More Than Just Tumor Destruction: Immunomodulation by Thermal Ablation of Cancer

Sebastian P. Haen,1, 2 Philippe L. Pereira,3 Helmut R. Salih,1 Hans-Georg Rammensee,2 and Cécile Gouttefangeas2

1 Abteilung II fuer Onkologie, Haematologie, Immunologie, Rheumatologie, und Pulnologie, Medizinische Universitaetsklinik, Otfried Mueller Straße 10, 72076 Tuebingen, Germany
2 Abteilung Immunologie, Interfakultaeres Institut fuer Zellbiologie, Auf der Morgenstelle 15, 72076 Tuebingen, Germany
3 Klinik fuer Radiologie, Minimalinvasive Therapien, und Nuklearmedizin, SLK-Kliniken Heilbronn, Am Gesundbrunnen 20-26, 74078 Heilbronn, Germany

Correspondence should be addressed to Sebastian P. Haen, sebastian.haen@med.uni-tuebingen.de

Received 29 June 2011; Accepted 25 August 2011

Academic Editor: Nejat Egilmez

Copyright © 2011 Sebastian P. Haen et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Over the past decades, thermoablative techniques for the therapy of localized tumors have gained importance in the treatment of patients not eligible for surgical resection. Anecdotal reports have described spontaneous distant tumor regression after thermal ablation, indicating a possible involvement of the immune system, hence an induction of antitumor immunity after thermoinduced therapy. In recent years, a growing body of evidence for modulation of both adaptive and innate immunity, as well as for the induction of danger signals through thermoablation, has emerged. Induced immune responses, however, are mostly weak and not sufficient for the complete eradication of established tumors or durable prevention of disease progression, and combination therapies with immunomodulating drugs are being evaluated with promising results. This article aims to summarize published findings on immune modulation through radiofrequency ablation, cryoablation, microwave ablation therapy, high-intensity focused ultrasound, and laser-induced thermotherapy.

1. Introduction

The local application of high or low temperatures is frequently used to induce protein denaturation, tissue necrosis, and tumor destruction in order to curatively or palliatively treat localized primary or secondary tumors [1]. Thermal ablative procedures in clinical practice comprise radiofrequency (RF) ablation, microwave ablation therapy (MWA), high-intensity focused ultrasound (HIFU), and laser-induced thermotherapy (LITT) with the use of high temperatures, as well as cryoablation with induction of low temperatures. Primarily all these techniques were applied for the palliative treatment of patients not eligible for surgical resection or frail patients with a reduced functional reserve capacity and many comorbidities [2, 3]. Local thermal ablative methods present several advantages as compared with surgery which include less damage to surrounding healthy tissue, greater patient comfort, for example, less pain and limitation in exercise due to wound healing, improved cosmetic results, and—in times of critical financial situations in the medical facilities—reduced cost and shorter periods of hospitalization [2, 4]. For selected patients, local thermoablative techniques have similar clinical outcomes as compared with historical controls of surgical resection [5–8]. However, except for early hepatocellular carcinoma, no large randomized clinical trial has been performed to directly compare thermoablation and surgical resection so far [9]. In clinical routine, thermal ablation techniques have gained further importance in the treatment of small tumors as an alternative to surgical resection. Their application is limited by the size of the tumor lesions since large tumors (＞4 cm) require more expanded treatment with an increased rate of complications and local recurrence [10, 11].

The choice of the most suitable thermal ablation modality depends on different premises. Tumors located in tissues with a high impedance like lung or bone can be better
treated with cryoablation or MWA [12–14]. Other factors for the assignment to an ablation modality depend on patient characteristics and comorbidities, on the physician's choice and availability of a certain method in a respective hospital, as well as on tumor location and relative position to other anatomic structures [1]. The clinical indications and characteristic features of each technique are summarized in Table 1.

The concept of thermal treatment for cancer is not new. The first patients with cerebral tumors were already treated with RF ablation in the early 20th century, but it took until the 1990s for RF ablation to become an accepted, commonly used treatment option for primarily unresectable tumor lesions in liver, kidney, bones, and lung [15]. During RF treatment, one or more RF applicators are placed into the target tissue and high-frequency alternating current is generated, leading to frictional heating above 60°C up to 100°C inducing coagulative necrosis [2, 16]. Higher temperatures would result in desiccation and subsequent increase in tissue impedance which limits further conduction of electricity into the tissue [12]. Recent studies have shown that the clinical outcome after RF ablation is comparable or even better in comparison to that of surgical resection. Consequently, RF ablation is currently being discussed as a possible new standard for elimination of metastatic liver lesions and oligofocal hepatocellular carcinoma (HCC) [5, 8, 17] and further as a curative treatment option in HCC and metastatic stages of colorectal carcinoma (CRC) when combined with surgery [18, 19]. Early-stage non-small cell lung cancer (NSCLC) can also be successfully treated with RF ablation. However, retrospective comparative analyses of survival have shown a strong tendency to increased survival benefits for NSCLC patients treated with surgery compared to RF ablation (46 versus 33 months, \( P = 0.054 \)) [20], limiting the application of RF ablation to patients with contraindications against surgery.

MWA represents a relatively new technique using electromagnetic waves to induce high temperatures of up to more than 100°C. Here also, an active microwave antenna is placed into the tumor. Since MWA does not require the conduct of electric current, temperatures above 100°C do not result in a decline of therapeutic efficacy [12]. This method could therefore be effectively applied in tissues with higher impedance like lung and bone [12]. In humans, MWA is currently mainly applied for the treatment of HCC [21, 22].

During HIFU, ultrasound beams of high energy are applied to focus acoustic energy on a well-defined region inducing tissue vibration. Although single ultrasound beams can penetrate tissue without causing significant heat, focussing beams from multiple directions into a selected region results in a temperature rise to over 60°C and subsequently in coagulative necrosis [23, 24]. HIFU also induces acoustic cavitation which represents a classical mechanical mechanism of tissue destruction. Acoustic cavitation (the expansion and contraction of gaseous nuclei in cells through acoustic pressure) leads to collapse of mitochondria, endoplasmic reticulum, as well as nuclear and cell membranes [25]. This procedure is the only noninvasive thermal technique and allows real-time imaging of the treatment progress by ultrasound (US) [25]. However, the clinical application of HIFU is limited since the size of the multidirectional ultrasound focus is confined by technical boundaries and a treatment time as short as possible is required for an accurate ablation [24]. HIFU has been applied for the treatment of breast, liver, pancreas, kidney, bone, prostate, and soft-tissue tumors [25–27].

Laser fibers placed into a tumor lesion are used for laser ablation where photon energy conduction induced heating can reach temperatures of over 50°C. The tissue penetration depth of laser light is only 0.4 mm which implies that multiple laser fibers have to be positioned into a tumor to ensure optimal tissue destruction [28]. However, this limited penetration can facilitate the monitoring and accuracy of the ablation. This technique is experimentally used for the treatment of breast, brain, liver, bone, and prostate tumors [29, 30]. More extensively used is the clinical exertion of laser photocoagulation in retinal diseases, here also leading to retinal scarring [31].

In contrast to all the techniques mentioned above, cryoablation utilizes not high, but extremely low temperatures that sink to −160°C [32]. Cryoablation involves the evaporation of liquid gases and is a purely thermal process which does not require application of electrical current, leading to a broader applicability in high impedance tissues like lung or bone. The extent of tissue destruction can be easily monitored by direct monitoring of ice-ball formation with all conventional imaging modalities [1]. Cryoablation is used in broad clinical application, even for the treatment of retinoblastoma in children [33]. In 1–6% of cases cryoablation causes a systemic inflammatory response syndrome (SIRS), termed as the cryoshock phenomenon which represents a potentially life-threatening complication [34–38] and limits its clinical application, especially for liver tumors [39].

The observation that spontaneous regression of untreated tumors can occur after thermoablation of distant tumor masses may indicate an involvement of immune activation upon thermoablation [39–42]. The initiation, maintenance, and termination of an effective antitumor immune response requires a complex interplay between cellular (immune cells including effector and regulatory subsets) and humoral components (cytokines, chemokines, antibodies). Various constitutive or inducible danger signals released by injured cells are known to play a determinant role in alarming the immune system against self-damage. In this danger model, cells dying by physiological processes such as apoptosis will be rapidly eliminated and ignored by the immune system whereas necrotic cells releasing their content in the extracellular space will trigger an immune response [43, 44]. In particular, heat shock proteins (HSP) constitute a group of molecular chaperones which stimulate the maturation of dendritic cells (DC) and carry antigenic peptides from their cells of origin inducing subsequent priming of antigen-specific T cells [45–48]. Local ablative treatment induces necrosis which may naturally modulate all of these parameters by inducing inflammatory processes finally leading to the development of an antitumor specific immune response. A growing series of reports describing inflammatory responses, antigen release
| Treatment                | Clinical indication: primary and secondary malignancies in liver, kidney, lung, and bone [2, 8, 17, 49] | Mechanism: application of alternating RF current through tip applicator placed around and in tumor tissue resulting in heat and coagulative necrosis | Cytokines | + | [51–54] Human | Immune Modulation |
|-------------------------|-------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------|-----------|---|----------------|-------------------|
| RF ablation             |                                                                                                 |                                                                                                 | Danger    | + | [55–61] Animal |                   |
|                         |                                                                                                 |                                                                                                 | Signals   | + | [62, 63] Human |                   |
|                         |                                                                                                 |                                                                                                 | Granulocytes | + | [64, 65] Animal |                   |
|                         |                                                                                                 |                                                                                                 | DC        | + | [54, 66] Human |                   |
|                         |                                                                                                 |                                                                                                 | T cells*  | + | [61] Animal  |                   |
|                         |                                                                                                 |                                                                                                 | Treg      | + | [67] Human   |                   |
|                         |                                                                                                 |                                                                                                 | B cells   | + | [66] Animal  |                   |
|                         |                                                                                                 |                                                                                                 | Antibodies* | + | [81] Human  |                   |
| Cryoablation            | Clinical indication: primary and secondary malignancies in liver, kidney, and prostate, as well as dermatologic and ophthalmologic tumors [4, 33, 79, 80]. | Mechanism: application of cold through gaseous evaporation at the tip of a cryoprobe. Repetitive freezing and thawing cycles lead to direct cellular damage through ice crystals, vascular and endothelial injury, and eventually thrombosis and ischemia [79, 82] resulting in coagulative necrosis and apoptosis at the ablation margin | Cytokines | + | [39, 83] Animal |                   |
|                         |                                                                                                                                                             |                                                                                                 | Danger    | + | [36, 84, 85] Human |                   |
|                         |                                                                                                                                                             |                                                                                                 | Signals   | ? |                    |                   |
|                         |                                                                                                                                                             |                                                                                                 | Granulocytes | + | [86] Animal |                   |
|                         |                                                                                                                                                             |                                                                                                 | NK cells | + | [83] Animal |                   |
|                         |                                                                                                                                                             |                                                                                                 | + | [87] Animal |                   |
|                         |                                                                                                                                                             |                                                                                                 | Monocytes/ Macrophages | + | [86, 88] Animal |                   |
|                         |                                                                                                                                                             |                                                                                                 | DC        | + | [69] Animal |                   |
|                         |                                                                                                                                                             |                                                                                                 | T cells | + | [38, 69, 79, 83, 89–98] Animal |                   |
|                         |                                                                                                                                                             |                                                                                                 | Treg      | + | [91] Animal |                   |
|                         |                                                                                                                                                             |                                                                                                 | Antibodies* | + | [86, 106–113] Animal |                   |
|                         |                                                                                                                                                             |                                                                                                 | + | [41, 87, 114–116] Human |                   |
| Microwave ablation therapy (MWA) | Clinical indication: mainly used for treatment of HCC, but also other primary and secondary malignancies of the liver [21, 22] | Mechanism: application of microwaves through tip applicator leading to coagulative necrosis [21] | Cytokines | ? | [117] Animal |                   |
|                         |                                                                                                                                                             |                                                                                                 | Danger    | + |                    |                   |
|                         |                                                                                                                                                             |                                                                                                 | Signals   | ? |                    |                   |
|                         |                                                                                                                                                             |                                                                                                 | Granulocytes | + | [118] Human |                   |
|                         |                                                                                                                                                             |                                                                                                 | NK cells | + | [119] Human |                   |
|                         |                                                                                                                                                             |                                                                                                 | Monocytes/ Macrophages | + | [119] Human |                   |
|                         |                                                                                                                                                             |                                                                                                 | DC        | ? |                    |                   |
|                         |                                                                                                                                                             |                                                                                                 | T cells | + | [22, 119, 120] Human |                   |
|                         |                                                                                                                                                             |                                                                                                 | Treg      | + | [22] Human |                   |
|                         |                                                                                                                                                             |                                                                                                 | B cells | ? |                    |                   |
|                         |                                                                                                                                                             |                                                                                                 | Antibodies | ? |                    |                   |
and uptake by professional APC and antitumor adaptive immunity shows that this can indeed be the case. This review aims to summarize findings on the modulation of the immune system through high- or low-temperature-induced thermal tissue ablation of cancer in animal tumor models and cancer patients. The respective techniques are presented in the order of common clinical use.

2. Radiofrequency Ablation

RF ablation has the broadest application in cancer treatment. It is therefore not surprising that most recent data relating to the activation of the immune system through thermoablation have been obtained using this method (Table 2).

2.1. Cytokines and Stress Response. Several groups have evaluated the systemic release of cytokines, chemokines, and various stress factors after RF ablation. Serum levels of proinflammatory cytokines like interleukins IL-1β, -6, and -8, as well as TNF were found to be either increased [51–54] or unchanged [133, 134]. In general, changes were modest and transient (several hours to days after ablation) [38, 52, 53, 134]. Moreover, IL-10 could be elevated in the serum postinterventionally [54, 133]. Over all, no case of severe SIR with multiorgan failure and coagulopathy, but significant increases in body temperature, mean arterial blood pressure, and concomitantly increased serum levels of adrenaline, nor adrenaline or C-reactive protein (CRP) have been reported shortly after RF ablation [53, 54, 133, 134].

In murine models, RF ablation induced strong upregulation of mRNA and/or protein levels of HSP-70, HSP-90, and glycoprotein 96 (gp96) as well as translocation of nuclear high-mobility group protein B1 (HMGB1) into the cytoplasm of tumor cells and into the intercellular space [55–57]. More specifically, increased HSP-70 expression was shown to occur at the margin of the ablation zone, the so-called transition zone, both in animals [55, 58, 59] and in human liver cells in vivo [62]. The time frame of maximal HSP-70 expression is described to be no more than 24 hours after ablation [56, 60, 62], the protein remaining upregulated in the necrosis surrounding tissue three days after RF ablation [61]. Several factors may influence local expression of heat shock proteins after RF: in rats receiving thermal ablation in different zones of the liver, the degree of HSP-70 expression was observed to be dependent on the relative spatial position of the ablated area to larger liver vessels since the blood stream of these vessels can nourish adjacent cells preserving cellular metabolism and, hence, expression
Table 2: Studies reporting immune modulation in cancer patients and animal models treated with RF ablation.

| Species       | Tumor Model | Immunologic effect                                                                 | References |
|---------------|-------------|-------------------------------------------------------------------------------------|------------|
| Human         | HCC (n = 1) | HSP-70, HSP-90 (cytoplasm, membrane)$\uparrow$                                   | [62]       |
|               | HCC (n = 8) | Activation of myeloid dendritic cells (blood)                                     | [51]       |
|               | HCC (n = 20)| CD4$^+$ and CD8$^+$ cells (blood)$\uparrow$                                 | [70]       |
|               |             | CD3$^+$CD56$^-$, CD56$^+$CD16$^+$ cells (blood)                                   |            |
|               |             | Activity of tumor-specific T cells$\uparrow$                                      |            |
|               | HCC (n = 37)| CD3$^+$CD56$^{dim}$ cells (blood)$\uparrow$                                    | [67]       |
|               |             | Activity of CD3$^+$CD56$^{dim}$ cells$\uparrow$                                  |            |
|               | HCC (n = 20)| Tumor-antigen specific T cells (blood)$\uparrow$                                | [77]       |
|               | RCC (n = 6) | CD3$^+$HLA-DR$^+$, CD4$^+$ and CD8$^+$ cells (blood)$\uparrow$                | [71]       |
|               |             | CD56$^+$CD16$^+$ cells (blood)$\downarrow$                                       |            |
| Liver metastases (n = 8) |           | Neutrophils (blood)$\uparrow$                                            | [66]       |
| HCC (n = 4)   |             |                                                                                   |            |
| Liver metastases (n = 6) |           |                                                                                   |            |
| HCC (n = 6)   |             |                                                                                   |            |
| Liver metastases of CRC (n = 10) |             |                                                                                   |            |
| Liver metastases (n = 9) |           |                                                                                   |            |
| HCC (n = 2)   |             |                                                                                   |            |
| Liver metastases (n = 13) |           |                                                                                   |            |
| HCC (n = 4)   |             |                                                                                   |            |
| Lung metastases (n = 4) | NSCLC (n = 10) |                                                                                   |            |
|               |             | IL-8, MIP-1$\alpha$, MIP-1$\beta$ (serum)$\uparrow$                              | [54]       |
|               |             | IL-10 (serum)$\uparrow$                                                          |            |
|               |             | CD4$^+$CD25$^+$Foxp$^+$ cells (blood)$\downarrow$                                 | [78]       |
|               |             | Neutrophils (blood)$\uparrow$                                                    |            |
| Liver metastases (n = 13) |           |                                                                                   |            |
| HCC (n = 4)   |             |                                                                                   | [133]      |
| Metastases (n = 29) ± chemotherapy |             |                                                                                   |            |
| Primary tumors (n = 26) |             |                                                                                   |            |
| Metastases (n = 16) |             |                                                                                   |            |
| HCC (n = 4)   |             |                                                                                   | [63]       |
| Metastases (n = 29) ± chemotherapy |             |                                                                                   |            |
| HCC (n = 4)   |             |                                                                                   | [63]       |
| RCC (n = 2)   |             |                                                                                   |            |

Mouse (BALB/c)  

| Tumor Model | Immunologic effect                                                                 | References |
|-------------|-------------------------------------------------------------------------------------|------------|
| CT26 hEpCam ± huKS-IL2 | Antitumor activity (splenocytes)$\uparrow$                                   | [74]       |
|             | Tumor growth (distant tumor)$\downarrow$                                           |            |
|             | Tumor growth (rechallenge)$\downarrow$                                             |            |
| C26         | CD4$^+$ cells (perinecrotic)$\downarrow$                                           | [65]       |
|             | Neutrophils (perinecrotic)$\downarrow$                                             |            |
|             | Neutrophils and lymphocytes (distant metastases)$\uparrow$                         |            |
| HCC BNL ± CCL3 | CD11c$^+$ cells (blood)$\uparrow$                                   | [73]       |
|             | CD11c$^+$ cells (tumor)$\uparrow$                                                  |            |
|             | CD4$^+$ and CD8$^+$ cells (tumor)$\uparrow$                                        |            |
|             | Tumor-specific cells (tumor)$\uparrow$                                             |            |
|             | Tumor growth (distant tumor)$\downarrow$                                           |            |
|             | Tumor growth (rechallenge)$\downarrow$                                             |            |
2.2. Cellular Immunity

2.2.1. Changes in Peripheral and Intratumoral Immune Cell Subsets. Postinterventional changes in peripheral leukocyte subsets have been observed by several groups and taken as evidence for the immune modulatory effect of RF ablation. Of note, antibody tools for cell subset identification, timepoints of observation, and patient cohorts differed between published studies. A decrease of circulating CD4+CD25+Foxp3+ regulatory T cells (Treg) was observed in patients 1 month after RF ablation of lung nodules [54]. In another study including 20 HCC patients, no significant changes in T-cell subsets were detected 1 month after RF (naïve or memory CD4+, CD8+) while increased percentages of activated T cells and circulating NK cells were noted in randomly selected patients from the study cohort [70]. The same group later described a marked expansion of CD3−CD56dim effector NK cells 1 week and 4 weeks after treatment [67]. Matuszewski and colleagues evaluated lymphocyte subpopulations after RF ablation of renal cell carcinoma (RCC) in 6 patients and found a globally increased proportion of activated T cells in the majority of patients (CD3+HLA-DR+) whereas effects on CD4+, CD8+, and NK (CD56−CD16+) cells varied among individuals and at different timepoints [71]. In patients with colorectal liver metastases, but not with RCC, a transient decrease in CD3+CD4+ T cells was noted shortly (day 2) after treatment [75]. Although these and further observations are heterogeneous, they collectively suggest an impact of RF ablation on various peripheral cell subsets, including T and NK cells [61], but also neutrophils, monocytes, B lymphocytes, and even DC [51, 54, 66, 75].

The assessment of tumor-infiltrating cells before and following RF ablation is intrinsically difficult in patients and available data have been obtained in various animal models. Most reports describe infiltration of immune cells in the transition zone hours to days after treatment. Granulocytes, macrophages, plasma cells, DC, CD3+, and CD4+ cells were found [49, 64, 68]. Interestingly, neutrophils and lymphocytes could also infiltrate distant, untreated metastases [65].

2.2.2. Antitumor Specific Responses. Few data addressing the adaptive immune response to tumors after RF ablation are available.

In a transplant-tumor model of VX2-hepatoma, rabbits were randomly assigned to treatment with RF ablation or to observation. Two weeks after RF ablation, the activation of HSP in these cells [60]. Further experiments in nude rats transplanted with human HCC also suggested a correlation between applied energy and level of expression of HSP-70 and -90 [55]. As we recently described, a significant systemic release of HSP-70 into the serum can also be detected one day after treatment in RF-treated cancer patients, but serum levels did not correlate with ablation volumes, histological tumor type, and other clinical or laboratory parameters [63].
of tumor-lysate specific T cells was detected and persisted over a postinterventional observation period of 6 weeks [68]. Animals in the RF-treated group had a significant survival increase [68].

Antigen-specificity of RF-induced antitumor T-cell responses was investigated in several reports. Dromi and coworkers used a murine urothelial carcinoma expressing the male minor histocompatibility antigen HY which was inoculated to female mice. T-cell responses against MHC-class I and class II HY-derived epitopes were significantly increased in the group of mice having received tumor RF ablation as compared to control animals. This was accompanied by an enhanced control of tumor growth, including upon rechallenge [49].

In a mouse model of OVA-expressing melanoma, adoptive transfer of splenocytes from RF-treated to naive mice led to a growth retardation of OVA+-, but not OVA--tumors after rechallenge, and to complete tumor elimination in 20% of the mice. The treatment could also induce long-lasting immunity since RF-treated mice surviving the first tumor inoculation were completely protected after a second challenge 70 days later [72]. Moreover, intratumor injection of tagged-OVA led to antigen uptake and maturation of CD11c+ cells in the tumor-draining lymphnode, albeit to a lesser extent than after cryoablation which was directly compared to RF ablation in this model [69].

In patients, HCC-reactive T cells were detected with IFNγ ELISPOT in 4/20 patients before RF ablation upon stimulation of PBMC with lysate of autologous tumor cells obtained either before or after treatment. One month after RF treatment, cellular reactivity was observed in 9/20 patients, strongly suggesting an in vivo immunization effect after RF-intervention [70]. Similar results were reported in two further cohorts of HCC and CRC patients [76].

Three recent publications have addressed the antigen-specificity of the RF-induced T-cell responses in patients. Napoletano and colleagues detected an increased IFNγ production upon stimulation with MUC-1-derived glycopeptides in 2 patients treated for liver metastases and also an increase in circulating CD3+CD19+ B cells. However, the specificity of antibodies was not studied [75]. Hiroishi and coworkers investigated CD8+ T-cell responses against MAGE-1, NY-ESO-1, and GPC3 antigens in patients with HCC and found that antigen-specific T cells were already detectable in samples obtained before RF ablation, and increased in approximately half of the patients [77]. Recently, we evaluated the occurrence of tumor-antigen specific T cells or antibodies after RF ablation in 55 cancer patients and found an increase in antigen-specific antibodies, and CD4+ or CD8+ T cells in several individuals receiving RF ablation alone or shortly after chemotherapy [78].

2.2.3. Combination Therapies. All the results presented above show that RF ablation is able to induce tumor-directed immunity; however, the observed therapeutic effects are limited. Combination therapies have therefore been already tested in preclinical models, with the aim to enhance antitumor responses and protection. For OVA-expressing melanoma, CTLA-4 blockade or Treg depletion (with anti-CD25 mAb) showed improvement in tumor control and enhanced induction of OVA-specific CD8+ T cells whereas CTLA-4 mAb application without RF ablation did not mediate the same effects [72].

The coadministration of the monocyte attracting chemokine ligand 3 and inflammatory protein-1α (CCL3/MIP-1α) [73], antibody-conjugated IL-2 [74] or even chemotherapy (liposomal doxorubicin) [59] also enhanced the effects of RF ablation. Finally, whereas intratumor injection of unloaded DC did not synergize with RF-treatment, application of tumor-lysate loaded DC was reported to abrogate tumor relapse in most animals. Interestingly, while vaccination with DC alone was ineffective with regard to survival benefits, the combination with RF ablation significantly improved the survival of tumor bearing mice [49, 57].

All these reports provide a strong rationale for testing the combination of RF therapy with immune-modulating agents in cancer patients. It has to be noted that—besides RF ablation—many patients currently receive additional therapies like chemotherapy which may also influence the development of tumor-specific immune responses as recently recognized [137].

2.3. Immune Response and Clinical Course. The relationship between occurrence of antitumor immunity after RF ablation and clinical outcome still remains elusive. In HCC patients with induced tumor-specific T-cell reactivity after RF ablation, the local- and distant-site recurrence was similar [70]. In contrast, Hiroishi and colleagues observed a correlation between the frequency of tumor-antigen specific T cells and a favorable tumor-free survival in HCC patients [77]. Here, it has to be noted that patients could additionally receive transarterial chemoembolization (TACE). We have recently observed a tendency to a better survival for patients who presented with at least a twofold increase of HSP-70 in the serum one day after treatment [63]. Since patient cohorts were small in all three studies, results need further confirmation.

3. Cryoablation

3.1. Special Premises of Cryoablation. While thermal techniques utilizing lethal high temperatures have been so far mostly described to stimulate immune responses, cryoablation has been described to exert both stimulatory and suppressive effects on the immune system. These particular features could be due to the specific physiological mechanisms of cold injury including (i) direct cellular damage through formation of ice crystals, and (ii) vascular and endothelial injury with potential ischemia [82]. Whereas most other thermoablative techniques are believed to induce essentially coagulative necrosis, apoptotic cells might be also present at the outer rim of the ablation zone after cryoablation. According to the danger model, apoptotic cells do not release their cellular content (antigens, HSP, and HMGB1) and induce immunological tolerance [43, 44].
It has been proposed that larger numbers of apoptotic cells might cause tissue protection and lead to immunosuppression while larger numbers of necrotic cells could serve as immunostimulators [4]. More recently, they showed that the cryoablation modality itself, that is, rate of freeze, influences both tumor growth and T cell recruitment [79]. Moreover, technique and rate of freezing cycles could play a role in the precise mechanisms of the watershed between immunosuppression and immunostimulation after cryoablation [79]. However, this model is questioned by more recent reports showing that apoptotic cells can also exhibit significant immunostimulatory capacity [138, 139]. One other influential factor for these contradictory observations could be the timepoint of immunomonitoring: early assessment might miss immune activation and antitumor activity. Interestingly, clinical improvement could be recorded rather late after cryotreatment (up to 10 weeks) [140, 141] which is in line with the new concept that assessment of tumor response upon immunotherapy should be performed later than after conventional cytostatic therapy [142].

Table 3 only presents recent immunological observations of the past decade. Many observations reporting immunosuppression by cryoablation were made earlier and are discussed below, but are not presented in the table.

3.2. Cytokines and Stress Response. Unlike the other thermoablative methods, cryoablation induces a cytokine release syndrome (SIR—1–6.4% of all cases, with a mortality rate of 0.2–4%) [34, 35], assimilated to the cryoshock phenomenon which is clinically manifested by thrombocytopenia, disseminated intravascular coagulation (DIC), and pulmonary failure [35–38]. Cryoshock is mainly limited to ablation of hepatocytes [39]. In sheep and rats, the frequency of SIR correlated positively with the extent of cryoablated liver tissue, animals with more than 35% of ablated tissue presenting an elevated risk of SIR [37]. Moreover, cryoablation leads to significant increases of serum IFNγ TNF, IL-6, and IL-12, but not IL-10 within several hours after intervention [36, 83, 84]. In a rat model, cytokine release after cryoablation, RF ablation, and LITT was compared. Between 1 and 6 hours after cryoablation, significantly elevated serum levels of IL-6 were observed. IL-10 serum levels were slightly, but not significantly elevated [38]. In patients, TNF and IFNγ could remain elevated for up to four weeks [85]. In a model of transgenic mice overexpressing HSP-70 only a slight increase of HSP-70 expression could be observed which proved to be tissue-protective against cryonecrosis in skeletal muscle cells. Here, is has to be noted that no complete cryoablation, but only cryolesioning of skin and skeletal muscle was performed [88]. To our knowledge, no data on HSP expression after intervention [41, 86, 114]. In contrast, Muller and colleagues treated osteosarcoma in mice with cryoablation and found a decrease of tumor-binding antibodies [113].

Another effect of cryoablation was detected by Ravindranath and colleagues who observed a release of gangliosides into the circulation of CRC patients after cryoablation but not after RF ablation or surgery. At the same time, the group also described increasing titers of anti-ganglioside IgM antibodies [116]. Since anti-ganglioside antibodies have inhibitory effects on primary tumors, such as the induction of complement mediated killing [143] or apoptosis [144], production of antitumor antibodies might be one of the mechanisms underlying the immune-mediated tumor rejection following cryoablation [116].

3.3. Antibodies. The earliest reports on immune modification after cryoablation described autoantibody production against ablated normal and tumor tissues in rabbits and monkeys, as well as in patients [41, 106–112, 114]. These antibodies were essentially IgG and IgM in the serum [41, 86, 114] and at the vicinity of the ablated lesion mainly IgG and IgA [115] appearing within two weeks after intervention [41, 86, 114]. In contrast, Muller and colleagues treated osteosarcoma in mice with cryoablation and found a decrease of tumor-binding antibodies [113].

3.4. Cellular Immunity

3.4.1. Changes in Peripheral and Intratumoral Immune Cell Subsets. In rats, significantly elevated peripheral leukocyte counts—especially CD3+ and CD4+ T cells—were detectable between 1 and 14 days after intervention [38, 89]. In humans, cryoablation led to an increase of circulating T cells in few patients [99, 100]. In a randomized trial, cryoablation—compared to conventional surgery—led to increased numbers of helper T cells and activated T cells [101]. In a cohort of patients with liver metastases, an increase of the Th1/Th2 ratio was observed in the peripheral blood [84] whereas Zhou and colleagues reported a decrease of circulating CD4+ CD25+Foxp3+ Treg after cryolesioning of HCC [105].

In tumor draining lymph nodes (TDLN), increased cellularity was observed both in T-cell (paracortical) and B-cell (germinal center) areas one week after treatment. Immunologic activity could remain increased over a time span of up to 10 weeks [90, 91]. Using a xenograft model of human melanoma in nude mice, Gazzaniga and coworkers further described a massive intravascular and peritumoral recruitment of leukocytes, essentially neutrophils and macrophages after cryoablation [86]. In a mouse mammary cancer model, the number of CD4+ T cells in TDLN was augmented. Interestingly, CD4+ CD25+ Treg were more numerous after low rate freeze [79].

3.4.2. Antitumor Specific Responses. Assessment of the immune modulation by cryoablation has yielded contradictory results. Older works have pointed out immunosuppressive effects: an increase of circulating immune effector cells was not of functional relevance for tumor rejection, rather, tumor outgrowth and increased metastasis was promoted. This indicated that cryoablation might mediate deleterious effects possibly by induction of suppressor T cells, today referred to as regulatory T cells, as well as delayed development of antitumor immunity [103, 104, 145]. In line with these findings, Machlenkin and colleagues did not observe cellular activation through cryotherapy as a monotherapy [92].
Table 3: Recent Studies reporting immune modulation in cancer patients and animal models treated with cryoablation.

| Species  | Tumor          | Model | Immunologic effect                                                                 | References |
|----------|----------------|-------|------------------------------------------------------------------------------------|------------|
| Human    | CRC ($n = 110$) |       | Gangliosides ($G_{M2}, G_{D1a}, G_{T1b};$ serum)†                               | [116]      |
|          |                 |       | Antiganglioside antibodies (serum)†                                              |            |
|          | HCC ($n = 111$) |       | CD4+CD25+Foxp3+ cells (blood, ablation zone surrounding tissue)†                 | [105]      |
| Prostate | $n = 20$        |       | IFNγ †, TNF (serum)†                                                              | [85]       |
|          | $n = 12$        |       | Tumor-specific T-cell responses (blood)†                                           | [85]       |
| Prostate | $n = 20$        | ± GM-CSF | Tumor-specific T-cell responses (blood)†                                     | [87]       |
|          | RCC ($n = 6$)   | + GM-CSF | Tumor-specific T-cell responses (blood)†                                           | [87]       |
|          | Liver metastases ($n = 12$) |       | IL-6, TNF (serum)†                                                                | [84]       |
|          | CCC ($n = 3$)   |       | Th1/Th2 ratio (blood)†                                                            |            |
| Mouse (BALB/c) | CRC | Colon-26 ± krestin | CD8+ antitumor T-cell reactivity (spleen) (†)                                   | [94]       |
|          |                |       | Number of metastases†                                                             |            |
|          |                | Colon-26 ± Treg depletion ± DC + BCG | Tumor-specific CD8+ T cells (spleen)†                                          | [96]       |
|          |                |       | Tumor growth (distant tumors)†                                                    |            |
|          |                | Colon-26 ± cyclophosphamide | Tumor-specific T cells (spleen, draining lymph nodes)† | [97]       |
| Mammary  | MT-901          |       | Tumor growth (rechallenge)†                                                       |            |
|          |                |       | IFNγ, IL-12 (serum)†                                                              | [83]       |
|          |                |       | Tumor-specific T cells (draining lymph nodes but not in spleen)†                  |            |
|          |                |       | NK cell activity (spleen)†                                                        |            |
|          |                |       | Tumor growth (rechallenge)†                                                       |            |
|          |                |       | T cells (draining lymph nodes)†                                                   |            |
|          |                |       | Tumor-specific T cells (draining lymph nodes)†                                    |            |
|          |                |       | Pulmonary metastases†                                                             |            |
|          |                |       | Pulmonary metastases (high-intensity freezing)†                                   | [79]       |
|          |                |       | Pulmonary metastases (low-intensity freezing)†                                    |            |
| Melanoma | B16-OVA ± imiquimod |       | Tumor-specific T cell proliferation†                                              | [98]       |
|          | B16-MO5 ± DC    |       | Tumor growth (rechallenge)†                                                       | [92]       |
| Mouse (C57BL/6) | Melanoma | B16-OVA ± CTLA4-mAb            | DC maturation and antigen uptake (TDLN)†                                        | [69]       |
|          |                 |       | Tumor growth (rechallenge)†                                                       |            |
|          |                 | B16-OVA ± CpG                     | DC (TDLN)†                                                                        | [135]      |
|          |                 |       | CD4+, CD8+ T cells (TDLN)†                                                        |            |
|          |                 |       | OVA-specific T cells (TDLN)†                                                      |            |
|          |                 |       | Tumor growth (rechallenge after peritumoral CpG administration)†                  |            |
| Mouse (NIH (S)-nu) | Melanoma | IIB-MEL-J (human) ± GM-CSF | Neutrophils (RB6-5CG*) (tumor-surrounding tissue)†                               | [86]       |
|          |                 |       | (tumor-surrounding tissue)†                                                       |            |
|          |                 |       | macrophages (F4/80*; tumor-surrounding tissue)†                                   |            |
|          |                 |       | DC (DEC205*; tumor-surrounding tissue)†                                            |            |
In contrast, other groups demonstrated immunologic activation in cryotreated animals (Table 3). Kimura and colleagues found an increased cytotoxic activity of peripheral lymphocytes and splenocytes against a murine leukemia virus-induced lymphoma [146]. Regression of distant metastases and resistance to tumor rechallenge was described by Bagley and colleagues who found that splenic lymphocytes isolated from sarcoma-bearing mice treated with cryoablation exhibited significantly increased cytotoxic activity against sarcoma cells as compared to those obtained from mice undergoing limb amputation [93]. Increased immunologic activity could be delayed up to ten weeks after intervention [140].

Urano and colleagues observed an increased activity of tumor-specific cytotoxic T lymphocytes (CTL) seven days after cryoablation in a mouse colon-carcinoma model. These effects were only observed after ablation of a single nodule while ablation of several lesions abrogated immune-related tumor regression. Here, a threshold of ablated tissue volume that governed immune stimulation or suppression was proposed [94]. Interestingly, tumor-specific effector cells isolated from TDLN but not from the spleen or peripheral blood secreted a higher amount of IFN-γ after treatment as compared to cells obtained following surgical resection.

In an OVA-expressing melanoma model, den Brok and colleagues observed an increase of antigen-loaded DC in draining lymph nodes both after cryoablation and RF-ablation. Of note, almost double as high cell numbers were observed compared to the induction through treatment with RF ablation [69]. Moreover, the numbers of infiltrating lymphocytes in TDLN were increased. These lymphocytes produced approximately 10-fold greater amounts of IFNγ upon stimulation with irradiated mammary adenocarcinoma cells after cryoablation than after surgical resection, delayed tumor growth and reduced the number of pulmonary metastases after adoptive transfer of TDLN cells of the cryoablated tumor [95]. The protection against a tumor rechallenge with B16-OVA cells was enhanced after cryoablation (50% surviving mice after 70 days) compared with the protective effect observed after RF ablation (20% surviving after 70 days) [79, 83, 95].

3.4.3. Combination Therapies. While Machlenkin and colleagues observed no clinical benefit with cryoablation alone, the combination with an intratumoral injection of immature DC induced robust activation of CD4+ and CD8+ CTL [92]. This synergistic effect was further improved after pretreatment with anti-CD4 or anti-CD25 mAb for Treg depletion [96].

In an OVA-expressing melanoma model, CTLA-4 blockade and depletion of regulatory T cells could further enhance cryoinduced tumor-specific T-cell responses [69]. Alternatively, concomitant injection of CpG 1668, a TLR 9 ligand, had a similar effect on T-cell recruitment. The route of adjuvant injection was crucial for immune induction, peritumoral CpG application showing to be superior to distant site. Although tumor growth was delayed after combination therapy, survival benefit was not superior to treatment with cryoablation alone [135].

Conditioning with cyclophosphamide injected one day before cryoablation led to increased IFNγ production of tumor-antigen specific CD4+ T cells in a mouse colon cancer model as detected in intracellular cytokine staining, enhanced survival and even some complete remission. Three out of four animals cured with the combination therapy also survived a tumor rechallenge with no macroscopically visible tumor upon autopsy [97]. Moreover, adoptive transfer of spleen and lymph node cells from surviving mice led to an improved survival in tumor-bearing mice. Depletion experiments showed that CD8+ effectors were responsible for tumor elimination indicating that immunological memory had developed [97].

In the same OVA-expressing melanoma model used by den Brok and colleagues, Redondo and coworkers observed a clear survival advantage for mice treated with cryoablation combined with repeated topical application of imiquimod as an adjuvant indicating that TLR-7 activation can enhance tumor-specific immune responses induced by thermal treatment [98].

To sum up, all these reports suggest that combination of cryoablation with check point blockade or immunoadjuvants is a promising approach in the treatment of cancer patients.

3.5. Immune Response and Clinical Course. In patients with hormone refractory prostate carcinoma, a combination of cryoablation with injection of GM-CSF as adjuvant was evaluated. T-cell reactivity against autologous tumor tissue lysates as determined in IFNγ ELISPOT was found to be weakly increased after therapy, and no correlation could be established between the breadth of the immune response and the clinical course as measured by analysis of PSA serum levels [85, 102]. Also in a small cohort of patients with RCC,
increased cytotoxic T-cell activity and increased antitumor serum antibodies in selected patients were observed which only weakly correlated with a favorable clinical response. Here also, GM-CSF was applied as an adjuvant [87].

4. Microwave Ablation

As with RF ablation, microwave ablation therapy (MWA) induces hyperthermia leading to coagulative necrosis. The clinical application of MWA is, however, more limited than the thermoablative methods discussed above and only few groups have evaluated the immunomodulatory effects of MWA (Table 4).

4.1. Cytokines and Stress Response. MWA was described to induce HSP-70 expression in normal kidney tissue lysates obtained from treated rats, as detected with specific ELISA. However, HSP-70 expression was significantly lower upon MWA as compared to animals treated with RF ablation and cryoablation [117].

4.2. Cellular Immunity. In a mouse tumor model of HCC, only 2/10 animals experienced tumor rejection upon rechallenge after MWA, suggesting an existing but suboptimal protective antitumor immunity [118]. However, the protective effect could be improved by intratumoral coadministration of GM-CSF loaded microspheres, and even more by intraperitoneal CTLA-4 blockade. The triple combination not only led to rejection of newly inoculated tumors, but was also effective in the rejection of established distant tumors. Splenocytes isolated from the treated mice killed hepatoma cells in vitro, but not an unrelated tumor cell line. In vitro depletion experiments using mAb could further show that cytotoxicity was mediated by T cells (both CD4+ and CD8+) and NK cells, confirming that antitumor immunity was induced upon combination therapy [118].

One month after MWA, 10 patients with hypersplenism that had developed as a result of portal hypertension exhibited a transient peripheral increase of T helper cells (CD3+CD4+) and B cells, but not of cytotoxic (CD3+CD8+) T cells [22]. In a larger cohort of patients suffering from HCC, immune cell infiltration was studied by immunohistochemistry analyses of biopsy tumor samples taken either before or at different timepoints (3–30 days) after MWA application. A markedly increased infiltration of lymphocytes (predominantly CD3+ T cells, CD56+ NK cells, and macrophages, but not of B cells) was detected after MWA inside the ablated lesions, in the adjacent normal tissue and in distant untreated lesions [119].

4.3. Immune Response and Clinical Course. The density of infiltrates of lymphocytes, macrophages, and CD56+ cells into MWA-treated liver tissue correlated inversely with the risk of local recurrence [119].

Zhou and colleagues performed a phase I clinical study in ten HCC patients with chronic hepatitis B by combining local microwave tumor ablation with immunotherapy, which was applied at 3 timepoints, that is, on the day of the MWA and then on days 11 and 100. Immature and mature monocyte-derived DC loaded with autologous tumor lysate were injected into the rim between the ablation zone and normal liver parenchyma and into the groin lymph nodes, respectively. Additionally, in vitro activated lymphocytes were applied intravenously. A modest and transient effect on peripheral T-cell subsets (decrease of CD4+CD25high—possibly Treg—and increase of CD8+CD28+—differentiated CD8+ T cells—was reported one month after treatment concomitant with a reduction in hepatitis B virus load observed in some patients, but analyses of the antitumor specific responses were not performed in this study. Of note, this clinical setting does not allow determining whether the observed effects were due to the MWA itself, to the immunotherapy regimen or to the combination of both treatments [120].

5. High-Intensity Focused Ultrasound (HIFU)

In addition to mere hyperthermia, HIFU also exerts nonthermal mechanical constraints (acoustic cavitation) on treated tissues that might contribute to and modulate its effects on the immune system [122] (Table 5).

5.1. Cytokines and Stress Response. In breast cancer patients, increased HSP-70 expression was detected on the cell membrane of treated cancer cells. HSP expression was mainly found in the central necrosis zone while only a few positively stained cells were observed in the periphery [122].

5.2. Cellular Immunity

5.2.1. Changes in Peripheral and Intratumoral Immune Cell Subsets. In patients with posterior uveal melanoma [125], pancreatic carcinoma [123], osteosarcoma, HCC, and RCC [126] that were treated with HIFU, increased percentages of CD4+ T cells and a higher CD4+/CD8+ ratio were observed [125, 126]. Another study observed only statistically significant higher NK cell percentages in the peripheral blood, while other leukocyte subsets remained stable [123].

In human breast cancer specimens collected 1-2 weeks after HIFU treatment, immunohistochemistry analyses showed a significant increase of T and B cells at the margin of the ablated region as compared to HIFU-untreated tumor samples. Interestingly, a subset of these cells were activated (CD57+) and expressed perforin and granzyme B, indicating the presence of activated cytotoxic effectors [27].

5.2.2. Antitumor Specific Responses. In a model of experimental neuroblastoma, reduced secondary tumor growth after HIFU treatment was observed, although involvement of immune cells was not evaluated further [136]. Zhang and coworkers immunized mice with a vaccine consisting of a lysate of the H22 hepatoma cell line either untreated or pretreated in vivo with HIFU. Ten days after vaccination, animals received a subcutaneous tumor challenge. Tumor growth was significantly delayed in mice vaccinated with previously HIFU-treated tumor cells.
Table 4: Studies reporting immune modulation in cancer patients and animal models treated with MWA.

| Species   | Tumor Model | Immunologic effect | References |
|-----------|-------------|--------------------|------------|
| Human     | HCC (n = 82) | CD3+ cells, CD56+ cells (treated and distant tumors)! | [119]      |
|           | HCC (n = 10) | CD68+ cells (treated and distant tumors)! | [119]      |
|           | ± DC         | Phase 1 study: CD4+CD25high cells (blood)! | [120]      |
|           |             | CD8+CD28– cells (blood)! | [120]      |
| Mouse     | HCC Hepa 1–6 | Activity of tumor-specific CD4+, CD8+ cells (spleen)! | [118]      |
| (C57BL/6) | ±GM-CSF     | NK1.1+ cells (spleen)! | [118]      |
|           | ±CTLA4-mAb  | Tumor growth (rechallenge)! | [118]      |

Table 5: Studies reporting immune modulation in cancer patients and animal models treated with HIFU.

| Species   | Tumor Model | Immunologic effect | References |
|-----------|-------------|--------------------|------------|
| Human     | Breast carcinoma (n = 23) | HSP-70 (membrane)! | [122]      |
|           | Breast carcinoma (n = 48) | CD3+, CD4+, CD8+ cells (tumor)! | [27]       |
|           | Pancreatic carcinoma (n = 15) | CD20+ cells (tumor)! | [123]      |
|           | Uveal melanoma (n = 5) | CD57+ cells (tumor)! | [124]      |
|           | Osteosarcoma (n = 6) | Activity of CD8+ cells (spleen)! | [125]      |
|           | HCC (n = 5) | Cytoplytic activity (spleen)! | [126]      |
|           | RCC (n = 5) | Tumor growth (rechallenge)! | [126]      |
| Mouse     | Neuroblasto ma | C1300 ± adriamycin | [136]      |
| (Ajax)    | HCC H22 ± DC | Tumor growth (rechallenge)! | [124]      |
| Mouse     | HCC | H22 ± tumor lysate vaccine | [121]      |

NK cell phenotype was not specified.

However, survival was not different between the vaccination groups [121]. In another model, the same group utilized a DC vaccine loaded with cell debris from HIFU-treated or -untreated tumor cells. While tumor growth was again reduced, no survival advantage could be observed. However, increased activity of CD8+ splenocytes could be detected in IFNγ ELISPOT [124]. These were supported by in vitro experiments showing activation of bone-marrow derived DC upon incubation with tumor lysates and increased tumor killing by splenocytes harvested from HIFU-treated animals [121].

In contrast, several studies describe increased numbers of peripheral T cells following HIFU, which did not exert enhanced antitumor immunity [123, 125, 126].

Several groups observed a loss in tumor antigen expression in ablated prostate [23] or breast carcinoma [147, 148] lesions after HIFU. Such downregulation would be expected to lead to a reduced recognition of tumor tissue through antigen-specific T cells. Further investigations will be needed to determine whether the HIFU method is generally appropriate for efficient induction of antitumor T-cell immunity in patients.

6. Laser Ablation

Laser-induced thermotherapy (LITT) is applied widely for photocoagulation in retinal diseases, where the release of proinflammatory cytokines [127] or the activation of retin-specific T cells after panretinal photocoagulation (PRP) [149] have been described. In cancer patients, however, laser ablation is still experimental, and only scarce publications have addressed the modulation of cellular immunity through LITT (Table 6).

6.1. Cytokines and Stress Response. In patients, laser ablation led to increased levels of IL-6 and TNF-receptor 1 in the serum of patients suffering from primary and secondary malignant lesions of the liver 72 hours after treatment. Changes in the level of other proinflammatory cytokines such as TNF and IL-1β were not observed in this study [130].

In a murine model of colorectal liver metastases, LITT was also shown to enhance expression of HSP-70 at the margin of the coagulated tissue, with cytoplasmic and nuclear expression in sublethally damaged mouse hepatocytes and extraparenchymal cells. In tumor cells, this upregulation was
detected between 12 hours and 7 days after intervention, with a peak at 24 hours [131].

6.2. Cellular Immunity. In WAG rats, Isbert and colleagues induced two independent tumors in the left and right liver lobes. One of the two tumors was either ablated with LITT or surgically removed, and immune cell infiltration into the untreated remaining tumor was compared to that observed in an untreated control group. Expression of CD8, CD86, MHC-class II, and adhesion molecules was found to be increased between 1 and 10 days after LITT at the tumor invasion front as compared to resection or no treatment, indicating an influx of immune cells [128]. Moreover, the growth of the untreated tumors was found to be considerably reduced in LITT-treated animals.

Using a murine CRC model, Lin and coworkers observed an increased infiltration of CD3+ T cells into the tumor-host interface and into the tumor, as well as into the liver parenchyma and—to a certain extent—also into distant tumor lesions. Moreover, increased activation of splenocytes and tumor infiltrating lymphocytes was reported in ex vivo IFNγ ELISPOT without antigen restimulation [132]. Of note, these results were all obtained in animal models, and immune modulation after LITT has not been reported for cancer patients yet.

Taken altogether, the available results strongly suggest that laser therapy, as shown for other thermoablation methods, can stimulate antitumor immune effector cells in vivo [128, 132].

7. Conclusion and Perspectives: Implications for Anticancer Therapy

During the past two decades, numerous publications in animal models and patients have shown that local thermoablative techniques can induce or enhance tumor-specific immune responses that contribute to tumor control. Although the sequential mechanisms involved are not yet fully elucidated, several pieces of the puzzle have been identified: thermal treatment induces necrosis and can (i) lead to local inflammation, release of danger signals—for example, heat shock proteins—which may even been detected systemically; (ii) stimulate the recruitment and activation of immune effector cells, including DC, at the vicinity and most probably inside the damaged tumoral tissue. Both processes occur rapidly, that is, within a few hours to days following intervention; (iii) activate antitumor adaptive immunity, including CD4+ T cells, and antibody production which can contribute to local tumor elimination, control distant tumors including micrometastases, and establish long lasting antitumor immunological memory [69, 72, 83, 93, 97, 121]. The source of tumor-associated antigens for inducing specific T cells may be either necrotic dying cells [138, 150, 151] or sublethally damaged cells [62, 63]. Besides these direct mechanisms, the removal of tumor tissue leads to depletion of Treg and more generally may overcome local immunosuppression shifting favorably the balance towards effective antitumor immunity [61, 96, 105].

Hence, thermoablation can trigger physiological cascades necessary and sufficient for a protective immune response. Obviously, several methods can be applied successfully, suggesting that the key element is the induction of local necrosis, which can be achieved by using different settings and temperatures. High temperatures (RF ablation, MWA, HIFU, and LITT) seem rather to sustain antitumor activity whereas both immunomodulatory and immunosuppressive effects have been reported upon cryoablation. Of note, the lesions induced by high-temperature thermoablation are probably not solely of necrotic nature but may also exhibit apoptotic cells [16, 152, 153]. Whether opposite immunological outcomes are hence related to a different balance between apoptosis, necrosis, and secondary necrosis, with apoptotic cells acting more in a tolerizing or immunosuppressive fashion and necrotic cells more immunogenic, is at the moment unclear [138, 139]. Because necrosis induction can be easily visualized during thermoablative intervention, controlled necrosis might be an ideal tool for inducing enhanced immunogenic cell death [154]. Interestingly, local hyperthermia between 40°C and 44°C has been also been described to modulate immunity, as reviewed elsewhere [155].

However, it should be noted that the reported effects of thermoablation alone on the immune system are generally modest, suggesting that such treatment as a monotherapy is in general not capable of inducing sufficient immune responses for full tumor protection [97, 118]. Thermotherapy should be therefore most effective in case of a limited

| Species | Tumor | Model | Immunologic Effect | References |
|---------|-------|-------|-------------------|------------|
| Human   | CRC (n = 4) | | IL-6, TNF-R1 (serum)! | [130] |
|         | HCC (n = 3) | |                   |            |
|         | Other (n = 6) | |                   |            |
| Mouse (CBA) | Liver metastases of CRC | MoCR | HSP-70 (cytoplasm, nuclear)! | [131] |
|         | Subcutaneous CRC tumors | MoCR | CD3+ cells (tumor-host interface)! | [132] |
|         |               |       | Spontaneous IFNγ production (spleen, lymph nodes, tumor, and distant tumors)! |            |
| Rat (WAG) | CRC | CC531 | CD8, CD86, MHC-II, CD11a, and ICAM1 expression (invasion front of distant tumors)! | [128] |
of interests to declare.

None of the authors has any commercial interests or conflicts of interests to declare.

In summary, thermal ablation represents a promising component for cancer immunotherapy in the treatment of small or subclinical tumor lesions which can be attacked by the patient’s immune system. By controlled induction of physiological stress, it offers the possibility of letting the “natural” immune response develop in its whole by breaking self-tolerance. So, thermal ablation of cancer provides a therapeutic implementation of the danger model. However, the induced antitumor immunity is weak and probably not sufficient alone to eradicate established tumors, but it can synergize with some chemotherapies and immunomodulating strategies. Selecting the appropriate thermoablative method and finding optimal combinations for individual patients will be an exciting challenge for the upcoming years.

Abbreviations

CD Cluster of differentiation  
CRC Colorectal carcinoma  
CT Computed tomography  
CTLA Cytotoxic T-lymphocyte antigen  
DC Dendritic cell  
HCC Hepatocellular carcinoma  
HIFU High-intensity focused ultrasound  
HMGB1 High-mobility group protein B1  
HSP Heat shock protein  
ICAM Intercellular adhesion molecule  
IFN Interferon  
IL Interleukin  
LITT Laser-induced thermo therapy  
mAb Monoclonal antibody  
MIP Macrophage inflammatory protein  
MRI Magnetic resonance imaging  
MWA Microwave ablation therapy  
n.a. Not applicable  
NSCLC Nonsmall cell lung cancer  
RCC Renal Cell Carcinoma  
RF Radiofrequency  
TDLN Tumor draining lymph node  
TLR Toll-like receptor  
TNF Tumor necrosis factor  
US Ultrasound.

Conflict of Interests

None of the authors has any commercial interests or conflicts of interests to declare.

Acknowledgments

The authors wish to thank Lynne Yakes for editorial assistance. Work of the authors was supported by the Deutsche Forschungsgemeinschaft (DFG, Grant no. DFG RA 369/7-1), the Else-Übelmesser Stiftung, the Studienstiftung des deutschen Volkes, the Deutsche Jose Carreras Leukemia Foundation, and Hölle & Hüttner AG, as well as the fortune Program of the Eberhard Karls University of Tuebingen (Grant no. 1530-0-0). Sebastian P. Haen is supported by the Deutsche José Carreras Leukemia Foundation.

References

[1] H. Webb, M. G. Lubner, and J. L. Hinshaw, “Thermal ablation,” *Seminars in Roentgenology*, vol. 46, no. 2, pp. 133–141, 2011.

[2] P. L. Pereira, “Actual role of radiofrequency ablation of liver metastases,” *European Radiology*, vol. 17, no. 8, pp. 2062–2070, 2007.

[3] K. Katsanos, F. Ahmad, R. Dourado, T. Sabharwal, and A. Adam, “Interventional radiology in the elderly,” *Clinical Interventions in Aging*, vol. 4, no. 1, pp. 1–15, 2009.

[4] M. S. Sabel, “Cryo-immunology: a review of the literature and proposed mechanisms for stimulatory versus suppressive immune responses,” *Cryobiology*, vol. 58, no. 1, pp. 1–11, 2009.

[5] R. Lencioni, D. Cioni, L. Crocetti et al., “Early-stage hepatocellular carcinoma in patients with cirrhosis: long-term results of percutaneous image-guided radiofrequency ablation,” *Radiotherapy*, vol. 234, no. 3, pp. 961–967, 2005.

[6] A. R. Gillams and W. R. Lees, “Five-year survival in 309 patients with colorectal liver metastases treated with radiofrequency ablation,” *European Radiology*, vol. 19, no. 5, pp. 1206–1213, 2009.

[7] A. B. Thumar, E. J. Trabulsi, C. D. Lallas, and D. B. Brown, “Thermal ablation of renal cell carcinoma: triage, treatment, and follow-up,” *Journal of Vascular and Interventional Radiology*, vol. 21, no. 8, pp. S233–S241, 2010.

[8] A. Zemlyak, W. H. Moore, and T. V. Bilfinger, “Comparison of survival after sublobar resections and ablative therapies for stage I non-small cell lung cancer,” *Journal of the American College of Surgeons*, vol. 211, no. 1, pp. 68–72, 2010.

[9] M. D. Lü, M. Kuang, L. J. Liang et al., “Surgical resection versus percutaneous thermal ablation for early-stage hepatocellular carcinoma: a randomized clinical trial,” *National Medical Journal of China*, vol. 86, no. 12, pp. 801–805, 2006.

[10] E. Paulet, C. Aubé, P. Pessaux et al., “Factors limiting complete tumor ablation by radiofrequency ablation,” *CardioVascular and Interventional Radiology*, vol. 31, no. 1, pp. 107–115, 2008.

[11] B. C. Allen and E. M. Remer, “Percutaneous cryoablation of renal tumors: patient selection, technique, and postprocedural imaging,” *Radiographics*, vol. 30, no. 4, pp. 887–900, 2010.

[12] A. S. Wright, L. A. Sampson, T. F. Warner, D. M. Mahvi, and F. T. Lee, “Radiofrequency versus microwave ablation in a hepatic porcine model,” *Radiology*, vol. 236, no. 1, pp. 132–139, 2005.

[13] R. A. McTaggart and D. E. Dupuy, “Thermal ablation of lung tumors,” *Techniques in Vascular and Interventional Radiology*, vol. 10, no. 2, pp. 102–113, 2007.

[14] J. Eradat, F. Abtin, A. Gutierrez, and R. Suh, “Evaluation of treatment response after nonoperative therapy for early-stage...
Clinical and Developmental Immunology

non-small cell lung carcinoma,” Cancer Journal, vol. 17, no. 1, pp. 38–48, 2011.

[15] A. E. Siperstein and A. Gitomirski, “History and technological aspects of radiofrequency thermoablation,” Cancer Journal, vol. 6, no. 4, pp. 5293–5303, 2000.

[16] S. Claussen, S. M. Krober, B. Kosan et al., “Pathomorphologic evaluation of pulmonary radiofrequency ablation: proof of cell death is characterized by DNA fragmentation and apoptotic bodies,” Cancer, vol. 113, no. 11, pp. 3121–3129, 2008.

[17] J. P. Guenette and D. E. Dupuy, “Radiofrequency ablation of colorectal hepatic metastases,” Journal of Surgical Oncology, vol. 102, no. 8, pp. 978–987, 2010.

[18] E. Y. L. Leung, C. S. D. Roxburgh, E. Leen, and P. G. Horgan, “Combined resection and radiofrequency ablation for bilobar colorectal cancer liver metastases,” Hepato-Gastroenterology, vol. 57, no. 97, pp. 41–46, 2010.

[19] T. T. Cheung, K. K. Ng, K. S. Chok et al., “Combined resection and radiofrequency ablation for multifocal hepato-cellular carcinoma: prognosis and outcomes,” World Journal of Gastroenterology, vol. 16, no. 24, pp. 3056–3062, 2010.

[20] S. R. Kim, H. J. Han, S. J. Park et al., “Comparison between surgery and radiofrequency ablation for stage I non-small cell lung cancer,” European Journal of Radiology. In press.

[21] T. Seki, M. Wakabayashi, T. Nakagawa et al., “Ultrasonically guided percutaneous microwave coagulation therapy for small hepatocellular carcinoma,” Cancer, vol. 74, no. 3, pp. 817–825, 1994.

[22] Y. Q. Duan, Y. Y. Gao, X. X. Ni, Y. Wang, L. Feng, and P. Liang, “Changes in peripheral lymphocyte subsets in patients after partial microwave ablation of the spleen for secondary splenomegaly and hypersplenism: a preliminary study,” International Journal of Hyperthermia, vol. 23, no. 5, pp. 467–472, 2007.

[23] G. J. I. H. Van Leenders, H. P. Beerlage, E. T. Ruijter, J. J. M. C. H. De La Rosette, and C. A. Van De Kaa, “Histopathological changes associated with high intensity focused ultrasound (HIFU) treatment for localised adenocarcinoma of the prostate,” Journal of Clinical Pathology, vol. 53, no. 5, pp. 391–394, 2000.

[24] K. Fischer, W. Gedroyc, and F. A. Jolesz, “Focused ultrasound as a local therapy for liver cancer,” Cancer Journal, vol. 16, no. 2, pp. 118–124, 2010.

[25] J. E. Kennedy, “High-intensity focused ultrasound in the treatment of solid tumours,” Nature Reviews Cancer, vol. 5, no. 4, pp. 321–327, 2005.

[26] G. Kramer, G. E. Steiner, M. Gröbl et al., “Response to sublethal heat treatment of prostatic tumor cells and of prostatic tumor infiltrating T-cells,” Prostate, vol. 58, no. 2, pp. 109–120, 2004.

[27] P. Lu, X. Q. Zhu, Z. L. Xu, Q. Zhou, J. Zhang, and F. Wu, “Increased infiltration of activated tumor-infiltrating lymphocytes after high intensity focused ultrasound ablation of human breast cancer,” Surgery, vol. 145, no. 3, pp. 286–293, 2009.

[28] A. H. H. Tan and P. J. Gilling, “Free-beam and contact laser soft-tissue ablation in urology,” Journal of Endourology, vol. 17, no. 8, pp. 587–593, 2003.

[29] U. Lindner, J. Trachtenberg, and N. Lawrentschuk, “Focal therapy in prostate cancer: modalities, findings and future considerations,” Nature Reviews Urology, vol. 7, no. 10, pp. 562–571, 2010.

[30] Z. Zhao and F. Wu, “Minimally-invasive thermal ablation of early-stage breast cancer: a systemic review,” European Journal of Surgical Oncology, vol. 30, no. 12, pp. 1149–1155, 2010.

[31] K. Kriechbaum, M. Bolz, G. G. Deak, S. Prager, C. Scholada, and U. Schmidt-Erfurth, “High-Resolution Imaging of the Human Retina In Vivo after Scatter Photocoagulation Treatment Using a Semiautomated Laser System,” Ophthalmology, vol. 117, no. 3, pp. 545–551, 2010.

[32] D. Chiu, L. Niu, F. Mu et al., “The experimental study for efficacy and safety of pancreatic cryosurgery,” Cryobiology, vol. 60, no. 3, pp. 281–286, 2010.

[33] J. W. Kim, D. H. Abramson, and I. J. Dunkel, “Current management strategies for intraocular retinoblastoma,” Drugs, vol. 67, no. 15, pp. 2173–2185, 2007.

[34] M. L. Weaver, D. Atkinson, and R. Zemel, “Hepatic cryosurgery in treating colorectal metastases,” Cancer, vol. 76, no. 2, pp. 210–214, 1995.

[35] J. K. Seifert and D. L. Morris, “World survey on the complications of hepatic and prostate cryotherapy,” World Journal of Surgery, vol. 23, no. 2, pp. 109–114, 1999.

[36] J. K. Seifert, M. P. France, J. Zhao et al., “Large volume hepatic freezing: association with significant release of the cytokines interleukin-6 and tumor necrosis factor α in a rat model,” World Journal of Surgery, vol. 26, no. 11, pp. 1333–1341, 2002.

[37] W. C. Chapman, J. P. Debelak, T. S. Blackwell et al., “Hepatic cryoablation-induced acute lung injury: pulmonary hemodynamic and permeability effects in a sheep model,” Archives of Surgery, vol. 135, no. 6, pp. 667–672, 2000.

[38] M. C. Jansen, R. van Hillegersberg, I. G. Schoots et al., “Cryoablation induces greater inflammatory and coagulative responses than radiofrequency ablation or laser induced thermotherapy in a rat liver model,” Surgery, vol. 147, no. 5, pp. 686–695, 2010.

[39] G. Gravante, G. Sconocchia, S. L. Ong, A. R. Dennison, and D. M. Lloyd, “Immuno-regulatory effects of liver ablation therapies for the treatment of primary and metastatic liver malignancies,” Liver International, vol. 29, no. 1, pp. 18–24, 2009.

[40] W. A. Soanes, R. J. Ablin, and M. J. Gonder, “Remission of metastatic lesions following cryosurgery in prostatic cancer: immunologic considerations,” Journal of Urology, vol. 104, no. 1, pp. 154–159, 1970.

[41] R. J. Ablin, W. A. Soanes, and M. J. Gonder, “Elution of in vivo bound antiprostatic epithelial antibodies following multiple cryotherapy of carcinoma of prostate,” Urology, vol. 2, no. 3, pp. 276–279, 1973.

[42] R. F. Sanchez-Ortiz, N. Tannir, K. Ahrar, and C. G. Wood, “Spontaneous regression of pulmonary metastases from renal cell carcinoma after radio frequency ablation of the primary tumor: an in situ tumor vaccine?” Journal of Urology, vol. 170, no. 1, pp. 178–179, 2003.

[43] P. Matzinger, “Tolerance, danger, and the extended family,” Annual Review of Immunology, vol. 12, pp. 991–1045, 1994.

[44] P. Matzinger, “The danger model: a renewed sense of self,” Science, vol. 296, no. 5566, pp. 301–305, 2002.

[45] N. E. Blachere, H. Udono, S. Janetzki, Z. Li, M. Heike, and P. K. Srivastava, “Heat shock protein vaccines against cancer,” Journal of Immunotherapy with Emphasis on Tumor Therapies for the Treatment of Primary and Metastatic Liver Malignancies, vol. 50, no. 11, pp. 210–214, 1995.

[46] N. E. Blachere, Z. Li, R. Y. Chandawarkar et al., “Heat shock protein-peptide complexes, reconstituted in vitro, elicit peptide-specific cytotoxic T lymphocyte response and...
tumor immunity,” *Journal of Experimental Medicine*, vol. 186, no. 8, pp. 1315–1322, 1997.

[48] S. K. Calderwood, S. S. Mambula, and P. J. Gray Jr., “Extracellular heat shock proteins in cell signaling and immunity,” *Annals of the New York Academy of Sciences*, vol. 1113, pp. 28–39, 2007.

[49] S. A. Dromi, M. P. Walsh, S. Herby et al., “Radiofrequency ablation induces antigen-presenting cell infiltration and amplification of weak tumor-induced immunity,” *Radiology*, vol. 251, no. 1, pp. 58–66, 2009.

[50] T. Kinoshita, E. Iwamoto, H. Tsuda, and K. Seki, “Radiofrequency ablation as local therapy for early breast carcinomas,” *Breast Cancer*, vol. 18, no. 1, pp. 10–17, 2010.

[51] M. Y. Ali, C. F. Grimm, M. Ritter et al., “Activation of dendritic cells by local ablation of hepatocellular carcinoma,” *Journal of Hepatology*, vol. 43, no. 5, pp. 817–822, 2005.

[52] S. Evrard, C. Menetrier-Caux, C. Biota et al., “Cytokines pattern after surgical radiofrequency ablation of liver colorectal metastases,” *Gastroenterologie Clinique et Biologique*, vol. 31, no. 2, pp. 141–145, 2007.

[53] M. C. Jansen, S. van Wanrooy, R. van Hillegersberg et al., “Assessment of systemic inflammatory response (SIR) in patients undergoing radiofrequency ablation or partial liver resection for liver tumors,” *European Journal of Surgical Oncology*, vol. 34, no. 6, pp. 662–667, 2008.

[54] A. M. Fietta, M. Morosini, I. Passadore et al., “Systemic inflammatory response and downmodulation of peripheral CD25+Foxp3+ T-regulatory cells in patients undergoing radiofrequency thermal ablation for lung cancer,” *Human Immunology*, vol. 70, no. 7, pp. 477–486, 2009.

[55] G. Schueller, J. Kettenbach, R. Sedivy et al., “Expression of heat shock proteins in human hepatocellular carcinoma after radiofrequency ablation in an animal model,” *Oncology Reports*, vol. 12, no. 3, pp. 495–499, 2004.

[56] W. L. Yang, D. G. Nair, R. Makizumi et al., “Heat shock protein 70 is induced in mouse human colon tumor xenografts after sublethal radiofrequency ablation,” *Annals of Surgical Oncology*, vol. 11, no. 4, pp. 399–406, 2004.

[57] Q. Liu, B. Zhai, W. Yang et al., “Ablation of local cancer recurrence after radiofrequency ablation by dendritic cell-based hyperthermic tumor vaccine,” *Molecular Therapy*, vol. 17, no. 12, pp. 2049–2057, 2009.

[58] R. Rai, C. Richardson, P. Flecknell, H. Robertson, A. Burt, and D. M. Manas, “Study of apoptosis and heat shock protein (HSP) expression in hepatocytes following radiofrequency ablation (RFA),” *Journal of Surgical Research*, vol. 129, no. 1, pp. 147–151, 2005.

[59] S. A. Solazzo, M. Ahmed, R. Schor-Bardach et al., “Liposomal doxorubicin increases radiofrequency ablation-induced tumor destruction by increasing cellular oxidative and nitrate stress and accelerating apoptotic pathways,” *Radiology*, vol. 255, no. 1, pp. 62–74, 2010.

[60] N. Bhardwaj, J. Dormer, F. Ahmad et al., “Heat shock protein 70 expression following hepatic radiofrequency ablation is affected by adjacent vasculature,” *Journal of Surgical Research*. In press.

[61] V. K. Todorova, V. S. Klimberg, L. Hennings, T. Kieber-Emmons, and A. Pashov, “Immunomodulatory effects of radiofrequency ablation in a breast cancer model,” *Immunological Investigations*, vol. 39, no. 1, pp. 74–92, 2010.

[62] G. Schueller, J. Kettenbach, R. Sedivy et al., “Heat shock protein expression induced by percutaneous radiofrequency ablation of hepatocellular carcinoma in vivo,” *International Journal of Oncology*, vol. 24, no. 3, pp. 609–613, 2004.

[63] S. P. Haen, C. Gouttefangeas, D. Schmidt et al., “Elevated serum levels of heat shock protein 70 can be detected after radiofrequency ablation,” *Cell Stress and Chaperones*, vol. 16, no. 5, pp. 495–504, 2011.

[64] J. Hänsler, D. Neureiter, D. Strobel et al., “Cellular and vascular reactions in the liver to radio-frequency thermoablation with wet needle applicators: study on juvenile domestic pigs,” *European Surgical Research*, vol. 34, no. 5, pp. 357–363, 2002.

[65] M. W. Nijkamp, A. Borren, K. M. Govaert et al., “Radiofrequency ablation of colorectal liver metastases induces an inflammatory response in distant hepatic metastases but not in local accelerated outgrowth,” *Journal of Surgical Oncology*, vol. 101, no. 7, pp. 551–556, 2010.

[66] A. Rugghetti, H. Rahimi, P. Rossi et al., “Modulation of blood circulating immune cells by radiofrequency tumor ablation,” *Journal of Experimental and Clinical Cancer Research*, vol. 22, no. 4, pp. 247–250, 2003.

[67] A. Zerbini, M. Pilli, D. Laccabue et al., “Radiofrequency thermal ablation for hepatocellular carcinoma stimulates autologous NK-cell response,” *Gastroenterology*, vol. 138, no. 5, pp. 1931–1942, 2010.

[68] T. T. Wissniowski, J. Hänsler, D. Neureiter et al., “Activation of tumor-specific T lymphocytes by radio-frequency ablation of the VX2 hepatoma in rabbits,” *Cancer Research*, vol. 63, no. 19, pp. 6496–6500, 2003.

[69] M. M. G. M. den Brok, R. P. M. Sutmuller, S. Nierkens et al., “Efficient loading of dendritic cells following cryo and radiofrequency ablation in combination with immune modulation induces anti-tumour immunity,” *British Journal of Cancer*, vol. 95, no. 7, pp. 896–905, 2006.

[70] A. Zerbini, M. Pilli, A. Penna et al., “Radiofrequency thermal ablation of hepatocellular carcinoma liver nodules can activate and enhance tumor-specific T-cell responses,” *Cancer Research*, vol. 66, no. 2, pp. 1139–1146, 2006.

[71] M. Matuszewski, J. Michajłowski, I. Michajłowski et al., “Impact of radiofrequency ablation on PBMC subpopulation in patients with renal cell carcinoma,” *Urologic Oncology*. In press.

[72] M. H. M. G. M. den Brok, R. P. M. Sutmuller, R. van der Voort et al., “In situ tumor ablation creates an antigen source for the generation of antitumor immunity,” *Cancer Research*, vol. 64, no. 11, pp. 4024–4029, 2004.

[73] N. Iida, Y. Nakamoto, T. Baba et al., “Antitumor effect after radiofrequency ablation of murine hepatoma is augmented by an active variant of CCl3 chemokine ligand 3/macrophage inflammatory protein-1α,” *Cancer Research*, vol. 70, no. 16, pp. 6556–6565, 2010.

[74] E. E. Johnson, B. H. Yamane, I. N. Buhtoianov et al., “Radiofrequency ablation combined with KS-II.2 immuno-cytokine (EMD 273066) results in an enhanced antitumor effect against murine colon adenocarcinoma,” *Clinical Cancer Research*, vol. 15, no. 15, pp. 4875–4884, 2009.

[75] C. Napoletano, F. Taurino, M. Bi et al., “RF strongly modulates the immune system and anti-tumor immune responses in metastatic liver patients,” *International Journal of Oncology*, vol. 32, no. 2, pp. 481–490, 2008.

[76] J. Hänsler, T. T. Wissniowski, D. Schuppan et al., “Activation and dramatically increased cytolytic activity of tumor specific T lymphocytes after radio-frequency ablation in patients with hepatocellular carcinoma and colorectal liver metastases,” *World Journal of Gastroenterology*, vol. 12, no. 23, pp. 3716–3721, 2006.
autoantibody response and comparison with iso-immunization,” *Immunology*, vol. 14, no. 2, pp. 149–158, 1968.

[109] S. Shulman, P. Bronson, C. Riera, E. J. Brandt, and C. Yan-torno, “Studies in cryo-immunology. 3. The immunoglobulin nature of the antibody response,” *Immunology*, vol. 14, no. 4, pp. 541–551, 1968.

[110] C. E. Blackwood and I. S. Cooper, “Response of experimental tumor systems to cryosurgery,” *Cryobiology*, vol. 9, no. 6, pp. 508–515, 1972.

[111] R. I. Ablin, “Cryosurgery of the rabbit prostate. Comparison of the immune response of immature and mature bucks,” *Cryobiology*, vol. 11, no. 5, pp. 416–422, 1974.

[112] R. I. Ablin, R. V. Jagodzinski, and C. Pros, “Cryosurgery of the monkey (macaque) prostate. I. Humoral immunologic responsiveness following cryostimulation,” *Cryobiology*, vol. 13, no. 1, pp. 47–53, 1976.

[113] L. C. Muller, M. Micksche, S. Yamagata, and F. Kerbschlaumer, “Therapeutic effect of cryosurgery of murine osteosarcoma—fluence on disease outcome and immune function,” *Cryobiology*, vol. 22, no. 1, pp. 77–85, 1985.

[114] R. I. Ablin, “Serum proteins in prostatic cancer. VI. Reduction of the suppressive (‘blocking’) properties of serum on in vitro parameters of cell mediated immunologic responsiveness following cryosurgery,” *Urologia Internationalis*, vol. 32, no. 1, pp. 65–73, 1977.

[115] H. Kogel, R. Grundmann, I. Fohltmeister, and H. Fichlmair, “Cryotherapy of rectal carcinoma. Immunological results,” *Zentralblatt fur Chirurgie*, vol. 110, no. 2-3, pp. 147–154, 1985.

[116] M. H. Ravindranath, T. F. Wood, D. Soh et al., “Cryosurgical ablation of liver tumors in colon cancer patients increases the serum total ganglioside level and then selectively augments antiangiolyside IgM,” *Cryobiology*, vol. 45, no. 1, pp. 10–21, 2002.

[117] F. Ahmad, G. Gravante, N. Bhardwaj et al., “Renal effects of microwave ablation compared with radiofrequency, cryotherapy and surgical resection at different volumes of the liver treated,” *Liver International*, vol. 30, no. 9, pp. 1305–1314, 2010.

[118] Y. Chen, S. Shen, B. Peng, and J. Tao, “Intratumoural GM-CSF microspheres and CTLA-4 blockade enhance the antitumour immunity induced by thermal ablation in a subcutaneous murine hepatoma model,” *International Journal of Hyperthermia*, vol. 25, no. 5, pp. 374–382, 2009.

[119] B. W. Dong, J. Zhang, P. Liang et al., “Sequential pathological and immunologic analysis of percutaneous microwave coagulation therapy of hepatocellular carcinoma,” *International Journal of Hyperthermia*, vol. 19, no. 2, pp. 119–133, 2003.

[120] P. Zhou, P. Liang, B. Dong, X. Yu, Z. Han, and Y. Xu, “Phase I clinical study of combination therapy with microwave ablation and cellular immunotherapy in hepatocellular carcinoma,” *Cancer Biology and Therapy*, vol. 11, no. 5, pp. 450–456, 2011.

[121] Y. Zhang, J. Deng, J. Feng, and F. Wu, “Enhancement of antitumor vaccine in ablative hepatocellular carcinoma by high-intensity focused ultrasound,” *World Journal of Gastroenterology*, vol. 16, no. 28, pp. 3584–3591, 2010.

[122] F. Wu, Z. B. Wang, Y. D. Cao et al., “Expression of tumor antigens and heat-shock protein 70 in breast cancer cells after high-intensity focused ultrasound ablation,” *Annals of Surgical Oncology*, vol. 14, no. 3, pp. 1237–1242, 2007.

[123] X. Wang and J. Sun, “High-intensity focused ultrasound in patients with late-stage pancreatic carcinoma,” *Chinese Medical Journal*, vol. 115, no. 9, pp. 1332–1335, 2002.

[124] J. Deng, Y. Zhang, J. Feng, and F. Wu, “Dendritic cells loaded with ultrasound-ablated tumour induce in vivo specific antitumour immune responses,” *Ultrasound in Medicine and Biology*, vol. 36, no. 3, pp. 441–448, 2010.

[125] D. F. Rosberger, D. J. Coleman, R. Silverman, S. Woods, M. Rondeau, and S. Cunningham-Rundles, “Immunomodulation in choroidal melanoma: reversal of inverted CD4/CD8 ratios following treatment with ultrasonic hyperthermia,” *Biotechnology Therapeutics*, vol. 5, no. 1-2, pp. 59–68, 1994.

[126] F. Wu, Z. B. Wang, P. Lu et al., “Activated anti-tumor immunity in cancer patients after high intensity focused ultrasound ablation,” *Ultrasound in Medicine and Biology*, vol. 30, no. 9, pp. 1217–1222, 2004.

[127] M. Shimura, K. Yasuda, T. Nakazawa et al., “Panretinal photocoagulation induces pro-inflammatory cytokines and macrophage thickening in high-risk proliferative diabetic retinopathy,” *Graefe's Archive for Clinical and Experimental Ophthalmology*, vol. 247, no. 12, pp. 1617–1624, 2009.

[128] C. Isbert, J. P. Ritz, A. Roggan et al., “Enhancement of the immune response to residual intrahepatic tumor tissue by Laser-Induced Thermotherapy (LITT) compared to hepatic resection,” *Lasers in Surgery and Medicine*, vol. 35, no. 4, pp. 284–292, 2004.

[129] R. J. Stafford, D. Fuentes, A. A. Elliott, J. S. Weinberg, and K. Ahrar, “Laser-induced thermal therapy for tumor ablation,” *Critical Reviews in Biomedical Engineering*, vol. 38, no. 1, pp. 79–100, 2010.

[130] R. Kallio, R. Sequeiros, H. M. Surcel, P. Ohtonen, H. Kiviniemi, and H. Syrjälä, “Early cytokine responses after percutaneous magnetic resonance imaging guided laser thermoablation of malignant liver tumors,” *Cytokine*, vol. 34, no. 5–6, pp. 278–283, 2006.

[131] M. Niftaham, V. Muralidharan, K. Su, C. Makcentui-Wilson, and C. Christophi, “Patterns of heat shock protein (HSP70) expression and Kupfer cell activity following thermal ablation of liver and colorectal liver metastases,” *International Journal of Hyperthermia*, vol. 21, no. 4, pp. 319–322, 2005.

[132] W. X. Lin, T. Fifis, C. Makcentui-Wilson et al., “Induction of Th1 Immune responses following laser ablation in a murine model of colorectal liver metastases,” *Journal of Translational Medicine*, vol. 9, no. 1, article 83, 2011.

[133] G. Schalte, D. Hennet, C. Waning, J. Tacke, R. Rossaint, and A. H. Mahnken, “Case study of hepatic radiofrequency ablation causing a systemic inflammatory response under total intravenous anesthesia,” *Korean Journal of Radiology*, vol. 11, no. 6, pp. 640–647, 2010.

[134] S. R. Schell, F. J. Wessels, A. Abouhamze, L. L. Moldawer, and E. M. Copeland, “Pro- and antiinflammatory cytokine production after radiofrequency ablation of unresectable hepatic tumors,” *Journal of the American College of Surgeons*, vol. 195, no. 6, pp. 774–781, 2002.

[135] S. Nierkens, M. H. den Brok, T. Roelsfson et al., “Route of administration of the TLR9 agonist CpG critically determines the efficacy of cancer immunotherapy in mice,” *PloS One*, vol. 4, no. 12, p. e8368, 2009.

[136] R. Yang, C. R. Reilly, F. J. Rescorla et al., “Effects of high-intensity focused ultrasound in the treatment of experimental neuroblastoma,” *Journal of Pediatric Surgery*, vol. 27, no. 2, pp. 246–251, 1992.
[137] L. Zitvogel, L. Apetoh, F. Ghiringhelli, and G. Kroemer, “Immunological aspects of cancer chemotherapy,” *Nature Reviews Immunology*, vol. 8, no. 1, pp. 59–73, 2008.

[138] S. Gallucci, M. Lolkema, and P. Matzinger, “Natural adjuvants: endogenous activators of dendritic cells,” *Nature Medicine*, vol. 5, no. 11, pp. 1249–1255, 1999.

[139] L. Zitvogel, O. Kepp, L. Senovilla, L. Menger, N. Chaput, and G. Kroemer, “Immunogenic tumor cell death for optimal anticancer therapy: the calreticulin exposure pathway,” *Clinical Cancer Research*, vol. 16, no. 12, pp. 3100–3104, 2010.

[140] A. Misao, K. Sakata, S. Saji, and T. Kunieda, “Late appearance of resistance to tumor rechallenge following cryosurgery. A study in an experimental mammary tumor of the rat,” *Cryobiology*, vol. 18, no. 4, pp. 386–389, 1981.

[141] K. Matsumura, K. Sakata, S. Saji, A. Misao, and T. Kunieda, “Antitumor immunologic reactivity in the relatively early period after cryosurgery: experimental studies in the rat,” *Cryobiology*, vol. 19, no. 3, pp. 263–272, 1982.

[142] J. D. Wolchok, A. Hoos, S. O’Day et al., “Guidelines for the evaluation of immune therapy activity in solid tumors: immune-related response criteria,” *Clinical Cancer Research*, vol. 15, no. 23, pp. 7412–7420, 2009.

[143] Y. Nishinaka, M. H. Ravindranath, and R. F. Irie, “Development of a human monoclonal antibody to ganglioside G(M2) with potential for cancer treatment,” *Cancer Research*, vol. 56, no. 24, pp. 5666–5671, 1996.

[144] K. Nakamura, M. Hanibuchi, S. Yano et al., “Apoptosis induction of human lung cancer cell line in multicellular heterospheroids with humanized antiganglioside GM2 monoclonal antibody,” *Cancer Research*, vol. 59, no. 20, pp. 5323–5330, 1999.

[145] K. Hayakawa, T. Yamashita, K. Suzuki et al., “Comparative immunological studies in rats following cryosurgery and surgical excision of 3-methylcholanthrene-induced primary autochthonous tumors,” *Gann, The Japanese Journal of Cancer Research*, vol. 73, no. 3, pp. 462–469, 1982.

[146] H. Kimura, “Comparative immunological studies on cryosurgery and surgical operation using Moloney murine sarcoma virus-induced primary tumors in BALB/c mice,” *Gann*, vol. 69, no. 4, pp. 507–515, 1978.

[147] F. Wu, Z. B. Wang, Y. D. Cao et al., “Changes in biologic characteristics of breast cancer treated with high-intensity focused ultrasound,” *Ultrasound in Medicine and Biology*, vol. 29, no. 10, pp. 1487–1492, 2003.

[148] F. Wu, Z. B. Wang, Y. D. Cao et al., “Heat fixation of cancer cells ablated with high-intensity-focused ultrasound in patients with breast cancer,” *American Journal of Surgery*, vol. 192, no. 2, pp. 179–184, 2006.

[149] T. R. Vrabec, R. N. Reber, L. E. Magargal, and L. A. Donoso, “S-antigen. Identification of human T-cell lymphocyte proliferation sites,” *Archives of Ophthalmology*, vol. 108, no. 10, pp. 1470–1473, 1990.

[150] B. Pulendran, “Immune activation: death, danger and dendritic cells,” *Current Biology*, vol. 14, no. 1, pp. R30–R32, 2004.

[151] H. J. Zeh and M. T. Lotze, “Addicted to death: invasive cancer and the immune response to unscheduled cell death,” *Journal of Immunotherapy*, vol. 28, no. 1, pp. 1–9, 2005.

[152] M. Nikfarjam, V. Muralidharan, and C. Christophi, “Mechanisms of focal heat destruction of liver tumors,” *Journal of Surgical Research*, vol. 127, no. 2, pp. 208–223, 2005.

[153] S. Chida, K. Okada, N. Suzuki, C. Komori, and Y. Shimada, “Infiltration by macrophages and lymphocytes in transplanted mouse sarcoma after irradiation with high-intensity focused ultrasound,” *Anticancer Research*, vol. 29, no. 10, pp. 3877–3882, 2009.

[154] P. K. Srivastava, “Hypothesis: controlled necrosis as a tool for immunotherapy of human cancer,” *Cancer Immun*, vol. 3, p. 4, 2003.

[155] P. Schildkopf, O. J. Ott, B. Frey et al., “Biological rationales and clinical applications of temperature controlled hyperthermia—implications for multimodal cancer treatments,” *Current Medicinal Chemistry*, vol. 17, no. 27, pp. 3045–3057, 2010.