


Defeating Cancers' Adaptive Defensive Strategies Using Thermal Therapies: Examining Cancer's Therapeutic Resistance, Ablative, and Computational Modeling Strategies as a means for Improving Therapeutic Outcome

Technology in Cancer Research & Treatment
Volume 17: 1-16
© The Author(s) 2018
Reprints and permission:
sagepub.com/journalsPermissions.nav
DOI: 10.1177/1533033818762207
journals.sagepub.com/home/tct


John M. Baust, PhD^{1,2}, Yoed Rabin, PhD³, Thomas J. Polascik, MD⁴,
Kimberly L. Santucci, PhD^{1,2}, Kristi K. Snyder, PhD^{1,2},
Robert G. Van Buskirk, PhD^{1,2,5}, and John G. Baust, PhD^{2,5}

Abstract

Background: Diverse thermal ablative therapies are currently in use for the treatment of cancer. Commonly applied with the intent to cure, these ablative therapies are providing promising success rates similar to and often exceeding “gold standard” approaches. Cancer-curing prospects may be enhanced by deeper understanding of thermal effects on cancer cells and the hosting tissue, including the molecular mechanisms of cancer cell mutations, which enable resistance to therapy. Furthermore, thermal ablative therapies may benefit from recent developments in computer hardware and computation tools for planning, monitoring, visualization, and education. **Methods:** Recent discoveries in cancer cell resistance to destruction by apoptosis, autophagy, and necrosis are now providing an understanding of the strategies used by cancer cells to avoid destruction by immunologic surveillance. Further, these discoveries are now providing insight into the success of the diverse types of *ablative therapies* utilized in the clinical arena today and into how they directly and indirectly overcome many of the cancers' defensive strategies. Additionally, the manner in which minimally invasive thermal therapy is enabled by imaging, which facilitates anatomical features reconstruction, insertion guidance of thermal probes, and strategic placement of thermal sensors, plays a critical role in the delivery of effective ablative treatment. **Results:** The thermal techniques discussed include radiofrequency, microwave, high-intensity focused ultrasound, laser, and cryosurgery. Also discussed is the development of thermal adjunctive therapies—the combination of drug and thermal treatments—which provide new and more effective combinatorial physical and molecular-based approaches for treating various cancers. Finally, advanced computational and planning tools are also discussed. **Conclusion:** This review lays out the various molecular adaptive mechanisms—the hallmarks of cancer—responsible for therapeutic resistance, on one hand, and how various ablative therapies, including both heating- and freezing-based strategies, overcome many of cancer's defenses, on the other hand, thereby enhancing the potential for curative approaches for various cancers.

Keywords

thermal ablation, cryosurgery, adjunctive therapies, cancer stem cells, cell death, computation tools

¹ CPSI Biotech, Owego, NY, USA

² Institute of Biomedical Technology, State University of New York at Binghamton, Binghamton, NY, USA

³ Department of Mechanical Engineering, Carnegie Mellon University, Pittsburgh, PA, USA

⁴ Division of Urology, Department of Surgery, Duke University Medical Center, Durham, NC, USA

⁵ Department of Biological Sciences, Binghamton University, Binghamton, NY, USA

Corresponding Author:

John M. Baust, PhD, CPSI Biotech, 2 Court St, Owego, NY 13827, USA.

Email: jmbaust@cpsibiotech.com



Abbreviations

CDL, CD⁺8 cytotoxic T lymphocytes; CT, computerized tomography; CSC, cancer stem cells; EMT, epithelial–mesenchymal transition; 5-FU, 5-fluorouracil; HIFU, high-intensity focused ultrasound; MCS, mobile; MRI, magnetic resonance imaging; Nd:YAG, neodymium: yttrium–aluminum–garnet; NK, natural killer;; PDT, photodynamic therapy;; RFA, radiofrequency ablation; SCS, stationary; TACE, trans-arterial chemoembolization; US, ultrasound

Received: June 19, 2017; Revised: December 19, 2017; Accepted: February 05, 2018.

Cancer: A Primary Target for Ablative Therapy

The present-day standards of care utilized extensively in cancer therapy do not, unfortunately, provide standards of cure. All existing *in situ* therapies, while applied with curative intent, only provide disease suppression for periods ranging from months to years in majority of patients. Despite more than 50 years of research, improvements in durable response to treatment outcomes have exhibited only modest change.¹ It is estimated that over 1.68 million individuals were diagnosed with cancer in the United States in 2016, the majority (86%) of which were aged 50 or older.² Further, it is estimated that 1630 Americans will die each day of cancer. At the research level, encouraging progress has been made toward providing the clinic with improved, even, curative, treatment strategies. However, translatable progress toward cure is often stifled by the observation that “cancer” represents a diverse group of more than 150 diseases linked to cellular genetic controls for which we presently lack sufficient knowledge to apply curative strategies. The problem, however, is more complex. Cancer is primarily a “disease” of aging, where its origin has evolutionary implications.^{3,4} Older tissues have a tendency for higher frequency errors in division, which leads to mutation. Furthermore, a weakened immune system with aging is subject to compromise of its surveillance and eradication functions. It follows that cancer, as a mortal disease, functions to prevent the inheritance of deleterious mutations within a population. Cancer continues to mutate and self-select for resistance to common therapies⁵ and to assure depletion through death of normal cells with or without mutagenic propensities. In other words, cancers are diseases of individuals who may ultimately require multiple customized/personalized treatment strategies for a given “class” of cancers (ie, prostate, breast, and so on).

Today, it is recognized that a cancer commonly represents an accumulation of mutations in a population of cells with genetic (patient) variability. A tumor is no longer considered a homogeneous mass of cells growing without control, thereby disrupting the architecture of both primary and numerous secondary (metastatic) sites. We have very recently come to realize that a tumor is a highly organized structure, dependent on numerous other supporting cells for its establishment, growth, and invasion. A hierarchy of intercellular commands provides for an orderly progression of the disease with accompanying

defensive strategies that compromise both natural immunity and additive therapeutic interventions (eg, radiation, chemotherapy).⁶ As such, a specific cancer in 1 individual may share select molecular similarities with that of another individual, but it also exhibits a unique biology that may well defeat a given generic/common therapeutic strategy when uniformly applied across a genetically heterogeneous patient population. This genetic diversity gives rise to differential sensitivity to most treatment options less so for excisional therapy¹ provided sufficient margins are provided in nonmetastatic patients.

As a result, the last decade of research has led to a paradigm shift in cancer-related knowledge and information. Today, 3 sentinel changes in our understanding of cancer are in process. First, cancer stem cells (CSC), initially reported in 1997,⁷ are now widely accepted as key elements of tumorigenesis as well as the “cell of origin” (a mutated tissue stem cell). Cancer stem cells are pivotal in (1) forming additional cancer cells and possibly secondary cancer loci, (2) forming noncancerous stromal (support) cells, (3) having a clear resistance to radiation⁸ and chemotherapy,⁹ and (4) establishing dormant states. To this end, CSCs are now recognized as the likely origin of a tumor and lend incredible resiliency to the disease. Second, tumor formation involves the recruitment of numerous noncancer support cells, which establish a microenvironment essential to tumor survival, growth, and ultimate metastasis. These tumor-associated cells include endothelial cells vital to blood vessel formation, fibroblasts to serve various support functions, cells of the immune system which assume a protective role for the cancer cells in masking cancer immunogenicity from circulating immune cells (eg, macrophages), nutritive serosal cells, and mesenchymal cells.¹⁰ Hence, the tumor is a protective neotissue environment that isolates its cells from the various defensive strategies of the body. Linked to each of the above is the growing body of evidence demonstrating that with successive therapeutic attempts, the cancer cells progressively acquire enhanced resistance to therapeutic modalities (eg, chemotherapy, radiation, and hormonal ablation). For example, exposure to successive bouts of cytotoxic drugs results in the survival of approximately 20% to 30% of the population of the cancer cells as only those cells in dividing stages succumb to the toxic exposure. With follow-up treatments, each additional dose results in tumor-associated fibroblasts, secreting a membrane protective protein (Wnt 16B) that enhances cancer cell

resistance with the certain long-term outcome of chemotherapeutic resistance.⁹ In addition, other defensive strategies are brought into play such as the upregulation of membrane protein pumps that function to transport the chemotherapeutic agent to the extracellular environment. Radiation induces the same Wnt 16B response plus the amplification of DNA repair/protective strategies and inhibition of apoptosis.⁸ These latter observations are problematic as the patient receives near-term growth suppression (remission) with likely return of malignant growth but with a further mutated cancer demonstrating refractoriness to the initial primary treatment. Following combinatorial approaches, such as mixed radiation schemata or multiple chemotherapeutic agents, variable benefit to a durable response is observed across the spectrum of cancer types. Hence, there is a critical unmet need to explore alternative strategies to treat cancer, characterized by nonrepetitive application and recruitment of multiple destructive paths in order to provide immediate destruction of targeted cancerous tissue while defeating the adaptive molecular defensive strategies of cancer cells. To this end, thermal ablation is now providing an attractive and effective means of treating cancer due to its ability to trigger thermal excursions in order to induce multiple stressors and forms of cell death.

Cancer Characteristics

To better understand the logic directing the strategic shift in cancer therapy, recognition of the principal characteristics—the hallmarks of cancer—is important. These features have evolved to assure successful tumor growth in the face of diverse, well-established, anti-tumor protective adaptations and therefore may compromise elements of emerging cancer therapeutics.¹¹ These hallmarks include (1) regulation of cell growth, (2) angiogenesis, (3) reprogrammed metabolism, (4) cell death resistance, (5) reproductive immortality, (6) metastatic qualities, (7) immunosuppression, and (8) microenvironment modification. An overview of each of these features is provided below.

Sustainable, Controlled Cell Growth

Arguably, the most fundamental trait of a cancer is sustained cell growth and controlled proliferation over long periods of time. Normal cells maintain precise control over cell proliferation through the production of growth factors that regulate the cell cycle to ensure tissue homeostasis. Cancer cells deregulate these control signals and establish a mitogenic signaling pathway that may include, in part, growth factor self-stimulation, hypersensitization, activation of downstream pathways, and recruitment of tumor-associated stromal cells to produce growth factors. Additionally, cancer cells are able to avoid excessive proliferative signaling through mechanisms such as senescence and apoptosis to prevent uncontrolled (explosive) growth so that division can continue, thereby allowing for sustained cancer growth over time. Through these and other mutagenic steps, cancer cells can change their destiny.¹²⁻¹⁵

Angiogenesis

Normal and cancer cells essentially share similar metabolic requirements, which are primarily supported by a vascular blood supply. Tumor cells also have the ability to alter metabolic pathways (discussed below). During embryogenesis, endothelial cells execute angiogenic programs to produce blood vessels. In adults, angiogenic processes are typically limited to wound repair and the female reproductive cycle. Cancer cells control an “angiogenic switch” through a series of growth factors that can induce new blood vessel formation (vascular endothelial growth factor [VEGF]), sustain a tumor-associated vascularity (Fibroblast Growth Factor [FGF]), and suspend growth (Thrombospondin-1 [TSP-1], angiostatin, etc). Hence, the blood supply to a tumor can be activated and then modulated to match the needs of the specific cancer.¹⁶⁻¹⁸

Reprogrammed Metabolism

The rapid growth observed in some tumors requires adjustments to the metabolic profile of the rapidly dividing cancer cells but not for those cells demonstrating slow growth. Cancer cells during periods of high growth rates switch metabolically from oxidative phosphorylation to aerobic glycolysis, which is about 18-fold less efficient in the production of Adenosine triphosphate (ATP) but provides essential substrate molecules (ie, amino acids and nucleosides) for cell growth. Cancer cells have also been shown to increase cell membrane levels of glucose transporters to facilitate increased glucose absorption.¹⁹⁻²²

Cell Death Resistance

Depending on physiological actions and the genetic control of these processes, cell numbers are controlled by 3 programmed processes: apoptosis, autophagy, and necrosis. Apoptosis is a gene-regulated process most prevalent in cancer management involving extrinsic (membrane-mediated), intrinsic (mitochondrial-mediated), and nuclear cell signaling pathways. Cancer cells evolve strategies to both limit and circumvent these modes of cell death. These may include the loss of the tumor-suppressor gene p53, downregulation of BAX and BIM, and upregulation of Bcl₂ and Bcl-xL. For cancer cells to survive and spread, they must maintain the ability to develop antiapoptotic strategies necessary to offset antitumor, proapoptotic signaling.²³⁻²⁵ Autophagy, which has common elements with apoptotic signaling pathways, may act independent of, or with, apoptosis to limit tumor growth. It acts as a limiting factor in early-stage cancer but may promote cancer growth in a later stage of the disease through the induction of reversible cancer cell dormancy during periods of exposure to chemotherapeutic agents, also known as the *autophagy paradox*. Necrosis is also a mode of cell death capable of paradoxical action. Cells that swell and rupture during necrosis release proinflammatory cytokines and chemokines that are responsible for the recruitment of an immune response. These inflammatory cells can be tumor promoting, as they stimulate angiogenesis and cell

proliferation. A prolonged inflammatory response is now known to be closely linked with tumorigenesis.

Reproductive Immortality

As a principle, tumor growth requires an unlimited potential for replication of the cancer cells. On the other hand, normal cells lack replicative immortality with limited passage capability (the Hayflick limit). One salient difference between normal and immortalized cells is telomerase activity. Telomerases, which add telomeres to the ends of DNA molecules, are absent in most nonimmortalized cells but present in cancer cells. Accordingly, telomeres are lost (shortened) in noncancerous cells during division but lengthened by telomerase activity in cancer cells. Telomerases are almost absent in most somatic cells but found in nearly 90% of cancer cells.²⁶⁻²⁸

Invasion and Metastasis

A tumor represents a local invasion of a tissue with cells in a malignant state. The tumor microenvironment provides several mechanisms to allow cancer cells to avoid immunodetection, survive, and invade. Several factors can be synthesized or produced by malignant cells, the immune system, or the stroma and can remodel the tumor microenvironment and the adaptive immune response, resulting in cancer dissemination. Some mechanisms include acquisition of aberrant immune-phenotypic traits that regulate interactions among tumor microenvironment components via immunosuppressive mediators, adaptability of malignant and immune cells to autocrine and paracrine stimulation or stress, or disrupting the balance between tumor-promoting and -suppressing functions.

As the malignancy progresses to higher pathological stages, cancer cells undergo numerous phenotypic changes (ie, down-regulation/mutation of cell–cell adhesion molecules such as E-cadherin and upregulation of N-cadherin) and initiation of the epithelial–mesenchymal transition (EMT) regulatory pathway—a classic wound-healing process that supports cell dissemination and resistance to apoptosis.^{29,30} Cancer stem cells can also upregulate extracellular proteolysis that degrades portions of the extracellular matrix, thereby allowing cancer cells to “detach” from a tumor mass and the cancer to more easily metastasize. During the metastasis process, the increased level of extracellular matrix protein expression on the CSC’s surface allows for increased attachment, establishment, and subsequent invasion and tumor formation at a new site. The ability of CSC’s to both increase attachment and selectively degrade extracellular matrix (ECM) molecules during metastasis has led to the *Two-Phase Expression Pattern Hypothesis* wherein 2 subsets of CSC’s, stationary (SCS) and mobile (MCS) are produced within a tumor.³¹ This hypothesis suggests that SCS is located in the interior of a tumor and can differentiate into MCS at the tumor–host interface, via the acquisition of transient EMT, enabling detachment, circulation, and subsequent establishment at another location to create a new tumor.³²

Immune Compromise

The body’s immune system operates a surveillance program designed to recognize and eliminate most cancer cells. Cancer cells, however, have numerous strategies designed to subvert this system. Cancer cells produce both highly and weakly immunogenic clones, the former of which triggers detection and destruction while the latter survives and escapes surveillance. Cancer cells can also secrete immunosuppressive agents (ie, transforming growth factor beta [TGF- β]) and can recruit inflammatory cells as cells resident to the tumor with immunosuppressive capabilities (ie, suppress the actions of cytotoxic lymphocytes).³³⁻³⁵ It is hypothesized that infiltrating CD⁺8 cytotoxic T lymphocytes (CTL) and natural killer (NK) cells are inhibited by the secretion of TGF- β and other immunosuppressive factors.

Establishment of a Microenvironment

Tumors are now recognized as malignant constructs containing diverse cell types with an ordered microanatomy. Although the cancer cells represent the foundation of the disease, the tumor represents a complex structure with diverse cell types that provide essential support to the cancer cells. This support is both regulated and responsive to tumor chronology.^{6,36-39}

Thermal Ablation Strategies

The plasticity of a cancer as represented by its hallmarks suggests that changing the cancer treatment paradigm will evolve with the development of knowledge regarding the biological basis for novel treatment options, together with the accumulation of evidence-based assurance that cancer survival strategies do not overcome the “best intent” of the therapy.

Historically, surgical excision has been the preferred therapy for most tumors. The excised tissue can be examined to determine the adequacy of excision and identify its histological characteristics, which may guide the need for further excision or adjunctive therapy, respectively. The results achieved by surgical excision have created a benchmark for all other therapies. However, not all tumors are suitable for excision due to location, extent of disease, potential complications, reconstruction problems, or associated diseases. Nor is excision necessarily more advantageous than other therapeutic approaches.⁴⁰ Thermal ablative strategies offer new promise in light of the above, since they rely on energy changes in the target and are applied as short duration monotherapies (without repetition) thereby limiting cancer’s adaptive capabilities to express defensive mutations. Furthermore, the destructive mechanisms of action involved with thermal ablative strategies include both physical and molecular insults resulting in the disruption of multiple defensive strategies, which are not cell cycle dependent and add a damaging structural (physical) element.

In effect, thermal ablation provides a *combinatorial challenge* to the tumor microenvironment not attained with traditional monotherapies. A variety of techniques are used for thermal

ablation and can be classified as additive (heat input) or subtractive (heat extraction). Additive thermal ablative approaches (heat or hyperthermia) include radiofrequency ablation (RFA), microwave, laser, and high-intensity focused ultrasound (HIFU), whereas the subtractive thermal approach category is comprised primarily of cryogenic technologies. The use of ablative techniques has been dependent upon progress in imaging technologies, such as computerized tomography (CT), intraoperative ultrasound (US), and magnetic resonance imaging (MRI), which provide visualization and the degree of guidance necessary for the use of percutaneous minimally invasive ablation.

Given the ever-growing interest in and use of ablation therapies to treat cancer, herein we discuss the various types of ablative therapy, outline a basis for adjunctive therapeutic approaches for utilizing thermal ablation in order to enhance the destructive effects, and overview advances in computational means and procedure planning in order to improve the therapeutic outcome.

Additive Thermal Ablative Approaches

Radiofrequency Ablation

Radiofrequency ablative energy deposition is commonly aimed at destroying tissue by heating it to temperatures above 50°C. The “thermal dose” to produce tissue coagulation is commonly cited at 50°C for approximately 5 minutes or 55°C for 5 seconds to induce nearly 100% epithelial cell kill. The degree of cell kill is both temperature and time dependent.⁴¹ Experimentation has shown significant differences in outcome based on the current, the distance from heat source and on the type of tissue.⁴²⁻⁴⁵ It is further suggested that some tumors may require a higher thermal dose, possibly achieved when approaching 90°C for a clinically relevant time scale.

Treatment by RFA energy requires a generator for the electric current, cables to connect the generator to thin needle electrodes placed in or around the tumor, and the availability of medical imaging (ie, US or CT) for electrode placement. The number of electrodes used may vary with the size of the tumor. The electrodes are diverse in structure and include monopolar, bipolar, and multitined designs, ranging in size from 13- to 17-gauge needles. Multitined structures increase the electrode surface area and improve the ability to deliver an adequate thermal dose. Although the typical target temperature for RFA is above 50°C, an adverse effect utilizing this technology is that the electrical impedance within the target region decreases with the increasing temperature, and independently with the distance from the electrode, which makes this application most practical for small tumors, 3 cm or less. To mitigate these adverse effects, the emergence of irrigated electrodes has had a substantial impact on reducing/slowing tissue impedance rise during application, thereby enabling improved delivery of a therapeutic dose further from the electrode tip. Current exits the body via large grounding electrode pads on the skin. The apparatus has evolved substantially in recent years with the trend of improved current generators and electrodes.⁴⁶

Radiofrequency ablation has been used for a large variety of tumor types, including those of the liver, lung, kidney, breast, and bone. Its applications in the lung, breast, and kidney appear to show an expanding trend.⁴⁷⁻⁴⁹ Palliative benefits of RFA in advanced cancers have also been described.⁵⁰ Percutaneous RFA treatment of liver tumors was initiated in the 1980s, where best results were obtained with small hepatocellular tumors (3 cm or smaller in diameter),⁵¹⁻⁵³ where short-term successful treatment may be expected in 70% to 90% of patients^{54,55}. Radiofrequency ablation has also been used for small (2 cm) localized breast cancers, inserting the RFA needles into the tumor under ultrasound guidance. The success rate in the treatment of early breast tumors is modest, and only a few patients have been treated in this way. As such, the efficacy of RFA in breast cancer remains uncertain. In the treatment of kidney tumors, however, radiofrequency ablation has been reported to yield long-term outcomes comparable to excision. To this end, RFA has been reported to have lower morbidity but a slightly higher recurrence rate compared to surgery.⁵⁶⁻⁶⁰ Although effective at ablating tissues if a threshold temperature is reached, the heat transferred into the tissue may dissipate into adjacent tissues and produce undesirable local effects, injuring adjacent tissues.^{54,55} The resulting damage postapplication often leads to a reduction in ablative zone precision and unwanted collateral damage, which is particularly of concern for sensitive neuronal structures. To this end, RFA as with many of the thermoablative modalities (microwave and HIFU) share a common core of potential complications or unintended consequences. Foremost includes incomplete tumor ablation due to any of a number of factors ranging from problems with disease localization, targeting, and energy delivery that may result in incomplete coverage of the entire tumor, inadequate critical temperature reached, or emergence of resistance. In addition, hemorrhage (immediate or delayed), fistula, injury to surrounding structures, pain, or infection can occur as a result of thermoablation. As with many forms of cancer treatment, the inability to achieve the desired goal may engender the need for further therapies.

When RFA has been utilized to treat larger lesions, especially those about 5 cm, the success rate is substantially lower, in the 50% to 70% range and sometimes even lower. The effectiveness of RFA for large tumors depends on its size and proximity to major blood vessels that serve as heatsinks and the presence of associated diseases such as cirrhosis.⁶¹ Radiofrequency ablation may be ineffective in larger lesions, where adjunctive therapy may provide a preferred solution. For example, the advantages of preceding RFA treatment with transarterial chemoembolization (TACE) has been reported.⁴⁶ Furthermore, combined RFA with concomitant percutaneous alcohol injection has shown better results than either of the techniques alone.⁶² Cytotoxic drugs and irradiation have also been utilized as adjuncts.⁶³⁻⁶⁵

Microwave Ablation

Microwave ablation creates heat through the excitation of water molecules, increasing kinetic energy and elevating the

tissue temperature. Most microwave-ablation devices operate at 2.45 GHz, delivering 60 W, and can produce tissue temperatures as high as 150°C. Newer devices operate at 915 Hz, use water-cooled antennas, and can deliver 80 W.⁶⁶ Microwave devices deliver energy via antenna(s), which are of various designs and offer several types of tissue-heating patterns.

Microwave ablation has been used for many types of tumors, including those of the liver, lung, breast, and bone.⁴⁸ When used for cancer of the liver, the results of microwave are similar to those achieved by radio frequency.⁶⁷ When applied in patients with lung cancer, microwave ablation under ultrasound guidance achieved a 50% to 60% five-year survival rate.^{68,69} Although effective, higher microwave power can cause injury to other tissues, especially the skin. Other reported injuries include liver abscess, perforation of the colon, tumor seeding, pleural effusion requiring thoracentesis, hemorrhage requiring arterial embolization, fever, and pain.⁷⁰ Common difficulty with microwave ablation and RFA is the nonuniform energy deposition of electromagnetic radiations, which may result in hot spots and difficulty to match the temperature field with the geometry of the tumor and criteria for thermal ablation success.

High-Intensity-Focused Ultrasound

High-intensity-focused ultrasound (HIFU) thermal ablative therapy uses ultrasound waves focused transcutaneously on the tumor, heating the target tissue, and producing necrosis. Treatment is guided by imaging via US or MRI to enable treatment targeting and monitoring. When used for breast tumors, a transducer is placed on the skin over the tumor. The high-frequency US waves in the range of 0.5 to 4.0 MHz are focused on the tumor. Focusing a beam of high-intensity US waves via acoustic lens results in discrete volumes of ablated tissue where the focal point of the beams converges. The thermal effect of HIFU is the primary mechanism of tissue destruction, whereby lipid membranes melt, proteins denature, ultimately resulting in coagulative necrosis. By shifting the focal point or “painting” the target, a 3-dimensional (3-D) volume of tissue can be destroyed. Cellular damage is determined by both the length of time and temperature the tissue receives. Cavitation, a process in which microbubbles form, expand, and interact with the US beam, also occurs to some degree during HIFU, and these cavitation bubbles cause reflection of the US beam. With inertial cavitation, these microbubbles can become quite hot, implode, and collapse, generating shock waves that can further damage tissue. However, the process of cavitation often produces less predictable damage in terms of targeted ablation. For effective application, it is recommended to achieve a temperature of 56°C or more for at least a second.⁴⁸ The results are dependent upon patient selection, ablation margin, treatment planning, treatment time, and related factors.⁷¹ When used for liver tumors, the need for accurate imaging for success in therapy is evident.⁷² One of the significant difficulties with HIFU ablation is the relatively small area in which the abovementioned conditions may be achieved instantaneously. The

common solution for this difficulty is a series of heating events, where the focal application is sequentially relocated to cover the entire tumor as would be done in HIFU application in the prostate.⁷³

Ryan et al compared pretreatment to posttreatment prostate biopsies taken at a mean of 14.1 months from men who underwent primary HIFU therapy.⁷⁴ A total of 51% of 45 men demonstrated posttreatment biochemical failure, and of those 77% had positive biopsies. An additional 25% of men without rising PSA (prostate specific antigen) were found to have cancer by biopsy performed during routine follow-up. Benign, unaltered tissue was seen in 29 of 30 biopsied prostates. Similarly, Biermann et al detected posttreatment prostate cancer in 44% of 25 men biopsied 6 months after primary HIFU.⁷⁵ In this study, tumors were identified within unaltered stroma suggesting incomplete ablation. At the Montreal Focal Therapy (2012) meeting,⁷⁶⁻⁷⁸ various participants expressed a number of concerns over HIFU including (1) a predefined uniform length of the HIFU does not match the prostate’s geometry, (2) distal coverage is uncertain due to an edge effect which may explain edge recurrence, (3) overtreatment where the sequential applications of heating overlap, (4) real-time observation is not possible and device-based indicators may not be accurate, and (5) unexplained adverse posttreatment pathological features.^{76,78,79} These features include a “reactive stroma” containing tumor glands and blood vessels in the margins and regrowth of smooth muscle, nerve tissue, androgen staining cells and myofibroblasts. The interest in minimally invasive HIFU therapy for localized prostate cancer is shared by both patients and physicians alike in that it is a repeatable, outpatient therapy with a purported low complication rate. Adjuvant therapy to boost the lethal effect of the ablative energy remains an unmet need and would likely improve cancer outcomes.

Laser Therapy

Interstitial laser therapy is a minimally invasive procedure that functions by a refraction of laser light on the tumor. In this treatment, light energy is absorbed by the tissue and converted into heat, which causes coagulative necrosis in part by damaging the endothelial cells of the microvasculature.⁸⁰ The technique requires the insertion of a fiber-optic probe percutaneously into the center of the tumor with guidance by fluoroscopy, US imaging, or MRI. Tissue temperature is measured by thermal sensors placed at the periphery of the tumor or by near real-time magnetic resonance thermometry. Laser energy is delivered until the temperature at the periphery exceeds 50°C. Two broad types of laser therapies are available: laser-induced interstitial thermotherapy and photodynamic therapy (PDT). Laser-induced interstitial thermotherapy is also referred to as interstitial laser photocoagulation or focal laser ablation and is a similar treatment to hyperthermia in its mechanisms of action. Laser-induced interstitial thermotherapy involves an optical fiber, which heats up and increases the temperature within the cancer cells. Although PDT is a nonthermal application, it is described here in brief for the completeness of

presentation. First, a photosensitive agent is injected intravenously, which tends to break down and become toxic under intense illumination. During application, strategically operated laser probes locally activate the photosensitive agent by strategic illumination.

Generally, laser for medical applications is generated from 3 sources: carbon dioxide (CO₂; infrared; wavelength of 10 600 nm), argon (488–518 nm), and neodymium:yttrium–aluminum–garnet (Nd:YAG; wavelength of 1060 nm). The CO₂ laser is used to destroy superficial tumors such as those involving the skin, oropharyngeal cavity, and cervix. The argon laser is excellent at superficial coagulation of organs and has been used in the eye, ear, dermatology, and various solid organs. The Nd:YAG laser is generally used to destroy cancer cells that are located in internal organs (ie, esophagus and colon). Interstitial laser therapy has been used for the treatment of early breast cancers and has shown success in 67% of patients.⁸¹ The treatment is best suited to invasive ductal cancers of no greater than 2 cm in diameter.⁸² The technique also has successful results in the treatment of fibroadenomas of the breast,⁸³ liver tumors, breast cancers metastatic in the liver,⁸⁴ and the palliative treatment of advanced breast cancers.⁸⁵ To focally ablate small prostate cancers, a 980-nm diode laser is commonly utilized (15 or 30 W). However, the laser itself has issues associated with precision which makes destroying and managing the perimeter of the cancer in order to achieve maximal cancer control difficult. Thus, therapies based on high-energy density, such as laser treatment, would benefit from adjuvant measures.

Subtractive Thermal Ablation

Cryoablation

Cryotherapy uses freezing to destroy tumors. The technique requires the use of a cryosurgical apparatus cooled by any one of a variety of cryogens, including nitrogen (gas, liquid, and supercritical), argon, nitrous oxide, or carbon dioxide. The apparatus range in type from small hand-held units to automated devices cooled by liquid nitrogen or pressurized gases, capable of cooling multiple probes during application to the tumor. Minimally invasive cryosurgical devices today are commonly based on the Joule-Thomson cooling of argon and 17- to 10-gauge cryoprobes.

Standard cryosurgical technique for prostate cancer requires that the tumor be frozen rapidly, thawed slowly, and then, in many applications, immediately exposed to a repeated freeze–thaw cycle. In the treatment of tumors, a tissue temperature of –40°C is typically recommended to be achieved in the repeated freeze/thaw cycle at the edge of the target region.⁸⁶ The need for the double freeze–thaw cycle is linked to the relatively slow rate of cooling associated with the edge of the frozen region that is less destructive than faster rates within the core of the lesion. The use of imaging techniques, such as US, CT, or MRI, which can visualize both cryoprobe placement and the freezing process, is critical for the minimally invasive procedure.

The mechanism of injury due to freezing features directs cell injury associated with ice crystal formation. Upon thawing, vascular stasis develops and the loss of circulation increases the certainty of tissue death. Throughout the frozen zone and especially in the periphery, where some cell survival may be expected because of the elevated freeze temperature, apoptosis (and possibly autophagy) contributes to cell death.⁸⁷ Cellular responses to a freeze–thaw cycle are complex and indicative of a nonhomogeneity of the target region, as not all cells freeze at the same temperature and rate, localized vascular effects may affect freezing, and the thermal history that each cell experiences is dependent on its relative location to the cryoprobe(s). To the pathologist, the tissue response to freezing ranges from inflammatory, as would follow minor freezing injury, to necrotic, which follows severe injury.⁸⁶

Cryosurgery has a wide range of uses in tumor treatment, virtually including every tissue and organ.⁸⁸ In the treatment of prostate cancer, the long-term disease-free survival (10-year follow-up) for low- to moderate-risk disease states has been reported at 80% and 75%, respectively.⁸⁹ These outcomes compare favorably with external radiotherapy and brachytherapy.⁹⁰ In 2008, the American Urological Association published a “Best Practice Policy Statement,” which acknowledged that cryosurgery was an accepted treatment for selected patients with early-stage disease as well as for salvage therapy.⁹¹ Recently, substantial interest has developed in focal or partial prostatic cryotherapy. The stimulus for this approach is the high incidence of erectile dysfunction after total gland cryosurgery. As with prostate, liver cancer has also been treated extensively using cryosurgery, with the best results being achieved in small single tumors of <3 cm in diameter. The short-term results of about 70% success are competitive with rRFA.⁹² Cryosurgery of kidney tumors, performed percutaneously or via laparoscopy, is an effective therapy yielding a 3-year cancer-specific survival rate of 98%.⁴² Cryoablation has also been used in the treatment of small breast cancers of no >1.5 cm, and it was found that the technique can eliminate the disease if no ductal carcinoma *in situ* exists.⁹³ Cryosurgery may also be used for palliative benefits in advanced breast cancers, providing pain relief, reduction in tumor size, and discharge.

The mechanisms of cryoablative damage are more comprehensively described when compared to mechanisms involved in high-temperatures therapy. Freezing imparts a varying but substantial level of physical damage on tumor cells. This variance is due to strength–duration relationships (ie, cooling rate, duration at nadir temperature, and rate of thaw) and to the “resistance” to freezing by some cells. Ice first forms in the spaces between cells and the capillary lumen but is not necessarily lethal. As the content of ice increases during continued cooling, physiological stresses have a cumulative impact. One key stressor, physiological “dehydration,” occurs as water is converted into ice resulting in freeze concentration of ions and low-molecular-weight agents (solute) within the unfrozen fraction, thereby resulting in log order elevations in tissue osmolality (ie, from ~350 to >8000 mOsm). This increase in solute levels is likely a major inducer of apoptosis. In addition, cell

metabolism is disrupted, resulting in increased levels of free radicals. A cascade of cell stressors adds to the ever-increasing level of apoptosis. Since the ablative process also includes tumor capillaries, blood flow is compromised. With rising levels of tumor hypoxia, cell death pathways shift from apoptosis to secondary necrosis. The freeze margin is of particular interest, as this tumor region experiences a lesser freeze insult. Present day procedures rely on a second freezing cycle to enhance lethality. In the future, however, it may prove advantageous to use freeze-sensitizing agents in concert with cryoablation.⁹⁴⁻⁹⁸

Adjunctive Thermal Ablative Strategies

The use of the modern monitoring techniques for ablation zone imaging (US, CT, or MRI), while essential to precision, are unable to predict the outcome of the procedure due to an inability to visualize the thermal gradient within the tissue during a procedure.⁹⁹⁻¹⁰² This results in uncertainty of a tissue target's exposure to lethal temperatures above or below the critical temperature. Accordingly, thermal ablation of cancer has 2 limitations: (1) tumor recurrence from cells surviving nonlethal conditions and (2) collateral morbidity associated with unintended thermal impact (hot or cold) on adjacent anatomical structures.¹⁰³⁻¹⁰⁵ To overcome these limitations, recent efforts have focused on the development of strategies to increase cancer destruction following exposure to temperatures traditionally thought to be nonlethal. The goal of these efforts is to yield complete cancer destruction within the targeted tissue and to enable physicians to more precisely predict/visualize the zone of lethality in real-time under US or other imaging modalities. In the area of cryosurgery, this has been described as "making ice lethal at $\sim 0^\circ\text{C}$." One such strategy is the use of adjunctive agents in combination with thermal ablation.^{64,65,86,106}

Adjuvants are designed to (1) enhance the physical effects of the ablation process, (2) activate cellular stress responses and attendant cell-death cascades, and/or (3) inhibit cell survival/repair cascades. To date, numerous *in vitro*, *in vivo*, and clinical studies have shown the potential benefit of adjunctive thermal ablative strategies. The majority of activity in this area has focused on the combination of cryosurgery and low-dose drugs or other chemical agents. This strategy is often referred to as cryosensitization. These studies have demonstrated beneficial outcomes when combining cryoablation with various classes of agents in several cancers including kidney, prostate, liver, skin, pancreas, lung, and colorectal cancer, among others.^{87,96,107-115} Several studies have demonstrated the benefits of the activation of membrane receptors using tumor necrosis factor alpha (TNF- α)^{110,116-118} and TNF-related apoptosis-inducing ligand (TRAIL)¹¹⁹ in combination with mild freezing ($> -10^\circ\text{C}$), resulting in near-complete ablation of prostate cancer in various models. Other studies have reported similar outcomes in renal cancer.¹²⁰ A similar strategy using the combination of chemotherapeutic agents and cryotherapy has also shown benefit.¹²¹ Le Pivert et al¹²¹ demonstrated enhanced prostate cancer destruction with the combination of

5-fluorouracil (5-FU) pretreatment and cryoablation in a murine model. Similar results have also been reported using low-dose (subclinical) 5-FU pretreatment (1-2 days) in combination with mild freezing (-15°C) in an *in vitro* prostate cancer cell model.¹²² Forest et al^{123,124} have also demonstrated the enhanced cancer death caused by cryo-chemo combination strategies in *in vitro* and *in vivo* lung cancer models. Recently, aggressive research is moving forward in the utilization of nanoparticles as both a potential primary and an adjunctive therapy. A diverse array of nanoparticles (gold, iron, lipid encasement, and so on) are being investigated as drug carriers, contrast agents, radiosensitizers, and photothermal agents, each of which may yield an effect focused on a tumor site. Early-phase clinical trials are mostly in a patient recruitment stage. However, 2 lysosomal spheres containing chemotherapeutic agents nanoparticle adjuvants have received USA Food and Drug Administration (FDA) clearance. To date, 6 categories of adjunctive agents have been described including (1) thermophysical adjuvants,^{109,125-130} (2) chemotherapeutics,^{120-124,131-133} (3) proinflammatory cytokines or vascular-based agents,^{116,118,134} (4) immunomodulators,^{113,135-139} (5) nanoparticles, and (6) nutraceutical-based sensitization.^{95-98,116,120} The list of categories serves as a general reference for classification, since many agents are nonexclusive and they may impact several different areas/ pathways within a cell. Numerous studies have been published on such combinatorial strategies while demonstrating the benefits of utilizing multitiered approaches to treat cancer. Given the success of cryosensitization strategies coupled with the desire to minimize side effects (both systemic and localized), many investigations and ongoing trials are now incorporating these as well as other agents.

Computation Tools and Thermal Modeling in the Service of Thermal Ablation

Although the ever-increasing power of computation means and advancements in information technology are rapidly affecting every facet of medicine, and despite feasibility demonstrations for a wide range of computation tools in the service of thermal ablation, these developments are only slowly translated into practical applications for the benefit of real-time monitoring, planning, and training. To this end, this section links the underlying principles of thermal damage with state-of-the-art computation means for monitoring, planning, and training of thermal surgery while mapping concurrent directions of development in the field.

Thermal ablation is dependent on the distribution of heat sources (high-temperature applications) or heat sinks (cryoablation) within a target area. In cryotherapy, heat sources may be used in tandem with heat sinks to better shape the frozen region¹⁴⁰ and to protect critical tissues such as the urethra during prostate cryoablation.^{141,142} The frozen region in cryotherapy can be identified and its shape can be reconstructed with the application of CT or MRI, using standard imaging-analysis software tools. Unfortunately, for the case of US—the most commonly used imaging modality in

cryotherapy—only partial freezing front identification can be achieved, since the US transducer serves as both emitter and receiver. As such, only the freezing front portion facing the transducer can be identified, which in turn casts shadow on the rest of the frozen region as well as on the area behind the frozen region. Notably, several pilot studies have demonstrated the feasibility of temperature field approximation within the frozen region for the case of CT and MRI¹⁴³⁻¹⁴⁵ while relying on freezing front reconstruction. In a recent study, Thaokar and Rabin¹⁴⁶ demonstrated that the unobservable portion of the freezing front during US imaging can also be predicted to a high degree of certainty, together with the temperature field within the frozen region and its vicinity. Although critical to the application of thermal therapy, unfortunately, medical imaging is not clinically used for deep-tissue thermal mapping.

Spatial Thermal Injury Distribution and Computerized Planning Strategies

The observation that different thermal destruction mechanisms may dominate different temperature ranges is key for planning and optimization of additive energy thermal ablation.¹⁴⁷⁻¹⁴⁹ For example, protein denaturation correlates strongly with cell death by heating and is increasingly of interest in focal thermal therapies of cancer and other diseases at temperatures that often exceed 50°C.¹⁵⁰

It is equally important to recognize that the temperature field is continuous and therefore that the thermal damage varies gradually with the distance from the energy source.¹⁵¹ For example, when an entire target region is planned to be heated above 70°C for a specific ablation application, there will be a surrounding region with an almost instantaneous thermal damage where temperatures are above 50°C, and there will be a second surrounding region where the tissue exhibits only partial hyperthermic damage (temperatures below 50°C), based on the variable local time of exposure (or variable thermal dose).¹⁵¹ The partially injured surrounding region may serve as a safety margin when critical tissues are not found in the vicinity of the target region.^{152,153} When critical tissues are adjacent to the target area, some portion of the partially injured region may be contained within the target region, trading partial injury with the need for preservation. Although the thermal damage threshold of 50°C and 70°C are presented here for illustrative purposes, they may vary among medical ablation applications and tissue types as overviewed earlier. Either way, planning the medical ablation procedure such that the core injured region will conform to the tumor shape while taking into account safety margins, considerations, and calls for computation means.

Similar to high-temperature applications, cryotherapy treatments are also associated with several distinct regions based on the extent of thermal injury.¹⁵⁴ For example, a single cryoprobe operation will create a core area with temperatures below the lethal temperature, where maximum freezing injury is achieved.¹⁵⁵ An area of partial freezing injury will surround it, which extends between the lethal temperature and the onset

of pure water freezing. A third region of hypothermic injury (below normothermic conditions but above freezing) may also develop, but due to the short duration of the cryotherapy procedure, its extent is most frequently insignificant. Similar to the challenge with high-temperature applications, the location of the partly cryoinjured region must be carefully planned to minimize unwanted damage while creating safety margins.¹⁵⁶ Given the complexities associated with the thermal environment, cell response to thermal exposure, and the desire to destroy a targeted region while minimally impacting surrounding tissues, procedural planning and cryoablation control can be aided by computation tools.

In order to account for the spatial distribution of the extent of thermal injury, the concept of a *defect region* associated with the thermal lesion has been developed.¹⁵⁶ For cryotherapy, the defect region includes areas internal to the target region having temperatures above a critical temperature—the planning isotherm (ie, areas within the target where insufficient thermal damage is caused) and areas external of the target region with temperatures below the planning isotherm (ie, areas outside the target where undesired thermal damage occurs). Prior to treatment, the entire target region may be considered a defect. As the thermal process progresses, increasing areas surrounding the cryoprobes are cooled, and the defect value gradually decreases. If cooling progresses long enough, external areas will also be cooled below the planning isotherm, resulting in the development of external defect. There will be a point in time in which the defect will reach a minimum value, balancing the portion of the external defect with the internal defect, which may serve as an indicator for termination of the cooling process.

When comparing different cryoprobe layouts, the one that results in the minimum overall defect may be considered superior. Either way, the defect region concept can serve as the basis for computer-generated cryotherapy planning,¹⁵⁷⁻¹⁶⁰ training,¹⁶¹⁻¹⁶³ and analysis of past procedures. Conceptually, the defect region concept is translational from cryotherapy to any thermal ablation application, by substituting the lethal temperature with the thermal dose for planning. Additionally, in defect region calculations, different tissues may receive different weights signifying their importance for planning,¹⁵⁶ which may be equally important in high-temperature applications.

Cryoablation Planning

Whether the threshold for successful thermal surgery is the thermal dose or a specific planning temperature, predicting the 3-D shape of the resultant lesion can only be accomplished by computational means. The lesion shape must be compared to the shape of the target region to evaluate the potential quality of the procedure, where better match means higher quality. The unmet need for planning and optimization of cryotherapy has been well recognized by the research community. Keanini¹⁶⁴ reported on a numerical optimization technique for prostate cryosurgery planning, with the goal of optimizing the number of cryoprobes, their diameter, and their active length. They

employed the simplex optimization method, which works well for linear problems,^{165,166} but it is associated with a prohibitively high computation cost. Baissalov et al¹⁶⁷ have presented a model for cryosurgery planning, based on a semi-empirical and only partially automated approach, which led to an operator-specific outcome. In a later report, Baissalov et al¹⁶⁸ demonstrated that it is possible to simultaneously optimize multiple cryoprobe placements and their thermal protocol, using a gradient-descent method. This method requires a large number of consecutive heat transfer simulations, making it impractical for clinical applications. Giorgi et al¹⁶⁹ have suggested a cryoprobe optimization approach based on an ant colony optimization algorithm, which is a bioinspired probabilistic technique for solving computational problems.¹⁷⁰

Rabin¹⁷¹ has identified 4 key aspects that must be addressed in order to make computerized planning of cryosurgery a practical reality: (1) a well-established set of criteria for planning, (2) a well-defined criterion for termination of cooling, (3) automation methods, and (4) clinically relevant runtime for automated planning (measured in seconds). It is suggested there that a special consideration should be given to 3D planning for prostate cancer treatment in realistic shapes. Orchestrated efforts to address those aspects have included the development of (1) an algorithm for interactive prostate model reconstruction,¹⁷² (2) a method to simulate the 3-D-shape evolution of the prostate as the cancer progresses,¹⁷³ (3) a weighted defect region as a criterion for cryosurgery optimization and cryoprobe termination,¹⁵⁶ (4) the robust force-field analogy algorithm for cryosurgery planning,^{156,157} (5) the low-cost bubble-packing algorithm for cryosurgery planning,^{157,160,174,175} (6) a rapid numerical technique for cryosurgery simulation,¹⁷⁶ and (7) its parallel implementation on a graphics processing unit.^{177,178} For example, current automated 3-D planning for prostate cryosurgery based on bubble packing, considering a urethral warmer and 14 cryoprobes in a variable insertion depth, is measured in 2 to 3 seconds,^{179,180} opening the door for a host of clinically relevant computation tools.

Computerized Training in Cryotherapy

Recently, efforts by Rabin and coworkers have led to the development of the first software prototype for cryosurgery tutoring.^{161-163,180} This prototype lists geometrical constraints on cryoprobe placement, displays a rendered shape of the prostate, simulates cryoprobe insertion, enables distance measurements as would be facilitated by US imaging, simulates the corresponding thermal history,¹⁷⁸ and evaluates the mismatch between the target region shape and a pre-selected planning isotherm in terms of defect values. The quality of a trainee planning is measured in comparison to a computer-generated plan, created for each case study using the bubble-packing algorithm.^{157,158} Although the tutoring level in this study aims only at geometrical constraints on cryoprobe placement and the resulting thermal history, this program creates a unique opportunity to gain insight into the process outside of the operation room. Validation of the computerized tutor has been performed

by collecting training data from surgical residents, having no prior experience or advanced knowledge of cryotherapy.^{161,162} In terms of match between a planning isotherm and the target region shape, results demonstrate medical residents' performance improved from 4.4% in a pretest to 37.8% in a posttest, following a single 50-minute training session (within 10% margins from a computer-optimized plan).

In order to create a virtual reality environment for computerized training and education, an US simulator has been further developed, which simulates synthetic ultrasound images and imaging artifacts created during prostate cryosurgery.¹⁷⁹ These studies have demonstrated that computerized training of cryosurgery is feasible, which would shorten the learning curve, while tailoring the educational experience to the trainee's goals and personal learning pace.

Uncertainty in Computer Modeling of Thermal Therapy

An outstanding question in mathematical modeling and computer simulations is related to how close computer predictions are to the thermal therapy outcomes. When thermal mapping in real time is of concern, one may wish to identify the location of a particular isotherm with the same certainty that medical imaging facilitates—typically measured in 1 to 2 mm.^{174,181,182} However, one must bear in mind that, with the exception of the freezing front in cryotherapy, there is no substitute to 3D mapping by computerized means.

Key sources of uncertainty in bioheat transfer simulations are associated with the detailed knowledge of the thermophysical properties of biological tissues, the anatomy, and with the vascular topology and blood flow rate. The classic bioheat equations developed by Pennes¹⁸³ is most frequently used for clinical applications, albeit yielding somewhat simplistic solutions. In general, this equation is good for simulations in areas characterized by a dense capillary network and low blood flow. Numerous scientific reports have been published studying the mathematical consistency and validity of the abovementioned classic equation while exploring various alternatives as reviewed by Charny¹⁸⁴ and Diller.¹⁸⁵ It is important to note that high-temperature applications result in an increased blood flow rate before critical damage to the vascular system, whereas cryoablation results in an opposing trend of decreased blood flow, until complete arrest upon freezing. Furthermore, the latent heat effect of freezing (ie, the amount of energy required for phase change) is typically an order of magnitude greater than the heating effect of caused by blood flow,¹⁸⁶ which frequently makes the classic bioheat equation a choice of practice for cryoablation applications. Either way, the effect of uncertainty in the thermophysical properties on the simulation outcome must also be evaluated by computation means.¹⁸⁷

Uncertainty in Temperature Data Interpretation

A unique source of uncertainty in measuring temperature during thermal ablation may come about through heat conduction by the temperature sensor itself.¹⁸⁸ Most frequently,

temperature sensors are embedded at the tip of long hypothermic needles and are strategically positioned by similar means to thermal probes. The hosting needle is made of stainless steel, which is characterized by 20-fold higher thermal conductivity compared to the surrounding tissues. This means that the thermal probe may create local cooling effect in the case of high temperature applications, by more effectively conducting heat away from the heated target region. Conversely, the temperature sensor may conduct heat to the target region during cryotherapy. This does not mean that temperature readings are necessarily in error, but it does mean that the temperature sensor may distort the local temperature where it is positioned, an effect that must be considered during interpretation of measured data.

Summary and Conclusions

Thermal ablation has established therapeutic benefit, yet for most cancers remains a tertiary or quaternary treatment option behind surgery, chemotherapy, and radiation. This is despite the fact that in many cancers, including prostate and renal cancer, cryoablation has been shown at least as effective, especially when taking into considerations of safety and minimizing side effects than many of today's preferred gold-standard treatments. The combination of the physical and molecular assault to a tumor elicited by cryoablation provides a unique, nonrepetitive, multimodal "1 to 2 punch" to destroy cancer. The combination of the physical and molecular assault to a tumor elicited by cryoablation, thereby overcomes many of the innate defensive strategies of the heterogeneous population of cancer and CSCs, which make up the complex microenvironment of a tumor. Additionally, many cryoablation strategies include the added benefits of minimally invasive procedures, which can be administered in the same-day outpatient setting. These can reduce complication rates and comorbidities compared to gold standard or other primary treatments, including surgery, radiation, and chemotherapy.^{189,190}

Whether applied as a monotherapy or in conjunction with sensitization/adjunctive agents, the therapeutic benefit of cryoablation results in both a molecular and a physical insult on a targeted tissue, making this form of thermal ablation an effective treatment paradigm for many cancers. Diverse thermal ablative strategies provide a combinatorial assault by overwhelming cancer cells' defensive responses not often attained with traditional monotherapies. Furthermore, the development of new computer hardware and computation means for visualization, training, planning, and real-time monitoring are providing new technological pathways to improve the efficacy and precision of today's thermal ablation devices and practices. Accordingly, a key challenge that computational tools can help alleviate in an effort to improve the outcome of thermal ablation is the correlation between the developing thermal field, the shape of the area to be treated, criteria for clinical success, and accepted best clinical practices.^{157,158,171} Together, the molecular, adjunctive and computational advances, coupled with advances in cryoablation device technology, including new and

more powerful cryogens, smaller cryoprobes and multimodal ablation probes, are poised to redefine the thermal ablation landscape. Given the positive outcomes and associated benefits, thermal ablation is well positioned to become a primary approach to treat cancer.

Acknowledgments

The authors wish to express their appreciation to Dr Andrew Gage, MD, for his diligent efforts in reviewing and editing this manuscript.

Declaration of Conflicting Interests

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: J. M. B., K. K. S., K. L. S., and R. V. B. are employees of CPSI Biotech. T. J. P. is an education/training consultant for Health-Tronics, Inc. Y. R. is the Editor of the Special Collection on Cryotherapy and an Associate Editor of TCRT.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: Support for this manuscript was provided in part by the NIH.

References

1. Coventry BJ, Ashdown ML. Complete clinical responses to cancer therapy caused by multiple divergent approaches: a repeating theme lost in translation. *Cancer Manag Res.* 2012;4:137-149.
2. American Cancer Society, Inc. *Cancer Facts & Figures 2016.* Atlanta, GA: American Cancer Society Inc; 2016.
3. Merlo LM, Pepper JW, Reid BJ, Maley CC. Cancer as an evolutionary and ecological process. *Nat Rev Cancer.* 2006;6(12):924-935.
4. Tian T, Olson S, Whitacre JM, Harding A. The origins of cancer robustness and evolvability. *Integr Biol (Camb).* 2011;3(1):17-30.
5. Chabner BA, Roberts TG, Jr. Timeline: chemotherapy and the war on cancer. *Nat Rev Cancer.* 2005;5(1):65-72.
6. Corn PG. The tumor microenvironment in prostate cancer: elucidating molecular pathways for therapy development. *Cancer Manag Res.* 2012;4:183-193.
7. Bonnet D, Dick JE. Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. *Nat Med.* 1997;3(7):730-737.
8. Ghisolfi L, Keates AC, Hu X, Lee DK, Li CJ. Ionizing radiation induces stemness in cancer cells. *PLoS One.* 2012;7(8):e43628.
9. Sun Y, Campisi J, Higano C, et al. Treatment-induced damage to the tumor microenvironment promotes prostate cancer therapy resistance through WNT16B. *Nat Med.* 2012;18(9):1359-1368.
10. Hanahan D, Coussens LM. Accessories to the crime: functions of cells recruited to the tumor microenvironment. *Cancer Cell.* 2012;21(3):309-322.
11. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell.* 2011;144(5):646-674.
12. Davies MA, Samuels Y. Analysis of the genome to personalize therapy for melanoma. *Oncogene.* 2010;29(41):5545-5555.
13. Lemmon MA, Schlessinger J. Cell signaling by receptor tyrosine kinases. *Cell.* 2010;141(7):1117-1134.

14. Wertz IE, Dixit VM. Regulation of death receptor signaling by the ubiquitin system. *Cell Death Differ.* 2010;17(1):14-24.
15. Witsch E, Sela M, Yarden Y. Roles for growth factors in cancer progression. *Physiology.* 2010;25(2):85-101.
16. Hanahan D, Folkman J. Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. *Cell.* 1996;86(3):353-364.
17. Baeriswyl V, Christofori G. The angiogenic switch in carcinogenesis. *Semin Cancer Biol.* 2009;19(5):329-337.
18. Ferrara N. Vascular endothelial growth factor. *Arterioscler Thromb Vasc Biol.* 2009;29(6):789-791.
19. DeBerardinis RJ, Lum JJ, Hatzivassiliou G, Thompson CB. The biology of cancer: metabolic reprogramming fuels cell growth and proliferation. *Cell Metab.* 2008;7(1):11-20.
20. Hardee ME, Dewhirst MW, Agarwal N, Sorg BS. Novel imaging provides new insights into mechanisms of oxygen transport in tumors. *Curr Mol Med.* 2009;9(4):435-441.
21. Jones RG, Thompson CB. Tumor suppressors and cell metabolism: a recipe for cancer growth. *Genes Dev.* 2009;23(5):537-548.
22. Semenza GL. HIF-1: upstream and downstream of cancer metabolism. *Curr Opin Genet Dev.* 2010;20(1):51-56.
23. Lowe SW, Cepero E, Evan G. Intrinsic tumour suppression. *Nature.* 2004;432(7015):307-315.
24. Adams JM, Cory S. The Bcl-2 apoptotic switch in cancer development and therapy. *Oncogene.* 2007;26(9):1324-1337.
25. Junttila MR, Evan GI. p53-a Jack of all trades but master of none. *Nat Rev Cancer.* 2009;9(11):821-829.
26. Blasco MA. Telomeres and human disease: ageing, cancer and beyond. *Nat Rev Genet.* 2005;6(8):611-622.
27. Ince TA, Richardson AL, Bell GW, et al. Transformation of different human breast epithelial cell types leads to distinct tumor phenotypes. *Cancer Cell.* 2007;12(2):160-170.
28. Artandi SE, DePinho RA. Telomeres and telomerase in cancer. *Carcinogenesis.* 2010;31(1):9-18.
29. Cavallaro U, Christofori G. Cell adhesion and signalling by cadherins and Ig-CAMs in cancer. *Nat Rev Cancer.* 2004;4(2):118-132.
30. Bex G, van Roy F. Involvement of members of the cadherin superfamily in cancer. *Cold Spring Harb Perspect Biol.* 2009;1(6):a003129.
31. Brabletz T, Jung A, Spaderna S, Hlubek F, Kirchner T. Opinion: migrating cancer stem cells - an integrated concept of malignant tumour progression. *Nat Rev Cancer.* 2005;5(9):744-749.
32. Kreso A, Dick JE. Evolution of the cancer stem cell model. *Cell Stem Cell.* 2014;14(3):275-291.
33. Teng MW, Swann JB, Koebel CM, Schreiber RD, Smyth MJ. Immune-mediated dormancy: an equilibrium with cancer. *J Leukoc Biol.* 2008;84(4):988-993.
34. Vajdic CM, van Leeuwen MT. Cancer incidence and risk factors after solid organ transplantation. *Int J Cancer.* 2009;125(8):1747-1754.
35. Bindea G, Mlecnik B, Fridman WH, Pages F, Galon J. Natural immunity to cancer in humans. *Curr Opin Immunol.* 2010;22(2):215-222.
36. Cho RW, Clarke MF. Recent advances in cancer stem cells. *Curr Opin Genet Dev.* 2008;18(1):48-53.
37. Schafer M, Werner S. Cancer as an overheating wound: an old hypothesis revisited. *Nat Rev Mol Cell Biol.* 2008;9(8):628-638.
38. DeNardo DG, Andreu P, Coussens LM. Interactions between lymphocytes and myeloid cells regulate pro- versus anti-tumor immunity. *Cancer Metastasis Rev.* 2010;29(2):309-316.
39. Nagy JA, Chang SH, Shih SC, Dvorak AM, Dvorak HF. Heterogeneity of the tumor vasculature. *Semin Thromb Hemost.* 2010;36(3):321-331.
40. Wilt TJ, Brawer MK, Jones KM, et al; Prostate Cancer Intervention versus Observation Trial (PIVOT) Study Group. Radical prostatectomy versus observation for localized prostate cancer. *N Engl J Med.* 2012;367(3):203-213.
41. Hildebrandt B, Wust P, Ahlers O, et al. The cellular and molecular basis of hyperthermia. *Crit Rev Oncol Hematol.* 2002;43(1):33-56.
42. Goel RK, Kaouk JH. Probe ablative treatment for small renal masses: cryoablation vs. radio frequency ablation. *Curr Opin Urol.* 2008;18(5):467-473.
43. Mertyna P, Hines-Peralta A, Liu ZJ, Halpern E, Goldberg W, Goldberg SN. Radiofrequency ablation: variability in heat sensitivity in tumors and tissues. *J Vasc Interv Radiol.* 2007;18(5):647-654.
44. Klossner DP CD, Baust JM, Van Buskirk RG, Gage AA, Baust JG. Thermal therapeutic options in the treatment of prostate cancer: Cellular responses to cryosurgery and hyperthermia. *Cryobiology.* 2006;53(3):438.
45. Baumann KW, Snyder KK, Baust JM, Van Buskirk RG, Baust JG. Monitoring the effects of dual thermal ablation on pancreatic cancer cell line PANC-1. *Cryobiology.* 2014;69(3):504-505.
46. Minami Y, Kudo M. Radiofrequency ablation of hepatocellular carcinoma: a literature review. *Int J Hepatol.* 2011;2011:104685.
47. Healey TT, Dupuy DE. Radiofrequency ablation: a safe and effective treatment in nonoperative patients with early-stage lung cancer. *Cancer J.* 2011;17(1):33-37.
48. Sharma R, Wagner JL, Hwang RF. Ablative therapies of the breast. *Surg Oncol Clin N Am.* 2011;20(2):317-339.
49. Milano MT, Zhang H, Usuki KY, Singh DP, Chen Y. Definitive radiotherapy for stage I nonsmall cell lung cancer: a population-based study of survival. *Cancer.* 2012;118(22):5572-5579.
50. Nazario J, Hernandez J, Tam AL. Thermal ablation of painful bone metastases. *Tech Vasc Interv Radiol.* 2011;14(3):150-159.
51. Livraghi T, Goldberg SN, Lazzaroni S, et al. Hepatocellular carcinoma: radio-frequency ablation of medium and large lesions. *Radiology.* 2000;214(3):761-768.
52. Yan K, Chen MH, Yang W, et al. Radiofrequency ablation of hepatocellular carcinoma: long-term outcome and prognostic factors. *Eur J Radiol.* 2008;67(2):336-347.
53. Gervais DA, Arellano RS. Percutaneous tumor ablation for hepatocellular carcinoma. *AJR Am J Roentgenol.* 2011;197(4):789-794.
54. Chen MH, Yang W, Yan K, et al. Treatment efficacy of radiofrequency ablation of 338 patients with hepatic malignant tumor and the relevant complications. *World J Gastroenterol.* 2005;11(40):6395-6401.

55. Rhim H, Lim HK, Kim YS, Choi D, Lee WJ. Radiofrequency ablation of hepatic tumors: lessons learned from 3000 procedures. *J Gastroenterol Hepatol.* 2008;23(10):1492-1500.
56. Olweny EO, Park SK, Tan YK, Best SL, Trimmer C, Cadeddu JA. Radiofrequency ablation versus partial nephrectomy in patients with solitary clinical T1a renal cell carcinoma: comparable oncologic outcomes at a minimum of 5 years of follow-up. *Eur Urol.* 2012;61(6):1156-1161.
57. Atwell TD, Schmit GD, Boorjian SA, et al. Percutaneous ablation of renal masses measuring 3.0 cm and smaller: comparative local control and complications after radiofrequency ablation and cryoablation. *AJR Am J Roentgenol.* 2013;200(2):461-466.
58. Matin SF, Ahrar K, Cadeddu JA, et al. Residual and recurrent disease following renal energy ablative therapy: a multi-institutional study. *J Urol.* 2006;176(5):1973-1977.
59. Thompson RH, Atwell T, Schmit G, et al. Comparison of partial nephrectomy and percutaneous ablation for cT1 renal masses. *Eur Urol.* 2015;67(2):252-259.
60. Wagstaff P, Ingels A, Zondervan P, de la Rosette JJ, Laguna MP. Thermal ablation in renal cell carcinoma management: a comprehensive review. *Curr Opin Urol.* 2014;24(5):474-482.
61. Salhab M, Canelo R. An overview of evidence-based management of hepatocellular carcinoma: a meta-analysis. *J Cancer Res Ther.* 2011;7(4):463-475.
62. Azab M, Zaki S, El-Shetey AG, et al. Radiofrequency ablation combined with percutaneous ethanol injection in patients with hepatocellular carcinoma. *Arab J Gastroenterol.* 2011;12(3):113-118.
63. Demanes DJ, Brandt D, Schour L, Hill DR. Excellent results from high dose rate brachytherapy and external beam for prostate cancer are not improved by androgen deprivation. *Am J Clin Oncol.* 2009;32(4):342-347.
64. Du C, Ying H, Zhou J, Hu C, Zhang Y. Experience with combination of docetaxel, cisplatin plus 5-fluorouracil chemotherapy, and intensity-modulated radiotherapy for locoregionally advanced nasopharyngeal carcinoma. *Int J Clin Oncol.* 2012;464-471.
65. Wang W, Shi J, Xie WF. Transarterial chemoembolization in combination with percutaneous ablation therapy in unresectable hepatocellular carcinoma: a meta-analysis. *Liver Int.* 2010;30(5):741-749.
66. Sun Y, Wang Y, Ni X, et al. Comparison of ablation zone between 915- and 2,450-MHz cooled-shaft microwave antenna: results in vivo porcine livers. *AJR Am J Roentgenol.* 2009;192(2):511-514.
67. Simo KA, Sereika SE, Newton KN, Gerber DA. Laparoscopic-assisted microwave ablation for hepatocellular carcinoma: safety and efficacy in comparison with radiofrequency ablation. *J Surg Oncol.* 2011;104(7):822-829.
68. Wolf FJ, Grand DJ, Machan JT, Dipetrillo TA, Mayo-Smith WW, Dupuy DE. Microwave ablation of lung malignancies: effectiveness, CT findings, and safety in 50 patients. *Radiology.* 2008;247(3):871-879.
69. Vogl TJ, Naguib NN, Gruber-Rouh T, Koitka K, Lehnert T, Nour-Eldin NE. Microwave ablation therapy: clinical utility in treatment of pulmonary metastases. *Radiology.* 2011;261(2):643-651.
70. Liang P, Wang Y, Yu X, Dong B. Malignant liver tumors: treatment with percutaneous microwave ablation—complications among cohort of 1136 patients. *Radiology.* 2009;251(3):933-940.
71. Schmitz AC, Gianfelice D, Daniel BL, Mali WP, van den Bosch MA. Image-guided focused ultrasound ablation of breast cancer: current status, challenges, and future directions. *Eur Radiol.* 2008;18(7):1431-1441.
72. Minami Y, Kudo M. Review of dynamic contrast-enhanced ultrasound guidance in ablation therapy for hepatocellular carcinoma. *World J Gastroenterol.* 2011;17(45):4952-4959.
73. Salgaonkar VA, Prakash P, Rieke V, et al. Model-based feasibility assessment and evaluation of prostate hyperthermia with a commercial MR-guided endorectal HIFU ablation array. *Med Phys.* 2014;41(3):033301.
74. Ryan P, Finelli A, Lawrentschuk N, et al. Prostatic needle biopsies following primary high intensity focused ultrasound (HIFU) therapy for prostatic adenocarcinoma: histopathological features in tumour and non-tumour tissue. *J Clin Pathol.* 2012;65(8):729-734.
75. Biermann K, Montironi R, Lopez-Beltran A, Zhang S, Cheng L. Histopathological findings after treatment of prostate cancer using high-intensity focused ultrasound (HIFU). *Prostate.* 2010;70(11):1196-1200.
76. Blana A, Rogenhofer S, Ganzer R, et al. Eight years' experience with high-intensity focused ultrasonography for treatment of localized prostate cancer. *Urology.* 2008;72(6):1329-1334.
77. Histological effects of Prostate therapies. *Montréal Conference on Focal Therapy for Prostate Cancer.* Montreal, Canada; 2012.
78. HIFU (Ablaterm): the French Hemi-ablation Study. *Montréal Conference on Focal Therapy for Prostate Cancer;* Montreal, Canada: HIFU (Ablaterm); 2012.
79. Van der Kwast T. Histological effects of prostate therapies. Paper presented at: Montreal Conference on Focal Therapy for Prostate Cancer, Montreal, Canada, 7-8 December 2012.
80. Robinson DS, Parel JM, Denham DB, et al. Interstitial laser hyperthermia model development for minimally invasive therapy of breast carcinoma. *J Am Coll Surg.* 1998;186(3):284-292.
81. Dowlatshahi K, Francescatti DS, Bloom KJ. Laser therapy for small breast cancers. *Am J Surg.* 2002;184(4):359-363.
82. van Esser S, Stapper G, van Diest PJ, et al. Ultrasound-guided laser-induced thermal therapy for small palpable invasive breast carcinomas: a feasibility study. *Ann Surg Oncol.* 2009;16(8):2259-2263.
83. Vogl TJ, Straub R, Zangos S, Mack MG, Eichler K. MR-guided laser-induced thermotherapy (LITT) of liver tumours: experimental and clinical data. *Int J Hyperthermia.* 2004;20(7):713-724.
84. Mack MG, Straub R, Eichler K, Sollner O, Lehnert T, Vogl TJ. Breast cancer metastases in liver: laser-induced interstitial thermotherapy-local tumor control rate and survival data. *Radiology.* 2004;233(2):400-409.
85. Akimov AB, Seregin VE, Rusanov KV, et al. Nd: YAG interstitial laser thermotherapy in the treatment of breast cancer. *Lasers Surg Med.* 1998;22(5):257-267.
86. Baust JG, Gage AA, Bjerklund Johansen TE, Baust JM. Mechanisms of cryoablation: clinical consequences on malignant tumors. *Cryobiology.* 2014;68(1):1-11.

87. Baust JG, Gage AA, Robilotto AT, Baust JM. The pathophysiology of thermoablation: optimizing cryoablation. *Curr Opin Urol.* 2009;19(2):127-132.
88. Gage AA, Baust JG. Cryosurgery for tumors. *J Am Coll Surg.* 2007;205(2):342-356.
89. Cohen JK, Miller RJ Jr, Ahmed S, Lotz MJ, Baust J. Ten-year biochemical disease control for patients with prostate cancer treated with cryosurgery as primary therapy. *Urology.* 2008; 71(3):515-518.
90. Long JP, Bahn D, Lee F, Shinohara K, Chinn DO, Macaluso JN Jr. Five-year retrospective, multi-institutional pooled analysis of cancer-related outcomes after cryosurgical ablation of the prostate. *Urology.* 2001;57(3):518-523.
91. Babaian RJ, Donnelly B, Bahn D, et al. Best practice statement on cryosurgery for the treatment of localized prostate cancer. *J Urol.* 2008;180(5):1993-2004.
92. Mala T. Cryoablation of liver tumours—a review of mechanisms, techniques and clinical outcome. *Minim Invasive Ther Allied Technol.* 2006;15(1):9-17.
93. Sabel MS, Kaufman CS, Whitworth P, et al. Cryoablation of early-stage breast cancer: work-in-progress report of a multi-institutional trial. *Ann Surg Oncol.* 2004;11(5):542-549.
94. Gage AA, Baust JM, Baust JG. Experimental cryosurgery investigations in vivo. *Cryobiology.* 2009;59(3):229-243.
95. Kimura M, Rabbani Z, Mouraviev V, et al. Role of vitamin D(3) as a sensitizer to cryoablation in a murine prostate cancer model: preliminary in vivo study. *Urology.* 2010;76(3):764 e714-720.
96. Santucci KL, Snyder KK, Baust JM, et al. Use of 1,25alpha dihydroxyvitamin D3 as a cryosensitizing agent in a murine prostate cancer model. *Prostate Cancer Prostatic Dis.* 2011; 14(2):97-104.
97. Baust JM, Klossner DP, Robilotto A, et al. Vitamin D(3) cryosensitization increases prostate cancer susceptibility to cryoablation via mitochondrial-mediated apoptosis and necrosis. *BJU Int.* 2012;109(6):949-958.
98. Baust JG, Bischof JC, Jiang-Hughes S, et al. Re-purposing cryoablation: a combinatorial ‘therapy’ for the destruction of tissue. *Prostate Cancer Prostatic Dis.* 2015;18(2):87-95.
99. Onik G, Porterfield B, Rubinsky B, Cohen J. Percutaneous transperineal prostate cryosurgery using transrectal ultrasound guidance: animal model. *Urology.* 1991;37(3):277-281.
100. Onik G, Rubinsky B, Zemel R, et al. Ultrasound-guided hepatic cryosurgery in the treatment of metastatic colon carcinoma. Preliminary results. *Cancer.* 1991;67(4):901-907.
101. Onik GM, Cohen JK, Reyes GD, Rubinsky B, Chang Z, Baust J. Transrectal ultrasound-guided percutaneous radical cryosurgical ablation of the prostate. *Cancer.* 1993;72(4):1291-1299.
102. Gilbert JC, Rubinsky B, Roos MS, Wong ST, Brennan KM. MRI-monitored cryosurgery in the rabbit brain. *Magn Reson Imaging.* 1993;11(8):1155-1164.
103. Caso JR, Tsivian M, Mouraviev V, Kimura M, Polascik TJ. Complications and postoperative events after cryosurgery for prostate cancer. *BJU Int.* 2012;109(6):840-845.
104. Pitman M, Shapiro EY, Hruby GW, et al. Comparison of biochemical failure definitions for predicting local cancer recurrence following cryoablation of the prostate. *Prostate.* 2012;72(16):1802-1808.
105. Saliken JC, Donnelly BJ, Rewcastle JC. The evolution and state of modern technology for prostate cryosurgery. *Urology.* 2002; 60(2 suppl 1):26-33.
106. Clarke DM, Baust JM, Van Buskirk RG, Baust JG. Addition of anticancer agents enhances freezing-induced prostate cancer cell death: implications of mitochondrial involvement. *Cryobiology.* 2004;49(1):45-61.
107. Baust JG, Gage AA, Clarke D, Baust JM, Van Buskirk R. Cryosurgery—a putative approach to molecular-based optimization. *Cryobiology.* 2004;48(2):190-204.
108. Gu XY, Jiang Z, Fang W. Cryoablation combined with molecular target therapy improves the curative effect in patients with advanced non-small cell lung cancer. *J Int Med Res.* 2011;39(5): 1736-1743.
109. Han B, Iftexhar A, Bischof JC. Improved cryosurgery by use of thermophysical and inflammatory adjuvants. *Technol Cancer Res Treat.* 2004;3(2):103-111.
110. Han B, Swanlund DJ, Bischof JC. Cryoinjury of MCF-7 human breast cancer cells and inhibition of post-thaw recovery using TNF-alpha. *Technol Cancer Res Treat.* 2007;6(6):625-634.
111. Hanai A, Yang WL, Ravikumar TS. Induction of apoptosis in human colon carcinoma cells HT29 by sublethal cryo-injury: mediation by cytochrome c release. *Int J Cancer.* 2001;93(4): 526-533.
112. Kuflik EG. Cryosurgery for skin cancer:30-year experience and cure rates. *Dermatol Surg.* 2004;30(2 pt 2):297-300.
113. Redondo P, del Olmo J, Lopez-Diaz de Cerio A, et al. Imiquimod enhances the systemic immunity attained by local cryosurgery destruction of melanoma lesions. *J Invest Dermatol.* 2007; 127(7):1673-1680.
114. Rodriguez-Bigas MA, Klippenstein D, Meropol NJ, Weber TK, Petrelli NJ. A pilot study of cryochemotherapy for hepatic metastases from colorectal cancer. *Cryobiology.* 1996;33(6): 600-606.
115. Yang WL, Addona T, Nair DG, Qi L, Ravikumar TS. Apoptosis induced by cryo-injury in human colorectal cancer cells is associated with mitochondrial dysfunction. *Int J Cancer.* 2003; 103(3):360-369.
116. Goel R, Swanlund D, Coad J, Paciotti GF, Bischof JC. TNF-alpha-based accentuation in cryoinjury—dose, delivery, and response. *Mol Cancer Ther.* 2007;6(7):2039-2047.
117. Goel R, Anderson K, Slaton J, et al. Adjuvant approaches to enhance cryosurgery. *J Biomech Eng.* 2009;131(7):074003.
118. Jiang J, Goel R, Iftexhar MA, et al. Tumor necrosis factor-alpha-induced accentuation in cryoinjury: mechanisms in vitro and in vivo. *Mol Cancer Ther.* 2008;7(8):2547-2555.
119. Clarke DM, Robilotto AT, Van Buskirk RG, Baust JG, Gage AA, Baust JM. Targeted induction of apoptosis via TRAIL and cryoablation: a novel strategy for the treatment of prostate cancer. *Prostate Cancer Prostatic Dis.* 2007;10(2):175-184.
120. Clarke DM, Robilotto AT, Rhee E, et al. Cryoablation of renal cancer: variables involved in freezing-induced cell death. *Technol Cancer Res Treat.* 2007;6(2):69-79.

121. Le Pivert P, Haddad RS, Aller A, et al. Ultrasound guided combined cryoablation and microencapsulated 5-fluorouracil inhibits growth of human prostate tumors in xenogenic mouse model assessed by luminescence imaging. *Technol Cancer Res Treat.* 2004;3(2):135-142.
122. Clarke DM, Baust JM, Van Buskirk RG, Baust JG. Chemo-cryo combination therapy: an adjunctive model for the treatment of prostate cancer. *Cryobiology.* 2001;42(4):274-285.
123. Forest V, Peoc' HM, Ardiet C, Campos L, Guyotat D, Vergnon JM. In vivo cryochemotherapy of a human lung cancer model. *Cryobiology.* 2005;51(1):92-101.
124. Forest V, Peoc' HM, Campos L, Guyotat D, Vergnon JM. Benefit of a combined treatment of cryotherapy and chemotherapy on tumour growth and late cryo-induced angiogenesis in a non-small-cell lung cancer model. *Lung Cancer.* 2006;54(1):79-86.
125. Koushafar H, Pham L, Lee C, Rubinsky B. Chemical adjuvant cryosurgery with antifreeze proteins. *J Surg Oncol.* 1997;66(2):114-121.
126. Koushafar H, Rubinsky B. Effect of antifreeze proteins on frozen primary prostatic adenocarcinoma cells. *Urology.* 1997;49(3):421-425.
127. Muldrew K, Rewcastle J, Donnelly BJ, et al. Flounder antifreeze peptides increase the efficacy of cryosurgery. *Cryobiology.* 2001;42(3):182-189.
128. Pham L, Dahiya R, Rubinsky B. An in vivo study of antifreeze protein adjuvant cryosurgery. *Cryobiology.* 1999;38(2):169-175.
129. Han B, Bischof JC. Direct cell injury associated with eutectic crystallization during freezing. *Cryobiology.* 2004;48(1):8-21.
130. Wang CL, Teo KY, Han B. An amino acidic adjuvant to augment cryoinjury of MCF-7 breast cancer cells. *Cryobiology.* 2008;57(1):52-59.
131. Ikekawa S, Ishihara K, Tanaka S, Ikeda S. Basic studies of cryochemotherapy in a murine tumor system. *Cryobiology.* 1985;22(5):477-483.
132. Mir LM, Rubinsky B. Treatment of cancer with cryochemotherapy. *Br J Cancer.* 2002;86(10):1658-1660.
133. Yuan F, Zhou W, Zhang J, et al. Anticancer drugs are synergistic with freezing in induction of apoptosis in HCC cells. *Cryobiology.* 2008;57(1):60-65.
134. Shenoi MM, Iltis I, Choi J, et al. Nanoparticle delivered vascular disrupting agents (VDAs): use of TNF-alpha conjugated gold nanoparticles for multimodal cancer therapy. *Mol Pharm.* 2013;10(5):1683-1694.
135. Udagawa M, Kudo-Saito C, Hasegawa G, et al. Enhancement of immunologic tumor regression by intratumoral administration of dendritic cells in combination with cryoablative tumor pretreatment and Bacillus Calmette-Guerin cell wall skeleton stimulation. *Clin Cancer Res.* 2006;12(24):7465-7475.
136. Gazzaniga S, Bravo A, Goldszmid SR, et al. Inflammatory changes after cryosurgery-induced necrosis in human melanoma xenografted in nude mice. *J Invest Dermatol.* 2001;116(5):664-671.
137. den Brok MH, Suttmuller RP, Nierkens S, et al. Synergy between in situ cryoablation and TLR9 stimulation results in a highly effective in vivo dendritic cell vaccine. *Cancer Res.* 2006;66(14):7285-7292.
138. Waitz R, Solomon SB, Petre EN, et al. Potent induction of tumor immunity by combining tumor cryoablation with anti-CTLA-4 therapy. *Cancer Res.* 2012;72(2):430-439.
139. Yuanying Y, Lizhi N, Feng M, et al. Therapeutic outcomes of combining cryotherapy, chemotherapy and DC-CIK immunotherapy in the treatment of metastatic non-small cell lung cancer. *Cryobiology.* 2013;67(2):235-240.
140. Rabin Y, Stahovich TF. Cryoheater as a means of cryosurgery control. *Phys Med Biol.* 2003;48(5):619-632.
141. Rabin Y, Stahovich T. The thermal effect of urethral warming during cryosurgery. *Cryo Letters.* 2002;23(6):361-374.
142. Cohen JK, Miller RJ, Shuman BA. Urethral warming catheter for use during cryoablation of the prostate. *Urology.* 1995;45(5):861-864.
143. Gilbert JC, Rubinsky B, Wong ST, Brennan KM, Pease GR, Leung PP. Temperature determination in the frozen region during cryosurgery of rabbit liver using MR image analysis. *Magn Reson Imaging.* 1997;15(6):657-667.
144. Hong JS, Wong S, Pease G, Rubinsky B. MR imaging assisted temperature calculations during cryosurgery. *Magn Reson Imaging.* 1994;12(7):1021-1031.
145. Sandison GA, Loye MP, Rewcastle JC, et al. X-ray CT monitoring of iceball growth and thermal distribution during cryosurgery. *Phys Med Biol.* 1998;43(11):3309-3324.
146. Thaokar C, Rabin Y. Temperature field reconstruction for minimally invasive cryosurgery with application to wireless implantable temperature sensors and/or medical imaging. *Cryobiology.* 2012;65(3):270-277.
147. Haen SP, Pereira PL, Salih HR, Rammensee HG, Gouttefangeas C. More than just tumor destruction: immunomodulation by thermal ablation of cancer. *Clin Dev Immunol.* 2011;2011:160250.
148. Chu KF, Dupuy DE. Thermal ablation of tumours: biological mechanisms and advances in therapy. *Nat Rev Cancer.* 2014;14(3):199-208.
149. Habash RW, Bansal R, Krewski D, Alhafid HT. Thermal therapy, part 1: an introduction to thermal therapy. *Crit Rev Biomed Eng.* 2006;34(6):459-489.
150. Qin Z, Balasubramanian SK, Wolkers WF, Pearce JA, Bischof JC. Correlated parameter fit of arrhenius model for thermal denaturation of proteins and cells. *Ann Biomed Eng.* 2014;42(12):2392-2404.
151. Dewey WC. Arrhenius relationships from the molecule and cell to the clinic. *Int J Hyperthermia.* 1994;10(4):457-483.
152. Lencioni R, Goletti O, Armillotta N, et al. Radio-frequency thermal ablation of liver metastases with a cooled-tip electrode needle: results of a pilot clinical trial. *Eur Radiol.* 1998;8(7):1205-1211.
153. Elias D, Cavalcanti A, Sabourin JC, Pignon JP, Ducreux M, Lasser P. Results of 136 curative hepatectomies with a safety margin of less than 10 mm for colorectal metastases. *J Surg Oncol.* 1998;69(2):88-93.
154. Gage AA, Baust J. Mechanisms of tissue injury in cryosurgery. *Cryobiology.* 1998;37(3):171-186.
155. Rabin Y, Julian TB, Olson P, Taylor MJ, Wolmark N. Evaluation of post cryosurgery injury in a sheep breast model using the

- vital stain 2,3,5-triphenyltetrazolium chloride. *Cryo Letters*. 1998;19(4):255-262.
156. Lung DC, Stahovich TF, Rabin Y. Computerized planning for multiprobe cryosurgery using a force-field analogy. *Comput Methods Biomech Biomed Engin*. 2004;7(2):101-110.
 157. Tanaka D, Shimada K, Rabin Y. Two-phase computerized planning of cryosurgery using bubble-packing and force-field analogy. *J Biomech Eng*. 2006;128(1):49-58.
 158. Tanaka D, Shimada K, Rossi MR, Rabin Y. Towards intra-operative computerized planning of prostate cryosurgery. *Int J Med Robot*. 2007;3:10-19.
 159. Tanaka D, Shimada K, Rossi MR, Rabin Y. Computerized planning of prostate cryosurgery with pullback operation. *Comput Aided Surg*. 2008;13(1):1-13.
 160. Tanaka D, Shimada K, Rossi MR, Rabin Y. Cryosurgery planning using bubble packing in 3D. *Comput Methods Biomech Biomed Engin*. 2008;11(2):113-121.
 161. Sehrawat A, Keelan R, Shimada K, Wilfong DM, McCormick JT, Rabin Y. Simulation-based cryosurgery intelligent tutoring system prototype. *Technol Cancer Res Treat*. 2016;15(2):396-407.
 162. Sehrawat A, Keelan R, Shimada K, Wilfong DM, McCormick JT, Rabin Y. Simulation-based cryosurgery training: variable insertion depth planning in prostate cryosurgery. *Technol Cancer Res Treat*. 2016;15(6):805-814.
 163. Joshi P, Sehrawat A, Rabin Y. Computerized planning of prostate cryosurgery and shape considerations. *Technol Cancer Res Treat*. 2017.
 164. Keanini B. Optimization of multiprobe cryosurgery. *J Heat Trans*. 1992;114(4):796-801.
 165. Vanderplaats GN. *Numerical optimization techniques for engineering design*. New York: McGraw-Hill, Inc; 1984.
 166. Kincaid D, Cheney W. *Numerical Methods*, 2nd edn. Pacific Grove, CA: Brooks Cole Publishing Co; 1996.
 167. Baissalov R, Sandison GA, Donnelly BJ, et al. A semi-empirical treatment planning model for optimization of multiprobe cryosurgery. *Phys Med Biol*. 2000;45(5):1085-1098.
 168. Baissalov R, Sandison GA, Reynolds D, Muldrew K. Simultaneous optimization of cryoprobe placement and thermal protocol for cryosurgery. *Phys Med Biol*. 2001;46(7):1799-1814.
 169. Giorgi G, Avalle L, Brignone M, Piana M, Caviglia G. An optimisation approach to multiprobe cryosurgery planning. *Comput Meth Biomech Biomed Engin*. 2013;16(8):885-895.
 170. Distributed Optimization by Ant Colonies, actes de la première conférence européenne sur la vie artificielle. Paper presented at: *actes de la première conférence européenne sur la vie artificielle*; 1991; Paris, France.
 171. Rabin Y. Key issues in bioheat transfer simulations for the application of cryosurgery planning. *Cryobiology*. 2008;56(3):248-250.
 172. Furuhashi T, Song I, Zhang H, Rabin Y, Shimada K. Interactive prostate shape reconstruction from 3D TRUS images. *J Comput Des Eng*. 2014;1(4):272-288.
 173. Sehrawat A, Shimada K, Rabin Y. Generating prostate models by means of geometric deformation with application to computerized training of cryosurgery. *Int J Comput Assist Radiol Surg*. 2013;8(2):301-312.
 174. Rossi MR, Tanaka D, Shimada K, Rabin Y. Computerized planning of cryosurgery using bubble packing: an experimental validation on a phantom material. *Int J Heat Mass Transf*. 2008;51(23-24):5671-5678.
 175. Rossi MR, Tanaka D, Shimada K, Rabin Y. Computerized planning of prostate cryosurgery using variable cryoprobe insertion depth. *Cryobiology*. 2010;60(1):71-79.
 176. Rossi MR, Tanaka D, Shimada K, Rabin Y. An efficient numerical technique for bioheat simulations and its application to computerized cryosurgery planning. *Comput Methods Programs Biomed*. 2007;85(1):41-50.
 177. Keelan R, Yamakawa S, Shimada K, Rabin Y. Computerized training of cryosurgery - a system approach. *Cryo Letters*. 2013;34(4):324-337.
 178. Keelan R, Zhang H, Shimada K, Rabin Y. Graphics processing unit-based bioheat simulation to facilitate rapid decision making associated with cryosurgery training. *Technol Cancer Res Treat*. 2016;15(2):377-386.
 179. Keelan R, Shimada K, Rabin Y. GPU-based simulation of ultrasound imaging artifacts for cryosurgery training. *Technol Cancer Res Treat*. 2017;16(1):5-14.
 180. A computerized tutor prototype for prostate cryotherapy: key building blocks and system evaluation. Paper presented at: *Proc. SPIE 10066, Energy-based Treatment of Tissue and Assessment IX*; 2017; San Francisco, CA.
 181. Rossi MR, Rabin Y. Experimental verification of numerical simulations of cryosurgery with application to computerized planning. *Phys Med Biol*. 2007;52(15):4553-4567.
 182. Thaokar C, Rossi MR, Rabin Y. A new method for temperature-field reconstruction during ultrasound-monitored cryosurgery using potential-field analogy. *Cryobiology*. 2016;72(1):69-77.
 183. Pennes HH. Analysis of tissue and arterial blood temperatures in the resting human forearm. *J Appl Physiol*. 1948;1(2):93-122.
 184. Charny KC. Mathematical models of bioheat transfer. In: Hartnett TFI J.P., Cho YI. ed. *Advances in Heat Transfer*. Waltham, MA: Academic Press; 1992: 119-156.
 185. Diller KR. Modeling of bioheat transfer processes at high and low temperatures. In: Hartnett TFI JP, Cho YI eds. *Advances in Heat Transfer*. Waltham, MA: Academic Press; 1992: 157-358.
 186. Rabin Y, Shitzer A. Numerical solution of the multidimensional freezing problem during cryosurgery. *J Biomech Eng*. 1998;120(1):32-37.
 187. Rabin Y. A general model for the propagation of uncertainty in measurements into heat transfer simulations and its application to cryosurgery. *Cryobiology*. 2003;46(2):109-120.
 188. Rabin Y. Uncertainty in temperature measurements during cryosurgery. *Cryo Letters*. 1998;19(4):213-224.
 189. Baust JM, Snyder KK, Katz J, Gage A, Van Burkirk RG, Baust JG. Investigation of neuronal cell stress response to transient freezing associated with cryosurgical ablation of the prostate. *Cryobiology*. 2010;61(3):364.
 190. Berrada MS, Bischof JC. Evaluation of freezing effects on human microvascular-endothelial cells (HMEC). *Cryo Letters*. 2001;22(6):353-366.