy were also observed (49, 50). Studies in both small and large animal models have demonstrated up to a 5.6 fold increase in intratumoral doxorubicin accumulation following RF ablation, with 1) the greatest amount of intratumoral doxorubicin occurs in the zone immediately peripheral to the central RF area, and 2) smaller amounts of doxorubicin were found in the central RF-coagulated area suggesting drug deposition in areas with residual, patent vasculature (50). These findings help explain why liposomal doxorubicin is likely to be complementary to RF ablation. The majority of the liposomes concentrated in a zone immediately peripheral to the area coagulated by RF heating and were within the region where non-lethal hyperthermia and increased destruction is observed (50). Additionally, the patchy penetration of liposomes into the zone of coagulation implies infiltration of chemotherapy into the coagulated focus (possibly through residual patent vessels) that may improve the completeness of tumor destruction.

Finally, these increases in intratumoral drug accumulation and tumor coagulation translated into gains in both animal survival/tumor growth studies and in preliminary clinical studies. D’Ippolito et al. reported increases in animal endpoint survival for rat R3230 adenocarcinoma tumors treated with combined RF/IV liposomal chemotherapy (28 days) compared to either RF or IV liposomal chemotherapy alone (18 days each) (49) [FIG. 5]. In a pilot clinical study combining RF ablation (internally-cooled electrode) with adjuvant liposomal doxorubicin, 10 patients with 18 intrahepatic tumors were randomized to receive either liposomal doxorubicin (20 mg Doxil) 24 hr prior to RF ablation or RF ablation alone (mean tumor size undergoing ablation was 4.0 ± 1.8 cm) (51). While no difference in the amount of tumor destruction was seen between groups immediately following RF, at 2-4 weeks, patients receiving liposomal doxorubicin had an increase in tumor destruction of 24% to 342% volumetric increase (median=32%) compared to a decrease of 76-88% for

treated with RF alone (a finding concordant with prior observations). Several additional and clinically beneficial findings were also observed only in the combination therapy group, including increased diameter of the treatment effect for multiple tumor types, improved completeness of tumor destruction particularly adjacent to intratumoral vessels, and increased treatment effect including the peritumoral liver parenchyma (suggesting a contribution to achieving an adequate ablative margin) [FIG. 6A-D].

Moving beyond RF ablation and Doxil – strategies to broaden applicability

While preliminary ‘proof-of-concept’ studies suggest significant potential clinical benefit when combining RF ablation with IV liposomal doxorubicin (51), continued research may provide additional gains in both tumor destruction and intratumoral drug delivery. Regardless, wider clinical applicability and potential gains will likely vary based upon adjuvant agent characteristics, including choice of chemotherapeutic agent and type of nanoparticle delivery vehicle and ablation characteristics.

Understanding mechanisms of synergy

Greater understanding of underlying mechanisms of cellular injury and interaction between thermal ablation and adjuvant therapies is one strategy that may allow development and use of more tailored adjuvant therapies. For combined RF ablation and Doxil, several mechanisms for increased tumor destruction have also been identified, most notably increased cell stress (in part, due to upregulation of nitrative and oxidative pathways) leading to apoptosis (52). Recently, Solazzo et al. performed immunohistochemical staining of rat breast tumors treated with RF ablation with and without adjuvant Doxil for markers of cellular stress (52). In the periablational rim surrounding the ablation zone, combination RF/Doxil increased markers of DNA breakage and oxidative and nitrative stress early (~4hr) after RF ablation, with subsequent co-localization staining for cleaved caspase 3 (a marker for apoptosis), suggesting that these areas later underwent apoptosis [FIG. 6]. N-acetylcysteine (NAC) was also administered in some animals, and reductions in both cellular stress pathways and apoptosis confirmed the causatory relationship between the two processes. Additionally, increased heat-shock protein production in a concentric ring of still-viable tumor surrounding the ablation zone, and immediately peripheral to the rim of apoptosis, was also been observed [FIG. 7A-B].

Adding additional adjuvant chemotherapies

As a next step, recent studies have focused on adding additional adjuvant chemotherapies that specifically target cellular stress pathways. Yang et al. combined RF ablation with IV liposomal paclitaxel, an agent with known pro-apoptotic and anti-heat shock protein effects in subcutaneous rat breast adenocarcinoma tumors (44). Combination RF-paclitaxel increased tumor coagulation and animal survival compared to RF alone, with even greater gains observed for RF-paclitaxel-Doxil. Interestingly, immunohistochemistry demonstrated reduced heat shock protein expression and increased apoptosis for treatment combinations that included paclitaxel [FIG. 8A-G]. Most recently, combining RF ablation with IV liposomal quercetin (a flavanoid agent with known anti-heat shock protein effects) also reduced heat shock protein expression and increased tumor coagulation and survival (44).

Based upon these results, it is becoming clear that judicious selection of the type of chemotherapy combined with thermal therapy is necessary to potentiate and optimize the tumoricidal effects occurring in the peripheral zone of hyperthermia created by RF heating, along with tailored regimens that are tumor and organ type-specific.

Modification of the nanoparticle agent

Modification of the nanoparticle agent is another direction of ongoing research that may permit even greater increases in tumor coagulation and cellular injury. In preliminary studies combining RF ablation with adjuvant liposomal doxorubicin, RF ablation combined with adjuvant empty liposomes also increased coagulation over RF alone but less than RF/Doxil (49). This suggests that the liposomal vehicle (such as the lipid components) itself interacts with sub-lethal hyperthermia in the periablational tumor to induce additional cellular injury or death. Similarly, increased lipid peroxidation has also been observed when RF ablation is combined with empty liposomes over RF alone (4, 35). Prior studies at low temperature hyperthermia (41-43°C) demonstrate increased cytotoxicity in the presence of unsaturated lipids (53-55), which has been ascribed to hyperthermia inducing enhanced oxidative stress and increased lipid peroxidation that could be amplified in the presence of highly oxidizable lipids. Also, the diffusible cytotoxic byproducts that form during lipid peroxidation (lipid hydroperoxides and lipid aldehydes) can also potentially enhance cell killing at the margins during thermal ablation therapy, and these species would also be expected to increase in cells treated with liposomal preparations containing GLA (gamma-linolenic acid) and arachidonic acid (56, 57). Modification of the liposome vehicle to include lipids with increased susceptibility to lipid peroxidation, and therefore greater potential for inducing oxidative damage, has also been performed. GLA-containing liposome preparations resulted in larger amounts of tumor coagulation with RF ablation compared to conventional phosphatidyl-choline preparations (4).

Similarly, modification of the nanoparticle agent size and type may permit even greater intratumoral drug deposition compared to conventional liposomal preparations. Traditionally, 100 nm sized liposomes have demonstrated optimal circulation times due to intravascular stability, higher amounts of drug encapsulation, and the ability to diffuse through the endothelial leakiness observed in tumors, and as such, many currently commercially-available liposomal drugs have been constructed in this size range (58-60). However, our own preliminary data suggests that use of smaller agents may increase the amount of periablational and interstitial drug deposition that can be achieved. In one experiment performed in Fischer 344 female rats (150 ± 10 gm), 40 animals (8 groups, $n=5$ per group) treated with RF ablation (1 cm active electrode tip, 21g needle, titrated to receive 70°C for 5 min) and without RF ablation of normal liver followed by injection (15 min post-ablation) of saline control, 20, 100, or 300 nm fluorescent beads (ultraclean polystyrene microspheres loaded with fluorescent dyes; FluoSpheres, Invitrogen, Grand Island, NY) and were sacrificed at 24 hr post-ablation. Histopathologic and fluorescent microscopy analysis demonstrated minimal uptake of beads in the central ablation zone, but significantly greater uptake in the periablational zone for smaller (20 nm) beads compared to larger sizes ($p<0.05$, FIG. 9A-B). While these preliminary studies with polystyrene microspheres do not differentiate between intra- and extracellular delivery, increased overall interstitial presence

suggests that many barriers to delivery of the drug have been overcome. Thus, continued investigation into use of smaller nanoparticle platforms is warranted. For example, as presented above, initial studies combined RF ablation with liposomal quercetin only partially suppressed protective HSP expression and increased tumor coagulation and apoptosis in the periablational rim. However, IHC co-localization of HSP70 and cleaved caspase 3 demonstrated that despite adjuvant liposomal quercetin, some HSP70 expression persisted in a rim of tissue beyond the zone of liposome deposition suggesting that improvements in drug delivery extending farther into periablational residual tissue may yield additional gains in tumor destruction (27). Therefore, incorporating alternative and even smaller nanoparticle delivery vehicle platforms, such as micelles, may be one potential way to overcome this delivery barrier.

Modification of liposome construction to increase release in low temperature (40-42°C) hyperthermic environments has also been a strategy to improve intratumoral release of nanoparticle contents to improve the available active amount of the chemotherapeutic agent (61, 62). Along these lines, Wells et al. tested a heat-sensitive liposomal drug delivery system in murine CT-26 tumors and documented a 2.5-fold increased liposome concentration in tumors exposed to 42°C compared to tumors at 31°C (63). Similarly, Shimose et al. have reported reduced tumor growth when combining thermosensitive liposomes encapsulated doxorubicin (TL-Dox) with conventional hyperthermia in primary and metastatic hamster osteosarcomas over conventional hyperthermia, alone or combined with non-TL doxorubicin (64). Much of this work has been done primarily at conventional lower levels of hyperthermia, and with administration paradigms that focus on using hyperthermia to facilitate liposomal drug release. A report by Gasselhuber et al. using modeling simulation of liposomal drug release in combination with modeling of RF tissue heating patterns suggests that maximum liposomal drug release occurs around the periablational zone (similar to what has been previously demonstrated with Doxil) (65). More recently, a low temperature sensitive liposomal doxorubicin formulation has been developed and is being tested clinically in combination with RF ablation (66). However, the optimal administration paradigm for this combination therapy is yet unclear. Based upon the timing of anti-HSP and proapoptosis effects that have been observed (4-12 hr) (52), it is far from clear that an additional early burst of drug release (0-1 hr), both spatially and temporally, will definitely increase tumor destruction compared to a slower, more prolonged drug release. In particular, the paradigm of tissue heating (both timing and variances in temperature ranges in the treatment zone) vary significantly between focal high-temperature RF tumor ablation and the low-temperature conventional hyperthermia that has been combined with thermosensitive liposomes in existing literature – such that, it is likely too early to assume that thermosensitive liposomal preparations will definitely be “better” than non-temperature sensitive agents. The need for additional studies identifying the optimal delivery vehicle characteristics and administration paradigms remains.

Pharmacokinetic characteristics, specifically blood circulation times and drug release rates, are also likely key to maximizing gains in combination therapy. Indeed, these are becoming the focus of greater study as different classes of liposomes become available. For example, early or rapid drug release patterns (such as that can occur with the thermally-labile liposomes) could saturate the system and result in greater amounts of the formally

encapsulated chemotherapeutic drug being released into the blood stream (and potentially very low levels of deposition in the periablational zone). In contrast, preparations with very long circulation or release times might result in greater delivery to the periablational zone, but with low or minimal release of active drug in the intratumoral interstitium insufficient to achieve required therapeutic levels. Additionally, the effect of known ablation-induced vascular changes on the rate of intratumoral nanoparticle drug accumulation has not yet been determined. It is possible that nanoparticle formulations, such as micelles, that are traditionally thought to have very short circulation times, may accumulate quickly in tissue around the periablational zone due to hyperthermia-related permeability changes. Finally, the hyperthermic effects generated by ablation may also reduce the required minimum amount of active drug to achieve cytotoxicity (49). For example, in early dosing studies, a range of single adjuvant IV liposomal doxorubicin doses (0.4-13.3 mg/kg) resulted in the same amount of RF tumor coagulation (35, 49). This suggests that traditional understanding of minimum required drug levels, and conversely associated systemic toxicity and side effects, that have been based upon conventional intravenous systemic administration paradigms may require modification in this setting.

One additional strategy to improve drug delivery and targeting has been to construct immunoliposomes by attaching exterior or surface ligands specifically targeting receptors or proteins that are found more commonly in on tumor cells (67), thereby potentially improving specificity (39), cytotoxicity (68) and intracellular incorporation and drug delivery (69). These techniques have been used for both chemotherapeutic agents and gene therapy (70) and for targeting molecules like epidermal growth factor receptor (EGFR) (69) and human epidermal growth factor receptor 2 (HER2) (71). Such immunoliposomes may be one method for selectively targeting persistently viable cells in the periablational zone that are exposed to non-lethal levels of hyperthermic injury and therefore express additional reactive or tumor-related biomarkers. Issues of improved targeting of a given molecular or protein target versus more global delivery of nanodrugs, particularly in the setting of heterogeneous and/or mutating tumor will need to be evaluated. However, targeting to global post-ablation responses such as HSP upregulation are particularly appealing.

As combination therapy is expanding to include a larger number of agents (or multiple agents) combined with ablative therapies, identifying the administration schedule that maximizes treatment efficacy for different tumors, drugs, and organ environments will be required. Initial animal studies on combined RF ablation and liposomal doxorubicin observed that the greatest tumor coagulation occurred when adjuvant liposomal doxorubicin was administered around the time of RF ablation (3 days pre- to 24 hr post-ablation, compared to 6 hr post-ablation for free doxorubicin) (49). This wide window of potential administration reflects improved circulation times and reduced reticuloendothelial clearance achieved with the development of PEG-ylated 'stealth' liposomes. Nevertheless, while many studies on combination RF ablation and Doxil have administered the adjuvant agent at the time of or immediately after ablation, this will likely vary for future combinations based upon complex drug-tumor-tissue heating interactions. For example, a recent study combining RF ablation with liposomal paclitaxel underscored the balance needed between the timing of delivery of the agent (i.e. providing sufficient time to sensitize tumor cells prior to RF) versus achieving maximal intratumoral concentrations (26). Waiting 15 min

post-RF ablation to give paclitaxel resulted in the greatest intratumoral drug accumulation, reflected matched peak drug circulation levels and hyperthermic tissue effects (i.e., greatest endothelial leakiness). Nevertheless, despite the fact that paclitaxel administered 24 hr before RF (paclitaxel-RF) resulted in increased but comparatively lesser amounts of intratumoral drug, more rapid coagulation was observed. This underscores that careful optimization of administration regimens, taking into account pharmacokinetic properties of the delivery vehicle, intratumoral drug release patterns, and ablation-induced effects on tumor vascularity, will be required as additional agents are developed.

Optimizing ablation parameters

Another strategy to maximize tumor destruction with combination therapy is to investigate and optimize ablation parameters. Current RF ablation protocols are device-specific and have been designed to maximize tumor destruction for a clinical environment, where optimization is a function of ablation time and coagulation (13, 72). However, achieving the greatest zone of sub-lethal hyperthermic injury, which is the area of maximum effect with adjuvant therapy, has not been incorporated into these protocols. For example, one commonly used commercially-available device uses a pulsing algorithm to maximize heating immediately around the electrode and in adjacent tumor (72), and additional modifications might yield a temperature profile in peripheral tumor that has an even wider range of tissue exposed to sub-lethal temperatures. Current animal studies demonstrate that increased tumor coagulation can be observed at a range of temperatures, and that there is a significant variability in the maximum 'critical' temperatures at the ablation margin, which varies based upon tumor and organ type. In preliminary studies, we have treated normal liver in female Fisher 344 rats with a range of thermal doses – modifying the time of ablation (0-10 min) and the electrode tip temperature (titrated to 55-90°C) over 9 data points (n=5 per group, total n=45). Animals were sacrificed 24 hr post-ablation, and outcome measures were tumor coagulation and IHC staining for HSP70 expression. We observed both increased tumor coagulation with increasing ablation time and temperature ($p<0.05$, FIG. 10A-C). Similarly, and more importantly, we also observed an increase in the rim thickness of the periablational zone of HSP70 with increasing RF times and tip temperatures. Given that this zone (as described above) can be specifically targeted with adjuvant therapies such as liposomal quercetin, these findings underscore that additional optimization of ablation protocols is required and can maximize the potential for tumor cellular injury in the setting of adjuvant therapy. In addition to modification of ablation parameters, adding metal particulate to the target tumor cells to increase the cytotoxic effects of the radiofrequency field is also being investigated. For example, several studies have reported increased apoptosis when administering tumor receptor-specific antibodies conjugated to gold nanoparticles in conjunction with exposure to radiofrequency fields (73, 74).

Optimizing administration paradigms

Most studies to date have used a single dose regimen when combining thermal ablation with adjuvant IV nanoparticle-based chemotherapeutics (35, 49, 51). However, the optimal administration paradigm of combination therapy has yet to be determined. Several factors will likely contribute to determining optimal administration (such as timing of

administration and multiple dosing regimens) and paradigms will likely vary based upon both chemotherapeutic mechanisms of action and ablation-induced tissue effects. In early animal studies with combined RF ablation and IV liposomal doxorubicin, the timing of a single dose (6.7 mg/kg) when administered from 48 hr before up to administration immediately after the time of ablation resulted in similar amounts of tumor coagulation (increased over RF ablation alone controls) (35). This finding likely reflects the long circulation time and stability of the agent used, which has a known circulating half-life of 48-72 hr. As was discussed in earlier sections, by comparison, IV liposomal doxorubicin results in relatively low levels intratumoral doxorubicin accumulation and no gross tumor coagulation/necrosis in control animals. Interestingly, administration of adjuvant IV liposomal doxorubicin 24 hr or greater after RF ablation did not result in the same gains in tumor coagulation, suggesting that synergistic effects occur in a time-sensitive window centered around the time of RF ablation (35). In another study, earlier increases in tumor coagulation were observed when IV liposomal paclitaxel was administered 24 hr prior to RF ablation compared to immediately after, highlighting that optimal administration times will likely vary based upon underlying chemotherapeutic mechanisms of action (26). In paradigms where the adjuvant agent is administered prior to ablation, intratumoral drug accumulation occurs in a heterogeneous manner with specific geographic areas of increased deposition. For example, while some drug accumulation occurs in the central ablation zone (akin to low levels of uptake seen in tumors that do not receive thermal ablation), the predominant cytotoxic effect in this area is high-temperature thermal ablation. One study suggests that several chemotherapeutic agents retain their activity even when exposed to such short courses of ablative temperatures (75). In contrast, a significantly greater amount of nanoparticle deposition is identified in the periablational rim. In this area, low-level sub-lethal hyperthermia affects tissue perfusion and vascular permeability in the periablational zone, and therefore, possibly also affects drug retention and release, though additional investigation is required.

Ultimately, the highest clinical gains are likely to be achieved by pursuing several different investigative lines in parallel to identify those variables that maximize tumor destruction. Similarly, as has been demonstrated in several prior studies, a single method of treatment (either tumor ablation or liposomal chemotherapy) will likely not sufficiently treat many types of cancers. To this end, treatment strategies that use a 'combination of combination therapies', targeting separate pathways of cellular function and stress, in combination with tailored nanoparticle delivery vehicles (such as liposomes with high lipid content, thermosensitive characteristics, or of various size), combined with a thermal ablation technique will likely yield the greatest degree of tumor destruction. Already, this has been observed in some studies, where combination 'triple therapy' with RF-paclitaxel-doxorubicin achieved greater amounts of tumor coagulation and animal survival than 'double' or 'single' therapy, we can anticipate that combining multiple therapies administered in parallel and each either contributing to a favorable treatment-tumor milieu or targeting individual cellular pathways will likely provide the most clinical benefit (26, 27). Similarly, in several more recent studies by Head et al. and Soundararajan et al., multipronged combination therapies (combining radiation and chemotherapy) have been reported using ^{186}Re radio-labeled doxorubicin-containing liposomes in conjunction with

RF ablation to improve treatment efficacy and reduce tumor growth compared to individual therapies or single combination therapies alone (76, 77). These early studies underscore the real clinical potential for pursuing a 'multi-hit' approach by incorporating multiple agents targeting a range of potentially susceptible pathways.

Selecting appropriate outcome measures

While mechanistic studies are critical to understanding and improving the synergies observed in combination therapy, recent studies have identified discordant results between tumor coagulation, changes in immunohistochemical markers for specific cellular pathways, and endpoint animal survival. This underscores the need to carefully address the most relevant endpoints. For example, when combining RF ablation with both IV liposomal doxorubicin and paclitaxel, Yang et al reported longer survival was more commonly seen with regimens that included liposomal doxorubicin compared to liposomal paclitaxel (with or without RF ablation), despite increases in apoptosis, and decreases in HSP70 production, and coagulation that would have seemed to favor paclitaxel (26).

The observed significant survival benefit for liposomal doxorubicin over liposomal paclitaxel is likely multifactorial. Both agents have different mechanisms of action, and doxorubicin was likely more effective in that particular tumor type (a breast adenocarcinoma, for which doxorubicin is a first-line therapy in humans). Increased coagulation and animal endpoint survival for RF/liposomal doxorubicin treatments compared to RF combined with paclitaxel despite its increased apoptosis, suggests that alternate mechanisms in addition to apoptosis are important in achieving slower tumor growth rates and/or complete tumor death following ablation. Thus, while early evaluation of coagulation diameter and immunohistochemistry markers can identify temporal differences between groups, the actual time it takes for a full effect to take place may vary. Hence, the determination of a full dose response curve likely needs to be tailored for each agent, and may make comparisons between different drugs even more difficult. Similarly, differences between intratumoral drug accumulation and coagulation demonstrate that identifying the drug concentration required for a threshold effect requires continued investigation.

Augmenting drug delivery with ablation techniques

Achieving favorable therapeutic effects while minimizing toxicity has proven challenging for many drugs particularly chemotherapeutics (78). While many compounds are shown to be tumoricidal in-vitro, less than optimal efficacy can be demonstrated in the clinic as barriers to efficient intratumoral delivery, limitations to uniform tumor drug uptake, and poor tumor retention retard the ability to achieve therapeutic dose concentrations within the boundaries of acceptable toxicity profiles. In short, unfortunately, intratumoral doses tend to be substantially lower than systemic doses. The problem is often further compounded by the systemic nature of intravenous delivery of chemotherapeutic drugs which have often been limited by unfavorable systemic toxicity and adverse events that commonly are the rate limiting barriers to achieving efficacious therapy (78, 79). As noted in multiple articles in this issue, the development of nanoparticle delivery platforms has been one such strategy to improve intratumoral drug delivery. However, despite many advancements in nanoparticle

development, including the creation of stealth liposomes, ligand targeting, and using nanoparticles that release their contents with specific cues, the percentage of administered dose that reaches the tumor can remain too low to achieve the desired or necessary effects (80). Therefore, additional adjuvant techniques to improve drug delivery and release are necessary.

In addition to ablation, a second backbone of the field of interventional oncology has been the development of image guided strategies to enhance selective tumor drug delivery, deposition, and retention. Classic examples of this have included direct tumoral injection of drugs such as ethanol and doxorubicin (25, 30), as well as selective intraarterial delivery of chemotherapy as is often practiced as chemoembolization (81, 82). Although these strategies have already been shown to deliver clinical benefit particularly for HCC and other liver tumors, nevertheless, direct injection strategies are often limited by the uneven diffusion of the drug throughout the target, and transcatheter therapies often increase the complexity and potential morbidity of delivering these drugs. Furthermore, chemoembolization is of limited utility to organs beyond the liver that cannot trap the excess particles in the sinusoids, placing the patient at risk for toxicity or emboli to the lung if invasive devices are not used to sequester the organ being treated (as is practiced for melphalan delivery to limbs for melanoma (83)). Thus, less-invasive methods for increasing drug deposition and uptake have been sought. To this end, we and other investigators have used thermal energy sources such as RF to markedly increase deposition of drugs such as liposomal doxorubicin into the tumor (as outlined in detail above) (4).

More recently, several additional technologies, some of which have tissue ablative effects, have been used to augment intratumoral drug deposition (through traditional hyperthermic effects), nanoparticle drug release, and intracellular uptake (through changes in membrane permeability). Two technologies in particular, high-intensity focused ultrasound (HIFU) (which can be administered transcutaneously and hence without inserting applicators into the patient) and irreversible electroporation (IRE) are being studied for the potential roles in improving intratumoral drug delivery (84, 85). A recent randomized animal study by Poff et al. studied the role of adjuvant HIFU in a squamous cell carcinoma (SCC7) in a CH3 murine model treated with a proteasome inhibitor, bortezomib (86). This study demonstrated that: a) the lower dose of bortezomib (1mg/kg) when administered following the administration of pulsed-HIFU could inhibit tumor growth compared to control groups of pulsed HIFU and drug alone to the same levels achieved by higher doses (1.5 mg/kg) of the drug. In addition to the primary endpoint, indices for apoptosis (i.e. programmed cell death) and decreased tumor vascularity were measured in an attempt to elucidate, at least in part, some of the mechanisms of the observed synergy. Indeed, Poff et al. show that the tumor growth delay was accompanied by increases in apoptosis 24 hr after combination therapy compared to the controls, although the pulsed-HIFU, however, did not further reduce blood vessels density beyond the reductions noted by the drug alone. Such early studies need additional validation to confirm their utility in clinically relevant sized tumors (3–7 cm) where volumetric coverage may be more challenging and for deeper seated tumors where energy penetration may prove more challenging. However, they highlight that using even short courses of adjuvant ablative techniques to concentrate liposomally-delivered drugs could potentially expand the clinical use of this and other chemotherapeutic agents that have previously

lacked efficacy due to an inability to achieve sufficient intratumoral drug concentrations. Additionally, selective intratumoral deposition of high drug concentrations could potentially allow an overall reduction of drug dosage (49), thereby reducing the potential for systemic toxicity, while maintaining delivery of high doses to the tumor target. Thus, nanoparticle delivery into ablated tumors has the unique potential to act as a focal targeting mechanism to guide the deposition of encapsulated agents, likely even more so for thermosensitive preparations (49, 87).

Finally, one advantage, in particular, of using adjuvant ablative techniques to improve drug delivery is their wider applicability in tumors and tissues where the damage of very high ablative specificity would make standard ablation risky, (such as in brain or pancreatic tumors) but where lower powered ablation or ablation localized only to central portions of the tumor without the need to achieving a complete ablation would be acceptable. Along these lines, several ablative technologies have low-powered “cousins” (i.e. low power ultrasound and reversible electroporation, respectively (42, 88-90)) that have been used to improve nanoparticle drug release and intracellular drug delivery as well, suggesting that, like RF-based systems, a low-powered modified protocol tailored to inducing those interstitial effects that maximize drug delivery and release rather than cell death may be equal or more beneficial in some cases. Similarly, traditional chemotherapy administration and dosing regimens have been developed for conventional systemic venous administration, where the administered dose is determined by a balance between effects of tumor growth and systemic side effects. As such, when incorporating both nanoparticle delivery vehicles combined with adjuvant ablation, optimal dosing patterns remain uncharacterized with new administration paradigms – in particular, higher drug dose delivery with these techniques and reduced systemic effects will likely change how these agents are administered.

Conclusion

The robust paradigm combining percutaneous image-guided thermal ablation therapies with evolving and improving nanoparticle-based chemotherapy platforms has already demonstrated early success in increasing tumor destruction and intratumoral drug accumulation in both experimental and pilot clinical studies. Strategies to further improve treatment efficacy in the form of optimization of the nanoparticle delivery vehicle and ablation parameters, and using additional agents to target specific hyperthermia-related cellular stress pathways are likely to provide additional benefit. Finally, given the increased intratumoral drug delivery and nanoparticle drug release that has been observed with combination therapy, the incorporation of ablation techniques as adjuvants to improve effects of primary nanoparticle based chemotherapies will also likely prove to be clinically relevant.

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References

1. Ahmed M, Brace CL, Lee FT Jr, Goldberg SN. Principles of and advances in percutaneous ablation. *Radiology*. Feb; 2011 258(2):351–69. [PubMed: 21273519]
2. Dupuy DE, Goldberg SN. Image-guided radiofrequency tumor ablation: challenges and opportunities--part II. *J Vasc Interv Radiol*. Oct; 2001 12(10):1135–48. [PubMed: 11585879]
3. Livraghi T, Meloni F, Goldberg SN, Lazzaroni S, Solbiati L, Gazelle GS. Hepatocellular carcinoma: radiofrequency ablation of medium and large lesions. *Radiology*. 2000; 214:761–8. [PubMed: 10715043]
4. Ahmed M, Goldberg SN. Combination radiofrequency thermal ablation and adjuvant IV liposomal doxorubicin increases tissue coagulation and intratumoural drug accumulation. *Int J Hyperthermia*. Nov; 2004 20(7):781–802. [PubMed: 15675672]
5. McGhana JP, Dodd GD 3rd. Radiofrequency ablation of the liver: current status. *AJR Am J Roentgenol*. Jan; 2001 176(1):3–16. [PubMed: 11133529]
6. Ahmed M, Goldberg SN. Thermal ablation therapy for hepatocellular carcinoma. *J Vasc Interv Radiol*. Sep; 2002 13(9 Pt 2):S231–44. [PubMed: 12354841]
7. Guenette JP, Dupuy DE. Radiofrequency ablation of colorectal hepatic metastases. *J Surg Oncol*. Dec 15; 2010 102(8):978–87. [PubMed: 21166002]
8. Seegenschmiedt M, Brady L, Sauer R. Interstitial thermoradiotherapy: review on technical and clinical aspects. *Am J Clin Oncol*. 1990; 13:352–63. [PubMed: 2198798]
9. Trembley B, Ryan T, Strohbehn J. Interstitial hyperthermia: physics, biology, and clinical aspects. *Hyperthermia and Oncology*. 1992; Vol 3:11–98. Utrecht: VSP.
10. Larson T, Bostwick D, Corcia A. Temperature-correlated histopathologic changes following microwave thermoablation of obstructive tissues in patients with benign prostatic hyperplasia. *Urology*. 1996; 47:463–9. [PubMed: 8638351]
11. Zevas N, Kuwayama A. Pathologic analysis of experimental thermal lesions: comparison of induction heating and radiofrequency electrocoagulation. *J Neurosurg*. 1972; 37:418–22. [PubMed: 4560953]
12. Goldberg SN, Gazelle GS, Halpern EF, Rittman WJ, Mueller PR, Rosenthal DI. Radiofrequency tissue ablation: importance of local temperature along the electrode tip exposure in determining lesion shape and size. *Acad Radiol*. 1996; 3:212–8. [PubMed: 8796667]
13. Appelbaum L, Sosna J, Pearson R, Perez S, Nissenbaum Y, Mertyna P, et al. Algorithm optimization for multitined radiofrequency ablation: comparative study in ex vivo and in vivo bovine liver. *Radiology*. Feb; 2010 254(2):430–40. [PubMed: 20093515]
14. Dodd GD 3rd, Soulen MC, Kane RA, Livraghi T, Lees WR, Yamashita Y, et al. Minimally invasive treatment of malignant hepatic tumors: at the threshold of a major breakthrough. *Radiographics*. Jan-Feb; 2000 20(1):9–27. [PubMed: 10682768]
15. Shimada K, Sakamoto Y, Esaki M, Kosuge T. Role of the width of the surgical margin in a hepatectomy for small hepatocellular carcinomas eligible for percutaneous local ablative therapy. *Am J Surg*. Jun; 2008 195(6):775–81. [PubMed: 18440487]
16. Livraghi T, Meloni F, Di Stasi M, Rolle E, Solbiati L, Tinelli C, et al. Sustained complete response and complications rates after radiofrequency ablation of very early hepatocellular carcinoma in cirrhosis: Is resection still the treatment of choice? *Hepatology*. Jan; 2008 47(1):82–9. [PubMed: 18008357]
17. Solbiati L, Livraghi T, Goldberg SN, Ierace T, DellaNoce M, Gazelle GS. Percutaneous radiofrequency ablation of hepatic metastases from colorectal cancer: long term results in 117 patients. *Radiology*. 2001; 221:159–66. [PubMed: 11568334]
18. Wood TF, Rose DM, Chung M, Allegra DP, Foshag LJ, Bilchik AJ. Radiofrequency ablation of 231 unresectable hepatic tumors: indications, limitations, and complications. *Ann Surg Oncol*. Sep; 2000 7(8):593–600. [PubMed: 11005558]
19. de Baere T, Elias D, Dromain C, Din MG, Kuoch V, Ducreux M, et al. Radiofrequency ablation of 100 hepatic metastases with a mean follow-up of more than 1 year. *AJR Am J Roentgenol*. Dec; 2000 175(6):1619–25. [PubMed: 11090390]

20. McGahan JP, Dodd GD 3rd. Radiofrequency ablation of the liver: current status. *AJR Am J Roentgenol.* Jan; 2001 176(1):3–16. [PubMed: 11133529]
21. Ahmed M, Liu Z, Lukyanov AN, Signoretti S, Horkan C, Monsky WL, et al. Combination radiofrequency ablation with intratumoral liposomal doxorubicin: effect on drug accumulation and coagulation in multiple tissues and tumor types in animals. *Radiology.* May; 2005 235(2):469–77. [PubMed: 15858089]
22. Horkan C, Dalal K, Coderre JA, Kiger JL, Dupuy DE, Signoretti S, et al. Reduced tumor growth with combined radiofrequency ablation and radiation therapy in a rat breast tumor model. *Radiology.* Apr; 2005 235(1):81–8. [PubMed: 15731375]
23. Grieco CA, Simon CJ, Mayo-Smith WW, DiPetrillo TA, Ready NE, Dupuy DE. Percutaneous image-guided thermal ablation and radiation therapy: outcomes of combined treatment for 41 patients with inoperable stage I/II non-small-cell lung cancer. *J Vasc Interv Radiol.* Jul; 2006 17(7):1117–24. [PubMed: 16868164]
24. Goldberg SN, Hahn PF, Tanabe KK, Mueller PR, Schima W, Athanasoulis CA, et al. Percutaneous radiofrequency tissue ablation: does perfusion-mediated tissue cooling limit coagulation necrosis? *J Vasc Interv Radiol.* Jan-Feb; 1998 9(1 Pt 1):101–11. [PubMed: 9468403]
25. Goldberg EP, Hadba AR, Almond BA, Marotta JS. Intratumoral cancer chemotherapy and immunotherapy: opportunities for nonsystemic preoperative drug delivery. *J Pharm Pharmacol.* Feb; 2002 54(2):159–80. [PubMed: 11848280]
26. Yang W, Ahmed M, Elian M, Hady el SA, Levchenko TS, Sawant RR, et al. Do liposomal apoptotic enhancers increase tumor coagulation and end-point survival in percutaneous radiofrequency ablation of tumors in a rat tumor model? *Radiology.* Dec; 2010 257(3):685–96. [PubMed: 20858851]
27. Yang W, Ahmed M, Tasawwar B, Levchenko T, Sawant RR, Collins M, et al. Radiofrequency ablation combined with liposomal quercetin to increase tumour destruction by modulation of heat shock protein production in a small animal model. *Int J Hyperthermia.* 2011; 27(6):527–38. [PubMed: 21846189]
28. Hakime A, Hines-Peralta A, Peddi H, Atkins MB, Sukhatme VP, Signoretti S, et al. Combination of radiofrequency ablation with antiangiogenic therapy for tumor ablation efficacy: study in mice. *Radiology.* Aug; 2007 244(2):464–70. [PubMed: 17641366]
29. Mertens JC, Martin IV, Schmitt J, Frei P, Bruners P, Herweg C, et al. Multikinase inhibitor sorafenib transiently promotes necrosis after radiofrequency ablation in rat liver but activates growth signals. *Eur J Radiol.* May 16.2011
30. Livraghi T. Role of percutaneous ethanol injection in the treatment of hepatocellular carcinoma. *Dig Dis.* 2001; 19(4):292–300. [PubMed: 11935089]
31. Shiina S, Tagawa K, Niwa Y. Percutaneous ethanol injection therapy for hepatocellular carcinoma: results in 146 patients. *AJR Am J Roentgenol.* 1993; 160:1023–5. [PubMed: 7682378]
32. Alexander DG, Unger EC, Seeger SJ, Karmann S, Krupinski EA. Estimation of volumes of distribution and intratumoral ethanol concentrations by computed tomography scanning after percutaneous ethanol injection. *Acad Radiol.* Jan; 1996 3(1):49–56. [PubMed: 8796640]
33. Arru M, Aldrighetti L, Gremmo F, Ronzoni M, Angeli E, Caterini R, et al. Arterial devices for regional hepatic chemotherapy: transaxillary versus laparotomic access. *J Vasc Access.* Jul-Sep; 2000 1(3):93–9. [PubMed: 17638234]
34. Homsí J, Garrett CR. Hepatic arterial infusion of chemotherapy for hepatic metastases from colorectal cancer. *Cancer Control.* Jan; 2006 13(1):42–7. [PubMed: 16508625]
35. Goldberg SN, Girnan GD, Lukyanov AN, Ahmed M, Monsky WL, Gazelle GS, et al. Percutaneous tumor ablation: increased necrosis with combined radio-frequency ablation and intravenous liposomal doxorubicin in a rat breast tumor model. *Radiology.* Mar; 2002 222(3):797–804. [PubMed: 11867804]
36. Gregoriadis, G., editor. *Liposomes as drug carriers.* Wiley; New York: 1988.
37. Senior JH. Fate and behavior of liposomes in vivo: a review of controlling factors. *Crit Rev Ther Drug Carrier Syst.* 1987; 3(2):123–93. [PubMed: 3542245]

38. Kemeny N, Huang Y, Cohen AM, Shi W, Conti JA, Brennan MF, et al. Hepatic arterial infusion of chemotherapy after resection of hepatic metastases from colorectal cancer. *N Engl J Med*. Dec 30; 1999 341(27):2039–48. [PubMed: 10615075]
39. Zhao H, Li GL, Wang RZ, Li SF, Wei JJ, Feng M, et al. A comparative study of transfection efficiency between liposomes, immunoliposomes and brain-specific immunoliposomes. *J Int Med Res*. May-Jun;38(3):957–66. [PubMed: 20819432]
40. Lasic, D.; Martin, F. *Stealth liposomes*. CRC Press; Boca Raton, FL: 1995.
41. Biswas S, Dodwadkar NS, Sawant RR, Torchilin V. Development of the Novel PEG-PE-Based Polymer for the Reversible Attachment of Specific Ligands to Liposomes: Synthesis and in vitro Characterization. *Bioconjug Chem*. Aug 29.
42. Schroeder A, Honen R, Turjeman K, Gabizon A, Kost J, Barenholz Y. Ultrasound triggered release of cisplatin from liposomes in murine tumors. *J Control Release*. Jul 1; 2009 137(1):63–8. [PubMed: 19303426]
43. Klibanov AL, Maruyama K, Torchilin VP, Huang L. Amphipathic polyethyleneglycols effectively prolong the circulation time of liposomes. *FEBS Lett*. Jul 30; 1990 268(1):235–7. [PubMed: 2384160]
44. Zhao H, Li GL, Wang RZ, Li SF, Wei JJ, Feng M, et al. A comparative study of transfection efficiency between liposomes, immunoliposomes and brain-specific immunoliposomes. *J Int Med Res*. May-Jun;2010 38(3):957–66. [PubMed: 20819432]
45. Vaage, J.; Barbara, E. Tissue uptake and therapeutic effects of stealth doxorubicin. In: Lasic, D.; Martin, F., editors. *Stealth Liposomes*. CRC Press, Inc; Boca Raton, FL: 1995.
46. Ranson MR, Carmichael J, O'Byrne K, Stewart S, Smith D, Howell A. Treatment of advanced breast cancer with sterically stabilized liposomal doxorubicin: results of a multicenter phase II trial. *J Clin Oncol*. Oct; 1997 15(10):3185–91. [PubMed: 9336354]
47. Gordon AN, Granai CO, Rose PG, Hainsworth J, Lopez A, Weissman C, et al. Phase II study of liposomal doxorubicin in platinum- and paclitaxel-refractory epithelial ovarian cancer. *J Clin Oncol*. Sep; 2000 18(17):3093–100. [PubMed: 10963637]
48. Rivera E, Valero V, Arun B, Royce M, Adinin R, Hoelzer K, et al. Phase II study of pegylated liposomal doxorubicin in combination with gemcitabine in patients with metastatic breast cancer. *J Clin Oncol*. Sep 1; 2003 21(17):3249–54. [PubMed: 12947059]
49. Ahmed M, Monsky WE, Girnun G, Lukyanov A, D'Ippolito G, Kruskal JB, et al. Radiofrequency thermal ablation sharply increases intratumoral liposomal doxorubicin accumulation and tumor coagulation. *Cancer Res*. Oct 1; 2003 63(19):6327–33. [PubMed: 14559820]
50. Monsky WL, Kruskal JB, Lukyanov AN, Girnun GD, Ahmed M, Gazelle GS, et al. Radiofrequency ablation increases intratumoral liposomal doxorubicin accumulation in a rat breast tumor model. *Radiology*. Sep; 2002 224(3):823–9. [PubMed: 12202721]
51. Goldberg SN, Kamel IR, Kruskal JB, Reynolds K, Monsky WL, Stuart KE, et al. Radiofrequency ablation of hepatic tumors: increased tumor destruction with adjuvant liposomal doxorubicin therapy. *AJR Am J Roentgenol*. Jul; 2002 179(1):93–101. [PubMed: 12076912]
52. Solazzo SA, Ahmed M, Schor-Bardach R, Yang W, Girnun GD, Rahmanuddin S, et al. Liposomal doxorubicin increases radiofrequency ablation-induced tumor destruction by increasing cellular oxidative and nitrate stress and accelerating apoptotic pathways. *Radiology*. Apr; 255(1):62–74. [PubMed: 20160000]
53. Spector AA, Burns CP. Biological and therapeutic potential of membrane lipid modification in tumors. *Cancer Res*. Sep 1; 1987 47(17):4529–37. [PubMed: 3304617]
54. Yoshikawa T, Kokura S, Tainaka K, Itani K, Oyamada H, Kaneko T, et al. The role of active oxygen species and lipid peroxidation in the antitumor effect of hyperthermia. *Cancer Res*. May 15; 1993 53(10 Suppl):2326–9. [PubMed: 8485720]
55. Kokura S, Yoshikawa T, Kaneko T, Iinuma S, Nishimura S, Matsuyama K, et al. Efficacy of hyperthermia and polyunsaturated fatty acids on experimental carcinoma. *Cancer Res*. Jun 1; 1997 57(11):2200–2. [PubMed: 9187121]
56. Kinter M, Spitz DR, Roberts RJ. Oleic acid incorporation protects cultured hamster fibroblasts from oxygen-induced cytotoxicity. *J Nutr*. Dec; 1996 126(12):2952–9. [PubMed: 9001361]

57. Working PK, Dayan AD. Pharmacological-toxicological expert report. CAELYX. (Stealth liposomal doxorubicin HCl). Hum Exp Toxicol. Sep; 1996 15(9):751–85. [PubMed: 8880211]
58. Mirahmadi N, Babaei MH, Vali AM, Dadashzadeh S. Effect of liposome size on peritoneal retention and organ distribution after intraperitoneal injection in mice. Int J Pharm. Jan 4; 383(1-2):7–13. [PubMed: 19729056]
59. Amselem S, Gabizon A, Barenholz Y. Optimization and upscaling of doxorubicin-containing liposomes for clinical use. J Pharm Sci. Dec; 1990 79(12):1045–52. [PubMed: 2079648]
60. Yuan F, Leunig M, Huang SK, Berk DA, Papahadjopoulos D, Jain RK. Microvascular permeability and interstitial penetration of sterically stabilized (stealth) liposomes in a human tumor xenograft. Cancer Res. Jul 1; 1994 54(13):3352–6. [PubMed: 8012948]
61. Yarmolenko PS, Zhao Y, Landon C, Spasojevic I, Yuan F, Needham D, et al. Comparative effects of thermosensitive doxorubicin-containing liposomes and hyperthermia in human and murine tumours. Int J Hyperthermia. 2010; 26(5):485–98. [PubMed: 20597627]
62. Kong G, Anyarambhatla G, Petros WP, Braun RD, Colvin OM, Needham D, et al. Efficacy of liposomes and hyperthermia in a human tumor xenograft model: importance of triggered drug release. Cancer Res. Dec 15; 2000 60(24):6950–7. [PubMed: 11156395]
63. Wells J, Sen A, Hui SW. Localized delivery to CT-26 tumors in mice using thermosensitive liposomes. Int J Pharm. Aug 11; 2003 261(1-2):105–14. [PubMed: 12878399]
64. Shimose S, Sugita T, Nitta Y, Kubo T, Ikuta Y, Murakami T. Effect of thermosensitive liposomal doxorubicin with hyperthermia on primary tumor and lung metastases in hamster osteosarcoma. Int J Oncol. Sep; 2001 19(3):585–9. [PubMed: 11494040]
65. Gasselhuber A, Dreher MR, Negussie A, Wood BJ, Rattay F, Haemmerich D. Mathematical spatio-temporal model of drug delivery from low temperature sensitive liposomes during radiofrequency tumour ablation. Int J Hyperthermia. 2010; 26(5):499–513. [PubMed: 20377363]
66. Poon RT, Borys N. Lyso-thermosensitive liposomal doxorubicin: an adjuvant to increase the cure rate of radiofrequency ablation in liver cancer. Future Oncol. Aug; 2011 7(8):937–45. [PubMed: 21823888]
67. Biswas S, Dodwadkar NS, Sawant RR, Torchilin V. Development of the Novel PEG-PE-Based Polymer for the Reversible Attachment of Specific Ligands to Liposomes: Synthesis and in vitro Characterization. Bioconjug Chem. Aug 29; 2011
68. Hertlein E, Triantafyllou G, Sass EJ, Hessler JD, Zhang X, Jarjoura D, et al. Milatuzumab immunoliposomes induce cell death in CLL by promoting accumulation of CD74 on the surface of B cells. Blood. Oct 7; 2010 116(14):2554–8. [PubMed: 20574049]
69. Mamot C, Drummond DC, Noble CO, Kallab V, Guo Z, Hong K, et al. Epidermal growth factor receptor-targeted immunoliposomes significantly enhance the efficacy of multiple anticancer drugs in vivo. Cancer Res. Dec 15; 2005 65(24):11631–8. [PubMed: 16357174]
70. Gao J, Sun J, Li H, Liu W, Zhang Y, Li B, et al. Lyophilized HER2-specific PEGylated immunoliposomes for active siRNA gene silencing. Biomaterials. Mar; 2010 31(9):2655–64. [PubMed: 20035999]
71. Yang T, Choi MK, Cui FD, Lee SJ, Chung SJ, Shim CK, et al. Antitumor effect of paclitaxel-loaded PEGylated immunoliposomes against human breast cancer cells. Pharm Res. Dec; 2007 24(12):2402–11. [PubMed: 17828616]
72. Goldberg SN, Stein M, Gazelle GS, Sheiman RG, Kruskal JB, Clouse ME. Percutaneous radiofrequency tissue ablation: optimization of pulsed-RF technique to increase coagulation necrosis. JVIR. 1999; 10:907–16. [PubMed: 10435709]
73. Glazer ES, Curley SA. Radiofrequency field-induced thermal cytotoxicity in cancer cells treated with fluorescent nanoparticles. Cancer. Jul 1; 2010 116(13):3285–93. [PubMed: 20564640]
74. Glazer ES, Zhu C, Massey KL, Thompson CS, Kaluarachchi WD, Hamir AN, et al. Noninvasive radiofrequency field destruction of pancreatic adenocarcinoma xenografts treated with targeted gold nanoparticles. Clin Cancer Res. Dec 1; 2010 16(23):5712–21. [PubMed: 21138869]
75. Ahrar K, Newman RA, Pang J, Vijjeswarapu MK, Wallace MJ, Wright KC, Dr. Gary J. Becker Young Investigator Award: Relative thermosensitivity of cytotoxic drugs used in transcatheter arterial chemoembolization. J Vasc Interv Radiol. Sep; 2004 15(9):901–5. 2004. [PubMed: 15361556]

76. Head HW, Dodd GD 3rd, Bao A, Soundararajan A, Garcia-Rojas X, Prihoda TJ, et al. Combination radiofrequency ablation and intravenous radiolabeled liposomal Doxorubicin: imaging and quantification of increased drug delivery to tumors. *Radiology*. May; 2010 255(2): 405–14. [PubMed: 20413753]
77. Soundararajan A, Dodd GD 3rd, Bao A, Phillips WT, McManus LM, Prihoda TJ, et al. Chemoradionuclide therapy with rhenium-186 labeled liposomal doxorubicin in combination with radiofrequency ablation for effective treatment of head and neck cancer in a nude rat tumor xenograft model. *Radiology*. 2011 [in press].
78. Moses MA, Brem H, Langer R. Advancing the field of drug delivery: taking aim at cancer. *Cancer Cell*. Nov; 2003 4(5):337–41. [PubMed: 14667500]
79. Jain RK. Delivery of molecular and cellular medicine to solid tumors. *Adv Drug Deliv Rev*. Mar 1; 2001 46(1-3):149–68. [PubMed: 11259838]
80. El-Kareh AW, Secomb TW. A mathematical model for comparison of bolus injection, continuous infusion, and liposomal delivery of doxorubicin to tumor cells. *Neoplasia*. Jul-Aug;2000 2(4):325–38. [PubMed: 11005567]
81. Llovet JM, Bruix J. Systematic review of randomized trials for unresectable hepatocellular carcinoma: Chemoembolization improves survival. *Hepatology*. Feb; 2003 37(2):429–42. [PubMed: 12540794]
82. Liapi E, Geschwind JF. Transcatheter and ablative therapeutic approaches for solid malignancies. *J Clin Oncol*. Mar 10; 2007 25(8):978–86. [PubMed: 17350947]
83. Grunhagen DJ, Brunstein F, Graveland WJ, van Geel AN, de Wilt JH, Eggermont AM. One hundred consecutive isolated limb perfusions with TNF-alpha and melphalan in melanoma patients with multiple in-transit metastases. *Ann Surg*. Dec; 2004 240(6):939–47. discussion 47-8. [PubMed: 15570199]
84. de Smet M, Heijman E, Langereis S, Hijnen NM, Grull H. Magnetic resonance imaging of high intensity focused ultrasound mediated drug delivery from temperature-sensitive liposomes: an in vivo proof-of-concept study. *J Control Release*. Feb 28; 2011 150(1):102–10. [PubMed: 21059375]
85. Chen D, Wu J. An in vitro feasibility study of controlled drug release from encapsulated nanometer liposomes using high intensity focused ultrasound. *Ultrasonics*. Aug; 2010 50(8):744–9. [PubMed: 20334887]
86. Poff JA, Allen CT, Traughber B, Colunga A, Xie J, Chen Z, et al. Pulsed high-intensity focused ultrasound enhances apoptosis and growth inhibition of squamous cell carcinoma xenografts with proteasome inhibitor bortezomib. *Radiology*. Aug; 2008 248(2):485–91. [PubMed: 18574138]
87. Dromi S, Frenkel V, Luk A, Traughber B, Angstadt M, Bur M, et al. Pulsed-high intensity focused ultrasound and low temperature-sensitive liposomes for enhanced targeted drug delivery and antitumor effect. *Clin Cancer Res*. May 1; 2007 13(9):2722–7. [PubMed: 17473205]
88. Ueno Y, Sonoda S, Suzuki R, Yokouchi M, Kawasoe Y, Tachibana K, et al. Combination of ultrasound and bubble liposome enhance the effect of doxorubicin and inhibit murine osteosarcoma growth. *Cancer Biol Ther*. Aug 15; 2011 12(4):270–7. [PubMed: 21613828]
89. Tsoneva I, Iordanov I, Berger AJ, Tomov T, Nikolova B, Mudrov N, et al. Electrodilivery of drugs into cancer cells in the presence of poloxamer 188. *J Biomed Biotechnol*. 2010; pii:314213. [PubMed: 20706647]
90. Schroeder A, Kost J, Barenholz Y. Ultrasound, liposomes, and drug delivery: principles for using ultrasound to control the release of drugs from liposomes. *Chem Phys Lipids*. Nov; 2009 162(1-2): 1–16. [PubMed: 19703435]

HIGHLIGHTS

- Combining image-guided thermal ablation with nanoparticle-based chemotherapy increases tumor destruction and intratumoral drug accumulation
- Strategies to further improve treatment efficacy include optimization of the nanoparticle delivery vehicle and ablation parameters
- Using additional agents to target specific hyperthermia-related cellular stress pathways are likely to provide additional benefit
- Incorporation of ablation techniques as adjuvants to improve effects of primary nanoparticle based chemotherapies will also likely prove to be clinically relevant

Figure 1. Schematic (A) and pictorial (B) representation of focal thermal ablation therapy

Electrode applicators are positioned either with image-guidance or direct visualization within the target tumor, and thermal energy is applied via the electrode. This creates a central zone of high temperatures in the tissue immediately around the electrode (which can exceed 100°C), and surrounded by more peripheral zones of sub-lethal tissue heating (<50°C) and background liver parenchyma. (reprinted with permission from Radiological Society of North America (1))

Figure 2. Method for combining thermal ablation with targeted drug delivery

Drugs are brought to the tumor site as part of normal circulation (left). Temperature elevations inside the ablation zone facilitate local drug release, which then accumulates in the sub-lethal region at the periphery of the ablation zone (middle). The net result is a larger zone of ablation than would be possible with ablation alone (right). (reprinted with permission from Radiological Society of North America (1))

Figure 3. Combining thermal ablation with adjuvant chemotherapy

A schematic diagram outlining the history of combining thermal ablation with adjuvant nanoparticle-based chemotherapy, including the role of mechanistic studies and several potential future areas of investigation.

Figure 4. Combination RF ablation and IV liposomal doxorubicin

Observed effects of combined RF/IV liposomal doxorubicin therapy (right) compared to RF alone (left, 12 min RF application,

1 cm internally-cooled electrode) in subcutaneous canine venereal sarcoma tumors. The central white zone (arrows) that corresponds to RF-induced coagulation is slightly larger (3 mm) in the combined therapy tumor, while the peripheral red zone is dramatically increased in size (0.21 to 0.93 cm). In the combined therapy tumor this red zone of increased tumor destruction is comprised of frank coagulative necrosis (reprinted with permission from Radiological Society of North America).

Figure 5. Kaplan-Meier Analysis of endpoint survival following treatment with RF and/or IV liposomal doxorubicin (Doxil)

Using tumor growth to a diameter of 3 cm as the survival end-point, greatest endpoint survival was observed with combined therapy, using liposomal doxorubicin and RF at either 70°C or 90°C. In addition, improved endpoint survival was noted using a higher RF thermal dose (90°C), when compared to combined treatment with a lower (70° C) RF dose (reprinted with permission from Radiological Society of North America).

Figure 6. Increased tumor destruction with combined RF and liposomal doxorubicin

82 year old male with an 8.2 cm vascular hepatoma. (a) CT image obtained immediately following RF ablation shows persistent regions of residual untreated tumor (white arrows) (Black zone = ablated region). (b) Two weeks following therapy there is interval increase in coagulation as the 1.5 cm inferior region of residual tumor and the 1.2 cm anteromedial portion of the tumor

no longer enhance (white arrowheads). A persistent nodule of viable tumor is identified (white arrow). This was successfully treated with a course of RF ablation. (c) CT image obtained immediately following RF ablation demonstrates the persistence of a large vessel (white arrows) coursing through the non-enhancing ablated lesion. (d) Two weeks post-therapy there is no enhancement throughout this region, and no vessel was seen on any of the three phases of contrast enhancement. No evidence of local tumor recurrence was identified at 48 months of follow-up (reprinted with permission from Am J Roentgen) (25).

Figure 7. Heat shock protein (HSP-70) expression after RF/liposomal doxorubicin therapy (A=4x, B=40x) Low and high power staining of tissues harvested 12 hours post-treatment with combined RF/liposomal doxorubicin therapy were stained for HSP70 (red) and cleaved caspase-3 (brown). A peripheral rim of heat shock protein expression external to the zone of apoptosis is evident (arrows) (reprinted with permission from Radiological Society of North America).

Figure 8. Apoptosis following combined RF ablation and liposomal chemotherapy

Photomicrographs show rim staining (arrows) of cleaved caspase-3 surrounding the zone of coagulation on sections from tumors excised at 4 hr (upper row, A-D) and 24 hr (lower row, E-H) at 4X magnification for **(A, E)** RF alone, **(B, F)** RF-DOX, **(C, G)** PTXL-RF, and **(D, H)** PTXL-RF-DOX. At 4 hr, RF-DOX demonstrated the strongest staining for cleaved caspase-3 expression.

At 24 hr, PTXL-RF demonstrated the greatest overall staining, for example, seen extending to the tumor margin in **(G)**

(reprinted with permission from Radiological Society of North America).

Figure 9. Effect of nanoparticle size on accumulation in the periablational zone

RF ablation $70^{\circ}\text{C} \times 5 \text{ min}$) was performed in normal liver in female Fischer 344 rats, followed by injection of fluorescent labeled beads (ultraclean polystyrene beads with different fluorescent-dyes). Images presented were obtained at 4X magnification (Nikon Eclipse TE300 Inverted Microscope with iVision software 4.0.14). Significantly greater bead deposition is demonstrated in the periablational zone for smaller 20 nm (excitation/emission wavelengths 505/515 nm) beads (A) compared to larger 100 nm (excitation/emission wavelengths 715/755 nm) beads (B). A few scattered beads seen within the ablation zone likely represent scattered patent residual microvessels.

Figure 10. Increasing thermal dose during RF ablation increases periablational HSP70 expression

RF ablation was performed in subcutaneously implanted R3230 breast adenocarcinoma tumors (1.5-1.8 cm in diameter). Temperature around the RF electrode tip was titrated to 70°C. RF ablation was applied for increasing durations of 2 min (A), 5 min (B), and 10 min (C). Progressively and significantly increasing HSP70 expression is observed in the periablational zone ($p < 0.05$, for all comparisons). This would afford a greater zone upon which liposomal quercetin can potentially act.