Heat has been used to treat tumors for thousands of years. There are reports of the Egyptians and Greek philosophers using such treatments as far back as 3000 BC and 500 BC respectively for various solid tumors. Albeit, in these cases, the treatment is not very controlled and consists of hot sticks or blades placed against tissue in order to thermally ablate the tumor. It is not until recent times that the application of heat through various mediums enables a more controlled, localized, and consistent method of treating tumors. While the therapeutic potential of this treatment becomes more apparent, the mechanisms related to its efficacy are only recently beginning to surface. This review discusses the evidence associated with the effects of localized heat on the hallmarks of cancer. Key literature describing modulations to vasculature, cell viability, DNA damage and repair, metabolism, immune system, and tumor metastasis in response to heat will be reviewed along with considerations for its optimal implementation in the clinic to enhance the efficacy of conventional treatments.

1. Introduction

Hyperthermia in the therapeutic setting can be defined as an artificial rise in temperature above healthy body temperature by various means. Therapeutic hyperthermia differs from fever as the former can be induced internally or externally, delivered locally, regionally, or whole-body, cover a large range of temperatures, and does not result in a change to the body’s set point temperature, while the latter is a systemic, immune response which slightly elevates the body’s set point temperature (≈1°C). 

The earliest known reported use of hyperthermia to treat tumors originates from Edwin Smith’s Surgical Papyrus. This Egyptian scroll dates back to approximately 1700 BC, however, it is believed that it is a copy of an even earlier text dated between 2500 and 3000 BC. Surgical cases are described in the papyrus providing some of the earliest documented evidence of medical practice. Curiously, case 39 in this translated text depicts the use of a “fire-drill,” which is rotated rapidly by hand to generate fire and is subsequently pressed against the breast tumor tissue.[5] Similar methods of ablating tumors with localized, extreme heat are also described in Greek and Roman literature from about 500 BC.[6] It was not until the late 19th century, however, where greater advances were made after the connection between cancer and fever was first identified. In these cases, cancer patients that subsequently contracted infections (artificially or not) were noted to have a better outcome than those that did not.[7–9] This newfound knowledge is famous for laying the foundations of cancer immunotherapy as we know it today; however, it is also worth noting that temperature elevations were also a determinant in the success of these treatments.[10–13] This work was then followed by clinical interest in whole-body hyperthermia, pioneered by Manfred von Ardenne and others, which explored its potential in combination with tumor-induced hypoxia.[14] Later, localized heat treatment for cancer gained more momentum and inducing temperature elevations via more controlled techniques began entering clinical practice.[15] Although clear advantages could be observed with these treatments, the mechanisms by which heat induces its anti-cancer effects were still relatively unknown. We are now beginning to understand that the effects of heat on cancer is highly complex, with evidence showing it can influence all hallmarks of cancer—for benefit or to its detriment. This review discusses the literature depicting the effects of localized heat on the hallmarks of cancer and how it can be utilized in the clinical setting to enhance the effects of conventional treatments.

2. Methods of Generating and Controlling Localized Heat in Cancer

Numerous techniques for generating localized heat in tumors have been explored, with the aim of obtaining greater control
over the thermal doses applied and limiting the side-effects associated with whole-body heating.\textsuperscript{[16]} Hereto, radiofrequency waves, ultrasound, and microwaves were the first localized thermal procedures explored in pre-clinical and clinical scenarios.\textsuperscript{[17–19]} Additionally, nanotechnology-based approaches have also shown early pre-clinical and clinical promise through the use of stimuli-responsive nanomaterials that generate heat in response to externally-applied magnetic fields or light.\textsuperscript{[20]}

2.1. Established Techniques for Localized Thermal Treatment

The use of high frequency currents became popular in the 20th century for treating tumors with heat.\textsuperscript{[17,19]} Radiofrequency waves (0.3–30 MHz) use electrodes placed within the tumor that expel an alternating electrical current which agitates the ions in tissue, resulting in the generation of heat by way of friction.\textsuperscript{[21–24]} Microwaves also utilize radiofrequency energy, but employs much higher frequencies (915–2450 MHz). This mode of treatment began clinical testing in the 1970s and exerts its effects via antennas positioned in the tumor that induce oscillations in the water molecules in tissues at the frequencies applied. These vibrations are also subsequently converted to heat through friction.\textsuperscript{[25,26]}

Ultrasound was first assessed for thermal applications in the clinic in the 1940s, while the jump to high-intensity focused ultrasound (HIFU) occurred in the 1990s. This procedure utilizes acoustic waves (0.5–3.5 MHz) generated by a transducer that invoke compression and rarefaction pressures on the target tissue medium, leading to local temperature elevations.\textsuperscript{[27–29]} Each of these techniques have been assessed in randomized trials, displaying promising results for multiple cancers.\textsuperscript{[30–32]} Although each technique has achieved clinical approval in some capacity, the most clinically established to date is radiofrequency hyperthermia, which is currently in use for multiple benign and malignant indications.\textsuperscript{[33–37]}

2.2. Nanotechnology-Based Techniques for Localized Thermal Treatment

Nanotechnology-based hyperthermia in cancer is a relatively young research discipline, although initial reports of this technique emerged in the 1950s and 60s.\textsuperscript{[38,39]} Despite an impressive amount of pre-clinical results being published, these technologies are still in the early stages of clinical evaluation and are not as clinically established as the previous techniques described.\textsuperscript{[40,41]}

Magnetic hyperthermia uses superparamagnetic iron oxide nanoparticles that can be stimulated with alternating magnetic fields to generate heat. This heat generation occurs through rapid changes in the nanoparticles polarity, resulting in heat by way of Néel (rotation of the nanoparticles internal magnetic moment) and Brownian (physical rotation of the nanoparticle) relaxation.\textsuperscript{[42,43]} Magnetic hyperthermia is clinically approved to treat glioblastoma in Europe and is also undergoing clinical investigation in prostate cancer in the United States.\textsuperscript{[44]} In the prospective, single-arm, phase II trial that led to its approval by the European Medicines Agency, iron oxide nanoparticles were injected directly into the tumors of glioblastoma patients and then exposed to six bi-weekly cycles of an external magnetic field with a fixed frequency of 100 kHz and an adjustable field from 2–15 kA m\(^{-1}\) in conjunction with radiation (notably, estimates on the safety threshold for magnetic field exposure ranges from 4.85–50 kA m\(^{-1}\) at a frequency of 100 kHz\textsuperscript{[40]}. Median overall survival was significantly prolonged in both primary and recurrent tumors against the comparable reference group in this trial.\textsuperscript{[45]} The unique properties of magnetic nanoparticles enable long-term placement of the heating source deep inside the tumor via image-guided intratumoral injection.\textsuperscript{[46–48]} Once residing in the tumor, no further invasive procedures are required and so multiple thermal cycles can be applied without further surgical intervention.\textsuperscript{[45]} This is an advantage on earlier techniques which -in the majority of cases- require invasive procedures each time they are applied for deep-seated tumors; hence, magnetic hyperthermia offers a less invasive option when multiple thermal cycles for deep tumors are required.\textsuperscript{[49,50]} Conversely, magnetic hyperthermia is still in its infancy with regard to clinical evaluation as randomized trials have yet to be undertaken for this therapy and therefore its true potential has yet to be fully elucidated.\textsuperscript{[40]}

Beyond magnetic-based thermal therapy, heating can also be achieved via laser light in a treatment known as photothermal therapy. Gold and other metallic nanoparticles can absorb light in the near-infrared spectrum (700–850 nm) due to their unique surface plasmonic resonance, leading to oscillations in their conductive band electrons and a generation of heat.\textsuperscript{[30,51]} This wavelength of light has relatively high tissue transparency and is minimally absorbed, alleviating side-effects observed with alternative light.\textsuperscript{[52]} Typically, pre-clinical studies have used laser power from 1–4 W cm\(^{-2}\), while pilot clinical studies have tested up to 6.5 W, delivered with an 18 mm optical fiber diffuser with a longitudinal treatment zone of 12.5 mm.\textsuperscript{[53,54]} As magnetic hyperthermia requires a large intratumoral concentration of nanoparticles to reach therapeutic temperatures (a maximum tissue concentration of 47 mg of iron per ml of tissue has been used in clinical testing\textsuperscript{[45,55]}), it is currently limited to intratumoral injection; photothermal therapy, by contrast, requires considerably less (a maximum concentration of 33.12 𝜇g of gold per gram of tumor tissue has been used in clinical testing\textsuperscript{[54]}) and so thermal efficacy can be achieved via intravenous injections of nanoparticles that subsequently accumulate in the tumor at sufficient concentrations to generate desirable temperature elevations.\textsuperscript{[53]} Nevertheless, photothermal therapy has two current hurdles: near-infrared light has limited ability to penetrate deep into tissues which means this approach requires invasive catheters to deliver light,\textsuperscript{[54,56–58]} and the treatment itself is yet to advance past pilot clinical testing.\textsuperscript{[59]}

2.3. Temperature Monitoring and Dosimetry

Thermal dose control and monitoring is essential for thermal therapies to ensure therapeutic temperatures are achieved within the tumor while also sparing healthy tissue from heat-induced damage.\textsuperscript{[60,61]} Temperatures are monitored in the clinic using temperature sensors strategically placed to cover the area of the target site, temperature source, surrounding healthy tissue and additional high-risk sites. For tumors requiring invasive monitoring (deeper than 1 cm, according to guidelines published by the European Society for Hyperthermic Oncology—ESHO),
image-guided catheters are utilized to enable internal placement of sensors. Sensors can also be combined with thermal imaging techniques such as magnetic resonance and infrared imaging to complement their readings in a noninvasive manner. For deep-lying tumors deemed unsuitable for invasive temperature monitoring, or to provide a greater insight to temperature distribution than sensors can provide alone, treatment planning is now increasingly used in the clinic to provide a simulated thermal map of the target site.

To normalize thermal doses across studies, the benchmark parameter is CEM43, which refers to the cumulative equivalent minutes the tumor is exposed to 43°C. Moreover, to account for the area of the tumor that is heated, CEM43TX is used—where X is a percentage commonly equal to 10, 50, and 90—and refers to the duration at which this temperature is exceeded in X% of the measured sites within the tumor. CEM43T90 has been the most commonly used parameter for determining the efficacy of heat in clinical trials and is also recommended in quality assurance guidelines published by ESHO for the clinical monitoring of superficial and deep hyperthermia. While this parameter has proven capable of predicting treatment outcomes in some clinical studies, it is not without its limitations and cannot account for the effect of combination therapies, heterogeneous temperature elevations common in hyperthermia, changes to blood perfusion and oxygenation in the tumor, inhibition to DNA repair, fractionated hyperthermia and thermotolerance, as well as being limited to hyperthermia temperature range making it unsuitable for thermal ablation (Figure 1). Hence, an updated parameter or combination of parameters to account for these various factors would greatly advance thermal dosimetry with this therapy.

Notably, for the purposes of maintaining consistency in this review, when citing thermal doses used in studies, the temperatures generated and their duration will be provided to account for the many studies which lack inclusion of CEM43 values.

Hyperthermia treatment is generally defined by temperatures of 40–45 °C, which can be broken down further to mild (40–42 °C) and moderate (42–45 °C) hyperthermia to account for their respective effects. While hyperthermia is utilized to sensitize tumors to radiation and chemotherapeutics in the clinic and not typically efficacious as a monotherapy, the intended purpose of thermal ablation is direct cell death via coagulative necrosis, and so employs higher temperatures (≥50 °C).

3. The Hallmarks of Cancer

The hallmarks of cancer were first proposed by Hanahan D. and Weinberg R. A. in 2000 and then updated by the same authors in 2011. These influential reviews described a framework in which the complexity of malignant transformation could be rationalized. As most malignant tumors have these features in common, the hallmarks offer a universal set of principles by which the biology of cancer can be understood and investigated. These hallmarks were listed as follows: evaded growth suppressor signaling, avoided immune destruction, enabled replicative immortality, induced angiogenesis, resisted cell death, reprogrammed energy metabolism, activated invasion and metastasis, and sustained proliferative signaling. Moreover, each of these hallmarks is facilitated by continual genomic instability and mutation and inflammation (Figure 2). Importantly, localized heat in tumors has been shown to influence the hallmarks of cancer in a variety of ways depending on the thermal doses applied, and the literature associated with this is discussed next.

First proposed in 2000 by Hanahan D. and Weinberg R. A., and updated by the same authors in 2011, the hallmarks of cancer provide a set of characteristics common to almost all malignant tumors.

4. The Effects of Localized Heat on the Hallmarks of Cancer

4.1. Localized Heat and Vasculature

Access to a blood supply is essential for the sustained progression of tumors. Blood provides a source of nutrients and oxygen for the tumor, while also acts as a means of waste removal. Additionally, vessels serve as a vehicle for metastatic expansion. Hence, the formation of blood vessels—or angiogenesis—is a key step in the growth and spread of cancer. Normally activated in the embryo during organogenesis and through restoration processes such as wound healing, cancer can hijack this carefully controlled mechanism and accelerate it for its own benefit. The hijacking of angiogenesis is known as the “angiogenic switch” and refers to a disruption in the balance of pro-angiogenic and anti-angiogenic factors in favor of rapid blood vessel formation. As angiogenesis occurs at a rate above normal physiological levels in a tumor, this results in the formation of aberrant, immature vasculature that is permeabilized and less efficient at thermoregulation than healthy tissue. Because of this, cancer cells are selectively sensitive to heat.
4.1.1. Decreased Vascular Perfusion

Due to this thermal sensitivity, lower temperatures are required to induce vascular stasis in tumors than healthy tissue. For instance, temperatures of 41–42 °C are prone to induce vascular stasis in rabbit tumors, whereas this effect manifests closer to 47 °C in healthy tissue (following one hour of treatment). In this study, vascular stasis was measured through microscopic measurements of microvessels derived from healthy and cancer tissues within rabbit ear clamps.[85] Additionally, temperatures of 42 °C or more for 30–60 min prove efficient at inducing stasis in most cancer models, while temperatures of 45 °C or more were necessary for the same effect in healthy tissues.[86] The mechanisms behind this thermally-induced stasis in tumors include endothelial cell damage, platelet and leukocyte adhesion, erythrocyte aggregation, and capillary thrombosis.[87–90] Consequently, blood circulation is disrupted and the tumor becomes nutrient deprived and increasingly hypoxic which, in turn, leads to acidosis.[91,92] While the induction of vascular stasis has inherent anti-tumor effects, it can also compromise combination therapies and should be accounted for when developing treatment plans. Hypoxic tumors are notoriously radioresistant due to reduced levels of reactive oxygen species (ROS) required for induction of DNA damage, and the consequential activation of pro-survival pathways (via hypoxia-inducible factor 1-HIF-1-signaling).[93,94] Moreover, antagonizing blood flow will also prevent the accumulation of drugs into the tumor; hence, inducing vascular stasis with heat can have detrimental effects on the efficacy of chemotherapy and radiation and should be carefully considered when developing treatment plans for combination therapies.

4.1.2. Elevated Vascular Perfusion

Another important dimension to this thermal-vascular effect, however, is that at slightly milder temperatures (approximately 40 °C), blood perfusion can increase in tumors.[86,87,92,95,96] Although this increased perfusion in tumors is not as profound as healthy tissue exposed to heat (twofold vs sixfold),[85] its impact will depend on the extensity and maturity of the tumor vascular system, as well as the elasticity (stiffness) of the tumor extracellular matrix (ECM). An increase in blood flow will oxygenate the tumor and increase sensitivity to radiation, while also enabling a greater concentration of drug to reach the tumor.[97–99] A recent clinical study in cervical cancer patients demonstrated that mild hyperthermia (up to 40 °C for one hour) could significantly increase blood flow into the tumor, which may explain why cervical cancer responds well to a combination of hyperthermia and radiation in trials.[100,101] Similarly, a study in breast cancer patients bearing liver metastasis could show a significant increase in blood flow in the hepatic artery following mild hyperthermia (up to 40 °C for 30 min) which resulted in a 34% increase in efficacy of paclitaxel and carboplatin compared to the drugs used alone.[102] It is also known that high pressures within stiff tumor microenvironments invoke compressions on blood and lymphatic vessels, mitigating blood perfusion.[103,104] In vivo studies have additionally shown that moderate hyperthermia or...
ablative temperatures (43 °C for 15 min or 52 °C for 3 min) can remodel the tumor ECM, thereby restoring compressed vessels and promoting blood perfusion—thus, enhancing the penetration of drugs and nanoparticles into tumors.\textsuperscript{105,106}

Curiously, as hypoxia itself triggers an angiogenic response, there may be a threshold where induction of strong vascular stasis may also independently induce angiogenesis and, therefore, subsequent increases in blood perfusion to the tumor. It has been hypothesized that the angiogenic trigger observed following a single, hour-long exposure to 46 °C is due to the presence of dormant cells undergoing autophagy which undergo a hormetic response after normal conditions (e.g., nutrient supply) have been restored.\textsuperscript{107} Additionally, hyperthermia-induced vascular stasis (43 °C for one hour) can lead to an increased expression of hypoxic markers (e.g., HIF-1), which, in turn, trigger angiogenic processes that can lead to increased tumor vascularization/perfusion and reduce hypoxia.\textsuperscript{108} Therefore, if the tumor is not sufficiently destroyed during treatment, a delayed pro-angiogenic response may also ensue in some cases following substantial vascular stasis, which can have both positive and negative implications. As previously discussed, elevation in blood flow can be beneficial to radiation and chemotherapies; unfortunately, however, equipping a tumor with a newly-developed blood supply has the potential to increase its growth and metastatic capabilities. This may explain why—albeit, in rare cases—tumors exposed to therapeutic heat can exhibit increased invasiveness and so this effect must be considered and monitored with extreme caution if the potential for strong vascular stasis is high.\textsuperscript{109–111}

Hence, if the aim is to use hyperthermia in combination with radiation, it may be necessary to apply moderate hyperthermia after radiation to avoid circulatory disruption which would otherwise reduce the levels of oxygen reaching the tumor and antagonize treatment efficacy. Mild hyperthermia, by contrast, may increase sensitivity to radiation when applied before or during treatment through increases in blood perfusion and tumor oxygenation. Similarly, if a pro-angiogenic response through strong vessel stasis or increased blood flow via ECM remodeling is possible, applying moderate hyperthermia or thermal ablation prior to radiation would be most beneficial in this regard. These considerations can also be applied for chemotherapy. Inducing vascular stasis may inhibit accumulation of the drug into the tumor and so moderate hyperthermia should be applied after the drugs circulation time is complete, while increased blood perfusion could increase tumor concentration of the drug when mild hyperthermia is applied during its circulation time. Moreover, the delayed antigenic response obtained through strong vascular stasis or elevated blood perfusion with ECM remodeling would be most beneficial to chemotherapy if evoked prior to its administration, giving time for the increased perfusion to be established in the tumor. By contrast, if hyperthermia or thermal ablation is used as a monotherapy and direct cytotoxic effects on the tumor are primarily desired, temperatures capable of inducing vascular stasis may be most suitable. Conversely, if local control is not fully established in these cases, strong vascular stasis has the potential to trigger angiogenesis in tumors and could lead to tumor growth over time, which should be monitored with great caution. Of course, the characteristics of the tumor (i.e., vascular profile and tumor stiffness) will play a major role when considering the thermal-induced vascular response generated following hyperthermia and ablation, but the literature does show some element of predictability (Figure 3).

Heat can have varied effects on the vascular system of a tumor depending on the temperatures generated. Mild hyperthermia (39–41 °C for 30–60 min) has been shown to increase blood perfusion as the tumor attempts to thermoregulate the elevated temperatures. It should be applied before or during RT and during CT circulation time to enable the increased blood flow to have the most beneficial effect. At moderate temperatures (42 °C or more for 60 min), the irregular tumor vasculature struggles to cope with these thermal doses and vascular stasis is induced. These temperatures should be applied after RT and CT circulation time, as reducing blood flow and oxygen entering the tumor will antagonize their efficacy. At greater temperatures again (43 °C or more for 60 min), the vascular stasis can become so extreme that hypoxia drives an angiogenic response leading to increases in tumor vasculature, blood perfusion and reoxygenation. Moreover, stiff tumors can experience ECM remodeling at these temperatures, which relieve the pressure on tumor vessels, thus enhancing perfusion. In this case, it would be best to apply these temperatures before CT and RT, to allow time for the formation/decompression of blood vessels in the tumor before treatment. Importantly, if complete local control is desired but not achieved, this increased angiogenesis may facilitate the tumors proliferation and expansion, which should be carefully considered and monitored during treatment. Abbreviations: CT, chemotherapy; RT, radiotherapy.

### 4.2. Localized Heat and Cytotoxicity

Mutations in the genome of healthy cells that lead to elevations in proliferation (via oncogenes) and attenuations in growth suppression (via tumor suppressor genes) are key, initial steps in the

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**Figure 3.** The effects of localized heat on tumor vascularity.
The process of malignant transformation. Accordingly, these transformed cells display a heightened resistance to cell death and the ability to replicate indefinitely, leading to the macroscopic growth of tumors.\textsuperscript{[112–114]} Anti-cancer therapies aim to overcome this accelerated growth by inducing cytotoxicity through a variety of different approaches.\textsuperscript{[115,116]} Interestingly, heat treatments can be tailored to induce alternative cytotoxic responses depending on the thermal doses applied to the tumor.

The evidence for hyperthermia (typically 42–43 °C for 60 min or more) compromising cellular functions in target cells has proven reasonably consistent across the literature. Such temperatures have been shown to damage cellular architecture through protein denaturation (i.e., misfolding and aggregation processes), leading to cell membrane and mitochondrial damage as well as disruption to many biochemical pathways, including transcription, replication, and DNA repair.\textsuperscript{[117–121]} Upregulation of intracellular ROS has also been noted at various temperatures,\textsuperscript{[122,123]} while thermal ablative temperatures (>50 °C for several minutes) may even damage DNA per se.\textsuperscript{[124]} Depending on the thermal dose, the response of tumor cells can be manifold, and cytotoxicity occurs if tumor cells are unable to activate or maintain an appropriate protective cellular response.\textsuperscript{[125]} One such family of proteins that play an important pro-survival role are heat shock proteins (HSP). Following thermal stress, general transcription, and translation becomes halted, while heat shock factors become selectively activated and bind to heat-shock elements on promoters of target genes, leading to HSP expression.\textsuperscript{[125]} HSP are highly conserved molecular chaperones that prevent protein aggregating and misfolding following cellular damage and inhibit cell death.\textsuperscript{[126]} It has been postulated that the induction of HSP does not occur if the temperature-based stress is too severe, with expression only observed in surviving cells.\textsuperscript{[127–129]} Although responsible for rectifying thermal injury, therapeutic hyperthermia—in the majority of cases—aims to overcome this thermodurability.\textsuperscript{[130]}

Depending on the thermal dose generated, different mechanisms of cell death can be induced. Temperatures of approximately 42–43 °C for 30–60 min have been shown to induce apoptosis (programmed cell death), whereas temperatures approaching thermal ablative doses—45 °C or higher for 30 min or more—result in cell death primarily through necrosis (cell swelling, lysis and uncontrollable release of intracellular contents) (Figure 4).\textsuperscript{[131–135]} The applied thermal dose is crucial from a clinical perspective as the primary mechanism of cell death is expected to have a significant impact on therapeutic outcome. For example, inducing high levels of necrosis within the tumor will result in an inflammatory response that could harm healthy tissue if not properly controlled and isolated to the desired location. On the other hand, if controlled, localized necrosis is achieved, the tumor will experience irreparable damage which can also contribute to an immune response against the tumor.\textsuperscript{[135]} Hyperthermia treatments (typically 40–45 °C for 60 min), by contrast, are used in the clinic for sensitizing cancers to chemotheraphy and radiation, and such treatment protocols have also been shown to contribute toward an anti-tumor immune response\textsuperscript{[139,140]} (see Section 4.5).

The primary mechanism of cell death following hyperthermia is temperature-dependent. Moderate temperature elevations (42–43 °C) for 30–60 min have been shown to induce apoptosis in cells, whereas temperatures above 45 °C for 30 min or more can kill cells primarily through necrosis. Higher temperatures are more effective at local control of tumors but carry a risk of off-target effects, while moderate temperatures are more associated with sensitization to CT and RT and are less likely to induce thermal damage to healthy tissue. Additionally, there is evidence to suggest
both thermal doses are capable of eliciting anti-tumor immune responses.

4.3. Localized Heat and DNA Repair and Damage

Maintaining genomic integrity is essential to the survival of healthy cells. DNA is susceptible to both single strand and double strand breaks (SSB and DSB) from exogenous (ultraviolet light, ionizing radiation, and carcinogens) and endogenous (ROS) sources, which, in turn, activate DNA damage responses (DDR) in cells.\[141\] The nature of the break determines the repair pathway activated. For SSB, there are three main pathways utilized: base excision repair (BER), nucleotide excision repair (NER), and mismatch repair (MMR). Likewise, DSB rely on two major mechanisms: non-homologous end joining (NHEJ) and homologous recombination (HR).\[151,152\] Importantly, mutations to genes involved in DDR occur in most cancers, enabling a high level of genomic instability to accumulate which facilitates the initiation and progression of cancer.\[144\] DDR significance can be particularly observed in hereditary cancers, where germline loss of function mutations to DNA repair genes predisposes the host to elevated lifetime risk of cancer development.\[145,146\] Additionally, tumors can become reliant on functionally intact repair mechanisms which compensate for the dysregulation of another and repair DNA lesions created by chemotherapy and radiation, enabling treatment resistance.\[147,148\] Hence, therapeutic interventions that target DNA repair serve two beneficial functions: first, inhibition of the compensatory DNA repair pathway in cancer (known as synthetic lethality) can result in cell death, and second, inhibition of DNA repair can overcome treatment resistance and act as a potential chemo/radio-sensitizer.\[147,149,150\]

Hyperthermia has proven capable of inhibiting many repair pathways.\[151,152\] However, much of the research in this space is based on indirect effects of repair inhibition, such as increased cell sensitivity to chemotherapy or radiation following hyperthermia as an indicator for DNA repair inhibition.\[153,154\] The molecular mechanisms behind these inhibitions remain elusive in most cases. Below is a summary of papers that hint at the molecular events associated with thermally-induced DNA repair inhibition (Table 1).

4.3.1. DNA Repair Inhibition

Table 1. Summary of literature detailing the molecular mechanisms behind heat-induced DNA repair inhibition.

| Experiment | Hyperthermia treatment | Repair pathway | Protein Effect | Ref. |
|------------|------------------------|----------------|---------------|------|
| In vitro: HeLa cells | 42 °C for 4 h | BER | OGG1 | Inhibition | [155] |
| In vitro: U373MG and U87MG cells | 45 °C for 30 min | BER | DNA polymerase β | Inhibition | [156] |
| In vitro: MCF7 cells | 41 °C for 1 h | BER | DNA polymerase β | Inhibition | [157] |
| In vitro: CHO cells | 41–46 °C for various durations | BER | DNA polymerase β | Inhibition | [158] |
| In vitro: Protein isolation from MOLT-4 cells | 44 °C for 30 min | NHEJ | DNA-PK subunits p470, Ku70, and Ku80 | Suppression in kinase activity | [159] |
| In vitro: U-1 cells | 45.5 °C for 15–30 min | NHEJ | DNA-PK subunit Ku80 | Aggregation | [160] |
| In vitro: DNA-PK-expressing SCID cells | 44 °C for 15 min | NHEJ | DNA-PK | Inhibition | [161] |
| In vitro: DNA-PK-expressing SCID cells | 44 °C for 15 min | NHEJ | DNA-PK subunits Ku80 and Ku70 | Inhibition | [162] |
| In vitro: HeLa U2OS, ES, and BRO cells | 41–42.5 °C for various durations | HR | BCRA2 and/or Rad51 | Inhibition | [163] |
| Ex vivo: Cervical squamous cell carcinoma biopsy | 41 °C for 90 min | HR | Rad51 | Inhibition | [163] |
| In vitro: BLM, HeLa, FaDu, and VH10-SV40 cells | 41–42 °C for 1 h or 43 °C for 15–30 min | HR | BRCA2 and Rad51 | Inhibition | [164] |
| In vitro: SW982, U2OS, SW872, and DLD1 cells | 41.8 or 43 °C for 90 min | HR | BRCA2 | Inhibition | [165] |
| In vitro: HeLa, U2OS, and BRO cells | 42 °C for 30 min | HR | BRCA2 | Inhibition | [166] |

Abbreviations: OGG1, 8-oxoguanine DNA glycosylase; DNA-PK, DNA-dependent protein kinase. Cell lines mentioned: HeLa, human cervical cancer; U373MG and U87MG, human glioma cells; MCF7, human breast cancer cells; MOLT-4, human leukemia cells; U-1, melanoma cells; SCID, mouse severe combined immunodeficient cells; U2OS, human osteosarcoma; BRO, human melanoma; BLM, human melanoma; FaDu, human head and neck cancer; VH10-SV40, p53-negative human fibroblasts; SW982, human synovial sarcoma; SW872, human liposarcoma; DLD1, human colorectal cancer; ES, mouse embryonic cells. BER, base excision repair; NHEJ, non-homologous end joining; and HR, homologous recombination.
transient nature of this inhibition (literature consistently shows that the levels of the repair mechanism of interest normalizes after a varied period of time following exposure to heat), the ability of the DNA repair system to compensate with an alternative pathway when one is compromised and the ability of chemotherapies and radiation to induce both SSB and DSB in DNA, thus not relying on one pathway for repair.\cite{151,152,162,172}

4.3.2. DNA Damage

A further consequence of heat-induced DNA repair inhibition is DNA damage. While the potential of mild-to-moderate hyperthermia (40–45 °C for 30–60 min) to directly induce DNA damage has been disputed in the literature, evidence for DNA damage through indirect mechanisms (inhibitions to DNA repair and replication) has been widely confirmed.\cite{131,173,175} Importantly, cells in S phase of cell cycle have repeatedly been shown to be most thermosensitive,\cite{130,176,177} and recent reports have shown that heat in this phase can induce SSB via inhibitions to topoisomerase-1. This phase of cell cycle is thought to be particularly susceptible to heat due to its more relaxed chromatin structure that exposes DNA and enzymes in the replication forks, making them vulnerable to various stresses.\cite{178,179} Moreover, these SSB can be subsequently converted to DSB following collisions in replication forks after cells overcome heat-induced cell cycle arrest.\cite{180} HeLa and human fibroblast cells in early S phase were shown to be most vulnerable to heat shock at 45.5 °C (but not 41 or 43 °C) for 30 min, which resulted in a senescence-like state marked by nuclear enlargement, elevated expression of β-galactosidase and p21\textsuperscript{CIP1} and persistent SSB generated through inhibitions to topoisomerase-1. It is thought that these SSB are subsequently converted to DSB during DNA replication which triggers this senescence-like phenotype. Following this, the study showed that the heat-induced senescence-like state in HeLa cells could be transient, and the cells could progress to mitosis four days after heat treatment; however, these cells have amplified centromeres resulting in a multipolar mitosis. Additional reports have also noted that on top of this S phase-specific effect, cells in G1 and G2 phase are also susceptible to DSB following heat stress. After hyperthermia at 45.5 °C for 30 min, MCF-7 cells in G1 and G2 phase experienced DSB (confirmed through γH2AX levels, neutral comet assay and terminal deoxynucleotidyl transferase incorporation assay), however the mechanism behind their induction are yet to be fully elucidated.\cite{182}

Inhibition of DNA repair mechanisms by hyperthermia is one of the major suggested mechanisms behind the chemosensitizing and radiosensitizing effects observed with this therapy. Hyperthermia (41–45 °C for various durations) can inhibit both SSB and DSB repair mechanisms which offers a promising platform for combination therapies. Moreover, heat has also been shown to induce DNA damage by antagonizing DNA repair and replication processes. In particular, the thermal-induction of SSB in S-phase cells via topoisomerase-1 inhibition has been observed at temperatures of 45.5 °C for 30 min, which can convert to DSB following subsequent DNA replication. S-phase cells are also the most radioresistant,\cite{118} which reflects the potent radiosensitizing effects observed with hyperthermia in the clinic (Section 5.2).

4.4. Localized Heat and Metabolism

Otto Warburg reported in the 1920s that cancer cells metabolize differently to healthy cells.\cite{183} While healthy, differentiated cells typically metabolize glucose through oxidative phosphorylation in the presence of oxygen and glycolysis under anaerobic conditions, most cancer cells predominantly metabolize glucose via the glycolytic pathway regardless of oxygen availability. A by-product of this aerobic glycolysis is a large amount of lactate which creates an acidic environment for the tumor. This is referred to as the “Warburg effect” and explains how cancers thrive under fluctuating levels of oxygen.\cite{184,185} This large consumption of glucose and acidic milieu are thought to potentiate invasiveness, impair anti-tumor immune responses and contribute toward drug resistance; hence, therapies that interfere with these metabolic processes may disturb invasion and immune suppression, sensitize the tumor to drugs and suppress tumor growth.\cite{186–188}

Most of the research published on heat and metabolism was undertaken in the mid-to-late 1900’s. This work was reviewed by Streffer C. in 1985 and 1988\cite{189,190} and led to the conclusion that metabolic activity (i.e., ATP turnover) is overall increased when cells are treated with moderate hyperthermia for 30-60 minutes. However, conflicting results (due to different thermal doses and in vitro–in vivo study variability) make it difficult to decipher whether metabolic processes become altered following treatment. Accordingly, it is difficult to draw comparisons with the direct effects of localized heat on metabolism in this regard. One previous study investigated the cell-dependent impact of hyperthermia on cell metabolism. Herein, a significant reduction of base line oxygen consumption rates were observed in two human pancreatic cancer cells (PANC-1 and AsPC-1) following five minutes treatment at 46 °C. A healthy pancreatic epithelial cell control, on the other hand, showed a significant increase in oxygen consumption following treatment.\cite{191} These results were not normalized for cell count or protein concentration, however. A more recent study measured changes in glycolysis and mitochondrial function following heat treatment of colorectal cancer cells. Hyperthermia at 42 °C for 60 min could induce significant increases in all measures of glycolysis (non-glycolytic acidification, glycolysis, glycolytic reserve, and glycolytic capacity) during the glycolytic stress test, while a significant increase in proton leakage was observed in the mitochondrial stress test.\cite{192} As no other changes were observed during the mitochondrial test, it could be surmised that the heat treatment may damage the mitochondrial membrane without having any influence on adenosine triphosphate synthase functional capacity in these cells.

Overall, little evidence is available on the effects of heat on cancer metabolism. Early reports, however, suggest that metabolic activity is elevated in cancer cells in response to moderate hyperthermia. Despite this, many questions remain unanswered and require further research. Identifying which metabolic processes are favored following localized heat treatments is essential to determine whether the tumor microenvironment is becoming more acidic and which molecular processes are active. This, in turn, will aid in decisions as to what combination therapies would be most applicable for each individual case.
4.5. Localized Heat and the Immune System

Cancer evolves sophisticated mechanisms to overcome the threat of the immune system, enabling its proliferation and metastatic growth. During the initial stages of tumor growth, an equilibrium exists where the most immunogenic cancer cells are eliminated by cytotoxic lymphocytes (CD8+ and natural killer (NK) T cells), leaving behind weakly immunogenic cells that proliferate and are less likely to be recognized and destroyed by the immune system. This early immune evasion occurs primarily through continual mutations to tumor antigens, which produce tumor variants resistant to immune recognition.[193,194] Eventually, the tumor can escape this equilibrium by creating an immune-tolerant microenvironment characterized by the recruitment of immunosuppressive cells (regulatory T cells, myeloid-derived suppressor cells), polarization of immune cells toward a pro-tumor phenotype (M2 macrophages) and upregulation of immune checkpoint proteins that counteract T cell responses (notably, programmed death-ligand 1 (PD-L1) and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4)), among others.[194–196] This immune evasion enables the tumor to proliferate unchecked, contributes to the formation of new blood vessels and facilitates metastasis.[197] Interest in the development of therapies to rewire the immune system to treat cancer has been massive in the last decade and epitomized by the awarding of the 2018 Nobel prize in Physiology or Medicine to James P. Allison and Tasuku Honjo for their discovery of the immune checkpoint proteins CTLA-4 and PD-1/PD-L1, respectively.[198] Currently, there are over 25 clinically-approved immunotherapies developed for 17 different cancers, with many more undergoing clinical and pre-clinical evaluation.[199]

As fever is a by-product of an immune reaction, it is self-evident that heat would induce an immunomodulatory effect. The literature regarding heat and the immune system varies largely depending on the heating methods and treatment durations used; what is well known, however, is that controlled, localized heat can elicit a systemic anti-tumor immune response.[200–203] The evidence associated with this immune stimulation strongly suggests an induction of dendritic cell (DC) maturation and cross-presentation to CD8+ T cells, while other findings hint at a heightened cytotoxicity profile of NK cells.[208,209]

4.5.1. Adaptive Immunity

Following temperature elevations in cells, HSP are upregulated as a form of thermotolerance (as discussed in Section 5.3). Mathematical modelling suggests that a temperature of 43 °C for 100 min is optimal to achieve maximum extracellular concentration of HSP.[205] Although their main role in cells is to maintain intracellular protein integrity following cellular exposure to stress,[126] they are also considered the main instigators of heat-driven anti-tumor immune responses. Injured or dying cancer cells actively and passively release HSP and other endogenous danger signals (known as damage-associated molecular patterns, or DAMP’s; examples include calreticulin, adenosine triphosphate and high mobility group B1) into the tumor microenvironment, where HSP can escort these DAMP’s and support antigen-presenting cell (APC) priming.[206,207] HSP also carry an inherent DC maturation capacity which may exacerbate this immunostimulatory effect.[208–211] This thermally-induced extracellular release of DAMP’s is defined as immunogenic cell death, which can stimulate a suppressed immune response.[212,213] DC can then home to the draining lymph node and cross-present tumor antigens to CD8+ T cells, enabling their effector function to expand and migrate to the tumor site and elicit a cytotoxic response.[201,214]

Numerous in vivo studies have confirmed this adaptive immune response by treating tumor-bearing rodents with moderate hyperthermia and then re-challenging them with the same tumors at a later time to observe if a memory response exists. Some notable examples of this response are discussed next. Mice with colorectal tumors generated on their femurs were treated with hyperthermia (42 °C for 30 min). A significant induction in apoptosis was achieved which coincided with a release of HSP70 into the tumor microenvironment. 24 h after heat treatment, mature dendritic cells (DC) were administered intratumorally, which resulted in a systemic anti-tumor immune response characterized by increases in lymphocyte, macrophage and eosinophil infiltration into the tumor. Notably, secondary tumor formation was also prevented in treated mice that were subsequently re-challenged one month later.[215] Hyperthermia treatment of mice melanoma models (four cycles of 43°C for 30 minutes) was also shown to increase inflammatory infiltrate and HSP expression in tumors. This treatment resulted in a strong reduction in tumor volume and resistance was also confirmed in these animals following tumor re-inoculation one-month post treatment. Importantly, this response was also shown to rely heavily on HSP72 antigen presentation to DC and subsequent cross-presentation of cytotoxic T lymphocytes in the draining lymph node.[216] Another notable study in this area used magnetic hyperthermia to treat mice with either B16 (melanoma) or CT26 (colon) tumors on both their left and right flanks with 43 °C for 30 min on their left flanks only. The left flank of mice with CT26 tumors was eliminated five days after hyperthermia treatment, while the right flank tumors experienced a significant growth delay compared to untreated animals; hence, achieving abscopal effects. Similarly, the B16 tumors experienced a significant reduction in tumor growth in both flanks against the untreated mice. Resistance was then assessed via treated mice that had their tumors excised three days after treatment and new tumors implanted in both flanks seven days later. The CT26 tumors did not re-establish on either flank, while the B16 tumors experienced a significant growth delay against the untreated animals, suggesting a memory response in both cases. Interestingly, this resistance to re-challenge did not occur at 45 °C for 30 min, implying that this response may be temperature-dependent. Moreover, inhibiting NK cell or T helper cell function resulted in no change in treatment efficacy or developed resistance, while the use of anti-CD8+ antibodies attenuated resistance, confirming CD8+ T cells were the main instigators of the anti-tumor immunity observed.[217]

While these examples describe the immunostimulatory effects of hyperthermia, the ability of ablative temperatures to induce anti-tumor immune responses has been reviewed extensively elsewhere.[218,219] Higher temperatures have been suggested as more efficient at instigating anti-tumor immune responses as they are more prolific at inducing immunogenic cell death,[220] although in vivo studies have repeatedly
confirmed that 42 °C for 30 min is effective for inducing significant DAMP expression.\textsuperscript{[221–223]} A number of reports have described adaptive responses with thermal ablation as a monotherapy via similar mechanisms to hyperthermia;\textsuperscript{[219,224]} however, in the majority of cases, ablative therapies only achieve durable anti-tumor immune responses when applied in combination with immunotherapies.\textsuperscript{[218,225]}

Therefore, based on the literature described, adaptive immunity can indeed be induced by moderate hyperthermia and the HSP70 family appear to be one of the principal sources of this response. It is apparent that HSP-bearing tumor DAMP’s can facilitate DC priming which, in turn, cross-prime CD8\textsuperscript{+} T cells and enable a cytotoxic response at both primary and metastatic tumor sites, while also maintaining this tumor rejection for months following treatment. Moreover, the extent of the immune stimulatory response may depend on the cancers immunogenicity and the temperatures generated, with 42–43°C seemingly favorable to 45°C (Figure 5). Likewise, thermoablative strategies have also shown potential to activate adaptive immune responses by DAMP upregulation, DC priming and CD8\textsuperscript{+} T cell activation, but adjuvant immunotherapy is required in the majority of cases to ensure a durable immune response. While these immunostimulatory effects are promising, clinical studies confirming these effects are required for both hyperthermia and thermal ablation to verify its clinical utility.

4.5.2. Innate Immunity

The impact of heat on innate immunity is poorly understood. There is some evidence, however, that thermal stress enhances NK cell cytotoxic function through direct and indirect means. Mild hyperthermia (\approx 39 °C) treatment of NK cells could directly augment their cytolytic effects,\textsuperscript{[226]} with clustering of the activating receptor NKG2D on the cell membrane thought to be the mechanism involved in facilitating its effector function.\textsuperscript{[227]} Additionally, cancer cells themselves have been sensitized to NK cytotoxicity through upregulations in NK-activating ligands (MICA and MICB; MHC class I polypeptide-related sequence A and B) following thermal stresses.\textsuperscript{[228]} Colon cancer cells were heated to 39.5 °C for six hours and displayed a heightened sensitivity to NK-mediated cell death via MICA expression, and this increased expression and sensitivity could be alleviated via siRNA treatment for HSF1—a regulator for MICA.\textsuperscript{[229]} Interestingly, both MICA and MICB genes share heat shock elements on their core promoter regions resembling that of HSP70.\textsuperscript{[229]} Numerous
reports have noted the ability of the HSP70 family to activate NK cells and facilitate their cytotoxic function.\textsuperscript{[230–233]} Renal cell carcinoma (ACHN) and prostate cancer (PC-3) cells were heated to 41.8 °C for three hours and assessed for their surface expression of HSP72. A transient increase in HSP72 was identified in ACHN cells, but not PC-3, and this expression peaked between 12–18 h post heat treatment, reverting to control levels after 48 h. Furthermore, no changes in NK sensitivity were observed in PC-3 cells, while ACHN underwent more than double the levels of cell lysis against controls treated with IL-2-activated NK cells. This effect could also be neutralized with treatment of anti-HSP72 prior to heat treatment.\textsuperscript{[234]} Likewise, patient-derived Ewing sarcoma cells treated with 41.8 °C for 200 min were shown to have an elevated surface expression of HSP72 for up to four days which corresponded to more than a twofold increase in lysis by IL-2-activated NK cells. This response was also cell-dependent, with several healthy and cancerous cells showing no increase in HSP72 following treatment. Again, this cell lysis could be inhibited by anti-HSP72 antibodies.\textsuperscript{[235]} Hence, elevations in HSP and other DAMP’s which get released into the tumor microenvironment. Some of these results have also translated to clinical studies. Of note, patients with either benign hyperplasia or adenocarcinoma of the prostate were treated with transrectal microwave hyperthermia consisting of six, bi-weekly treatments reaching 45 °C for 30 min at a time. Immunological parameters were assessed before and at multiple timepoints after treatment (up to six months) and compared against untreated controls. A significant increase in NK cell cytotoxicity was observed in adenocarcinoma patients that peaked two months post heat treatment, but no increase was observed for the benign hyperplasia group. Interestingly, the cancer patients were separated into responders and non-responders and a significant increase in NK cytotoxicity was noted in the responders versus non-responders.\textsuperscript{[236]} This prognostic potential of NK activity has also been reported in hepatocellular carcinoma patients treated with radiofrequency ablation (temperatures not provided).\textsuperscript{[237]} A recent report verified this clinical result in a rabbit model of hepatocellular carcinoma. Hereto, radiofrequency ablation (12–15 min at >50 °C) resulted in a significant increase in activated NK cells expressing NKG2D whose augmented cytotoxic activity could be mitigated with a neutralizing antibody for NKG2D function.\textsuperscript{[238]}

A variety of thermal doses (from mild hyperthermia to thermal ablation) have demonstrated capable of eliciting NK cell cytotoxic function against cancer. In these cases, enhanced NK cell activity is thought to be generated through elevated expression of its activating receptor NKG2D and its associated ligands on cancer cells, particularly MICA and MICB (Figure 5). Importantly, this response has been shown to be cell-dependent in in vitro studies and thought to be under the control of HSP, notably HSP72. While some clinical studies confirm this response, further studies are required to decipher whether this is cancer-dependent and determine the optimal thermal doses for eliciting this effect.

Following moderate hyperthermia for 30 minutes or short bursts of thermal ablation, tumor cells undergo necrosis and apoptosis which result in an upregulation of HSP that chaperone DAMP’s which get released into the tumor microenvironment. These DAMP’s are recognized by DC’s which internalize, process, and cross-present the antigens to CD8+ T cells in the draining lymph node. The cytotoxic T cells then migrate to the tumor and elicit their effector function. Additionally, hyperthermia and ablative therapies (at various temperatures and durations) can also upregulate the NK cell activation receptor NKG2D and its associated ligands MICA and MICB on cancer cells, enabling its cytotoxic function. Abbreviations: DC, dendritic cells; DAMP’s: damage-associated molecular patterns; CTL: cytotoxic T lymphocyte.

Both moderate hyperthermia and thermal ablation have been shown to activate innate and adaptive anti-tumor immune responses through NK and CD8+ T cells, respectively. While moderate hyperthermia has shown more examples of robust CD8+ T cell responses as a monotherapy compared to thermal ablation, these are only in vivo studies and must be verified in clinical scenarios to confirm the response and provide scope for enhancing cancer immunotherapies in various cancers. Similarly, mild-to-moderate hyperthermia and thermal ablation have also shown potential to generate innate responses through NK cell activation. This has translated to preliminary clinical data with thermal ablation where NK cells may also be a predictive marker for treatment response. Interestingly, in both innate and adaptive immune thermal-activation, HSP (notably HSP72) have been found to play a fundamental role in the immune stimulation observed, suggesting this too may be a marker for treatment response in this regard. Once further pre-clinical and clinical studies have confirmed the results previously reported, the scope for combining thermal treatments with immunotherapies to facilitate T cell (immune checkpoint inhibitors) and NK cell (cytokine activation therapy) responses can be fully appreciated.\textsuperscript{[219,239,240]}

### 4.6. Localized Heat and Metastasis

A number of studies have shown the potential of heat to inhibit the invasive potential of cancer. Heating melanoma and breast cancer cells in a water bath to between 43 and 47 °C for 30 min was shown to significantly inhibit cell invasiveness and expression of MMP-2, MMP-9, and TGF-β1 in a temperature-dependent manner.\textsuperscript{[241,242]} Likewise, photothermal therapy generating temperatures up to 48 °C for 10–20 min could selectively kill triple-negative breast cancer stem cells in vitro, while also inhibiting secondary mammosphere formation in these cells in a temperature-dependent manner. This treatment was additionally shown to significantly inhibit lymph node and lung metastasis in mouse models of this cancer (≥60 °C for 10 min).\textsuperscript{[243]} Many papers have also described the ability of nanoparticle-mediated hyperthermia to kill cancer stem cells and/or inhibit their renewal capacity.\textsuperscript{[244–246]} A similar in vivo study in 4T1 metastatic breast cancer models could also show that mild thermal ablation (46–48 °C for 30 min) increases long-term survival and inhibits metastasis against surgery alone.\textsuperscript{[247]}

Conversely, however, some reports exist which show that heat alone can have a detrimental effect on cancer spread. For example, pro-metastatic responses have also been reported in the clinic in a small number of patients (4 out of 96) receiving laser hyperthermia for liver metastasis with colorectal cancer\textsuperscript{[209]} as well as
cervical cancer patients treated at 42–43 °C for 30 min—where 4 out of 23 patients experienced distant metastasis versus 1 out of 23 in the control group.[110] This accelerated progression has also been reflected in a number of recent pre-clinical studies using thermal ablation.[248–250]

It is therefore apparent that moderate hyperthermia temperatures or higher have the potential to elicit an anti-metastatic response in cancer. The mechanisms behind this effect vary but can include a downregulation in metastatic protein expression, cytotoxic response to cancer stem cells or through abscopal effects (discussed in Section 4.5). It is also important to note, however, that cases exist where the opposite has been observed, where heat treatments have been shown to drive metastasis in cancer. Although literature in this regard is limited, available data would suggest that this effect may be due to resulting pro-inflammatory responses, increase in stemness and metastatic markers or induced angiogenesis following heating.[108,111,251,252]

5. Considerations to the Application of Localized Heat in Cancer

5.1. Summary of the Effects of Localized Heat on Cancer

There is a great deal of complexity when considering the broad effects of heat on tumors. The response is dependent on both the thermal doses applied and the specific tumor microenvironment in question. Below is a summary of the large array of reported effects associated with different thermal doses (Figure 6).

1) In vascularized tumors, increased blood perfusion occurs at mild hyperthermia doses (30–60 min), whereas vascular stasis becomes prevalent at moderate hyperthermia temperatures or greater for 60 min. Moreover, at moderate hyperthermia temperatures or higher, the resulting strong vascular stasis and ECM remodeling can lead pro-angiogenic
signaling and reduction in tumor pressure, respectively, culminating in increased blood perfusion.

2) Apoptosis occurs in cells treated with moderate hyperthermia for 30–60 min, while necrosis is the dominant mechanism of cell death at temperatures of 45 °C and above for 30 min or more.

3) DNA repair pathways are inhibited with mild and moderate hyperthermia at durations ranging predominantly from 15–90 min.

4) Heat-induced DNA damage occurs in cells exposed to moderate hyperthermia for 30 min due to inhibitions to DNA repair and replication signaling.

5) Cellular metabolism is elevated following exposure to moderate hyperthermia for 30–60 min.

6) NK and CD8+ T cell anti-tumor immune responses can be elicited with mild-to-moderate hyperthermia for 30 min or more or short bursts of thermal ablation.

7) Moderate hyperthermia for 30 min or short bursts of thermal ablation can inhibit cancer cell invasiveness and kill cancer stem cells. Conversely, these same thermal doses have also shown to accelerate cancer metastasis in rare cases.

Depending on the temperatures generated within a tumor, different responses can be induced. Each of these responses come with their own pros and cons and so thermotherapy must be strategically planned and tested to maximize treatment efficacy in each scenario. Mild hyperthermia can increase blood perfusion into tumors and inhibit DNA repair mechanisms, acting as a sensitizer for CT and RT. At these mild temperatures, however, there is no direct damaging effects on the tumor and sensitization requires an intact vascular system, which is not always the case. Moderate hyperthermia can directly induce apoptosis in cells and inhibit DNA repair which leads to DNA damage and provides scope for sensitizing the tumor to CT and RT. Apoptosis also leads to a release of DAMP’s which stimulate anti-tumor immune responses. On the other hand, vascular stasis and elevated metabolism can occur at these temperatures which may antagonize CT and RT efficacy if induced prior to their use. At temperatures moving from moderate hyperthermia to thermal ablation, irreversible damage is inflicted on the tumor resulting in necrotic cell death. This necrosis also releases DAMP’s which activates anti-tumor immunity. The high temperatures severely damage tumor vasculature and have also been shown to remodel the tumor ECM. Curiously, the resulting hypoxia and reduction in tumor stiffness from these effects can elicit vessel formation and alleviate tumor pressure, leading to increased blood perfusion which may sensitize to CT or RT or instigate further tumor growth if the entire tumor has not been sufficiently treated. Conversely, induction of vessel stasis before CT and RT can also inhibit their efficacy. Abbreviations: CT, chemotherapy; RT, radiotherapy; ECM: extracellular matrix.

Based on this summary and the literature detailed in Section 4, it is clear that localized heat can influence all hallmarks of cancer (Figure 7). Notably, these effects are temperature-dependent in the majority of cases (e.g., refs. [164, 181, 217]). Hence, optimizing the thermal dose applied and treatment protocol in each experimental scenario is essential to achieving optimal therapeutic outcomes. Of course, this implies that precise temperature doses must be applied to the tumor region, which remains a challenge for thermal techniques currently in clinical use due to the inaccessibility of deep tumors, heterogeneous temperature distributions associated with hyperthermia treatment, and the need for more advanced thermal modeling systems to establish greater control over dosimetry.[253–255]

Heat is capable of impacting all hallmarks of cancer—better or for worse. This plethora of possible effects provide a promising scaffold for combining heat with a wide range of therapies to enhance their response. The hallmarks of cancer, first described by Hanahan D. and Weinberg R. A in 2000[76] and then updated in 2011[77] are mentioned in black, while the relationship between each hallmark and heat are described in blue.

### 5.2. Combination Therapies

The myriad of effects described here above provide ample scope for combining localized heat treatment with established oncological regimens to elicit sensitizing effects.[256–258] The pre-clinical and clinical evidence for each modality are detailed next, along with considerations for optimal implementation.

#### 5.2.1. Heat and Radiation

One promising treatment approach utilizes hyperthermia as an adjunct to radiation. Heat has proven a strong radiosensitizer for a number of reasons:[29,259,260]

1) Increased blood perfusion and reoxygenated tumors.
2) DSB repair inhibition.
3) Preferential cytotoxicity of radioresistant cells.

A review published in 2015 analyzed 38 clinical studies constituting over 1700 patients in each treatment arm and reported an overall significant increase in complete tumor response with hyperthermia and radiation against radiation alone (54.9% vs 39.8% respectively).[140] The cancers involved in these trials included breast, rectum, bladder, cervix, head and neck, lung, anal canal, and melanoma (most successful trials are summarized in Table 2 below). A similar review analyzed 34 studies (>2000 patients in total) to determine the effect of this combination therapy on local, recurrent breast cancer and found complete tumor response could be increased by 22% when hyperthermia (microwave, radiofrequency, or ultrasound) treatment is added to radiation in these patients versus radiation alone.[261] From this clinical data, it was determined that melanoma, bladder, and cervical cancer are most sensitive to the addition of hyperthermia to radiation—in terms of complete response (i.e., loco-regional control).[198]

Importantly, hyperthermia dose and treatment protocols vary considerably across clinical studies. For example, recurrent and advanced breast cancers were heated using an external electromagnetic source generating tumor temperatures of either 43 °C for one hour or 42.5 °C for 30 min across five trials, summarized in a single manuscript.[262] The number of heat treatments (2–8), total radiation dose (28.8–50 Gy), and time interval between these treatments (<30 to >90 min) differed largely across the studies. Variability across clinical studies makes it difficult to compare treatment efficacy and establish optimal regimes for...
particular cancers, especially considering the number of variables involved with hyperthermia treatment. Conflicting opinions still exist with regards to time intervals between radiation and hyperthermia. The general consensus at present, however, is that hyperthermia and radiation should be performed as close together as possible, to maximize radiation-induced cell death before heat-induced DNA repair-inhibition normalizes within the tumor. This point is bolstered by a recent study in advanced cervical cancer patients which showed a dramatic increase in treatment efficacy when the intervals between radiation and hyperthermia were shorter (≤79.2 min) rather than longer (>79.2 min), resulting in a significant benefit in three-year in-field recurrence and five-year survival ($p = 0.021$ and $p = 0.015$, respectively). A further unresolved issue is the number of cycles of hyperthermia to administer, as the risk of thermotolerance increases with every consecutive treatment (see Section 5.3). One clinical study in recurrent breast cancer reported no significant difference between four and eight cycles of hyperthermia (41 °C for 60 min; applied within one hour of radiation and more than three days apart from the previous cycle). A similar conclusion was also reported in two studies published in the late 1980s and early 1990s, where two hyperthermia treatments were found to be equivalent to six treatments in a variety of tumors (42.5 °C for 45 min or 43 °C for 30 min; applied within one hour of radiation and at 3–7 day intervals).

From the meta-analysis described above and key clinical studies summarized in Table 2, it is clear that hyperthermia is a promising adjuvant to radiation for a number of cancers, displaying significant benefits to tumor response and even overall survival in some cases against radiation alone. At present, hyperthermia has been almost exclusively assessed in late stage or recurrent cancers, so its efficacy in early stage cancer is yet to be determined. From the clinical data described, a number of conclusions can be drawn. First, the sequence of hyperthermia and radiation does not appear significant to treatment outcome, however, the interval between these treatments must be as short as possible (<2 h) to ensure the strongest radiosensitizing effect. Next, the temperatures generated within the tumors in these studies suggest that inhibitions to DNA repair and preferential killing of radioresistant cells are the more likely radiosensitizing mechanisms involved in these cases. Accordingly, higher temperatures have been shown to provide better radiosensitizing...
effects than lower.\textsuperscript{265,272,273} Finally, the number of hyperthermia cycles varies between studies, it is difficult to determine a point where thermotolerance renders subsequent heat fractions obsolete, and whether this is cancer dependent. It is worth noting that most trials in this area aim to have 3–7 days between heat treatments to allow for the transient stress response to subside before subjecting the tumor to another cycle.\textsuperscript{274–276} This is certainly an area of research that requires further attention, however.

\subsection*{5.2.2. Hyperthermia and Chemotherapy}

Although not as auspicious as the wealth of clinical evidence available for hyperthermia combined with radiation, heat has been shown to benefit chemotherapy based on these proposed mechanisms:\textsuperscript{265,277,278}

1) Increased drug accumulation in tumors.
2) DNA repair inhibition.
3) Preferential cytotoxicity of chemoresistant cells.

Much like hyperthermia and radiation, the chemosensitizing effects of heat vary considerably depending on the thermal dose, time interval between treatments and the cancer itself; however, the varying mechanism of action of chemotherapies contribute an additional layer of complexity in this case.\textsuperscript{279,280} Of the drugs assessed during in vitro and in vivo studies, alkylating agents and platinum-based chemotherapies displayed the strongest enhancement when introduced simultaneously with hyperthermia (41.5–43.5 °C for 30 min); whereas pyrimidine analogues and vinca alkaloids experienced no potentiation.\textsuperscript{297,281} A similar study measured the chemosensitizing effects of fibrosarcoma mouse models heated to 41.5 °C for 30 min following intraperitoneal injections of gemcitabine (60 or 120 mg kg\textsuperscript{-1}), irinotecan (20 or 40 mg kg\textsuperscript{-1}), docetaxel (175 or 350 mg m\textsuperscript{-2}), paclitaxel (75 or 150 mg m\textsuperscript{-2}), or oxaliplatin (8.5 or 17 mg kg\textsuperscript{-1}). Gemcitabine, docetaxel, and irinotecan all experienced significant tumor growth delay at both low and high doses of the drugs when combined with heat. Oxaliplatin was sensitized at the higher dose only, while paclitaxel showed no benefit.\textsuperscript{282} A review published in 2016 evaluated the combination of hyperthermia and chemotherapy as a potential treatment for non-muscle-invasive bladder cancer. Under selective conditions, cisplatin, mitomycin C, gemcitabine, epirubicin, and doxorubicin could all display significant benefits to their efficacy when combined with heat (≥40–43 °C for one hour).\textsuperscript{283} Considering the clinical data available on this matter, some promising trials have been undertaken which evaluate the effects of hyperthermia and chemotherapy versus chemotherapy alone and are summarized in Table 3 below.\textsuperscript{284,277} It is not entirely clear why some drugs demonstrate significant enhancement following thermal therapy and some do not. Heat can induce drastic changes on the tumor microenvironment which may hinder or augment a drug’s efficacy in a myriad of ways. The variety of biological and therapy-based factors at play make it incredibly difficult to establish which pathways or mechanisms are involved in the response observed.\textsuperscript{285,287} Therefore, a lot of work is still required to elucidate the full potential of this combination therapy, although the clinical data available does hold promise. Like radiotherapy, it appears from the clinical data that a shorter time interval between chemotherapy and heat presents a better therapeutic outcome.\textsuperscript{286} However, this may be drug specific as some reports show treating with chemotherapy first, then hyperthermia at a later timepoint may be necessary to maximize a drug’s cytotoxic potential.\textsuperscript{288,289} With regards to thermal dosing, cycles of 42–43 °C for one hour have been repeatedly used in the clinical setting to potentiate chemotherapy. Based on

| Cancer                        | HT treatment                                      | RT treatment                                      | Interval | Result                                               | Ref.     |
|-------------------------------|---------------------------------------------------|---------------------------------------------------|----------|------------------------------------------------------|----------|
| Recurrent glioblastoma        | Magnetic HT: 6x one-hour sessions applied bi-weekly with a median peak temperature of 51.2 °C | Median dose of 30 Gy with 5x 2 Gy fractions per week | HT directly before or after RT fraction | Median survival from first recurrence: 13.4 months for HT and RT versus 6.2 months in RT alone | [45]     |
| Advanced cervical cancer      | Radiofrequency HT: 3x 60 to 90-min sessions applied weekly and aiming to reach temperatures of 42 °C (tumor temperature not recorded in majority of patients) | 46–50.4 Gy in daily fractions of 1.8–2 Gy | HT 3–4 h after RT fraction | Complete tumor response: 83% for HT and RT versus 57% for RT alone (p = 0.001) | [101]    |
| Recurrent breast cancer       | Microwave HT: 3x one-hour sessions applied seven days apart (50th percentile tumor temperature of 42.5 °C) | 28.8 Gy in 3.6 Gy fractions lasting two weeks | HT ≥ 90 min from RT fraction | Complete tumor response: 51/90 for HT and RT versus 17/59 for RT alone (p = 0.001) | [262]    |
| Recurrent or metastatic melanoma | Microwave or radiofrequency HT: 3x one-hour sessions four days apart aiming to reach 43 °C (although only 9% of patients reached this thermal dose) | 24 or 27 Gy in three fractions of 8 or 9 Gy four days apart | HT ≤ 30 min of RT fraction | Complete tumor response: 62% for HT and RT versus 35% for RT alone (p = 0.003) | [271]    |

Abbreviations: HT, hyperthermia; RT, radiotherapy; Gy, Grays; Ref, reference.
these temperatures, it appears that the predominant chemosensitivity mechanisms induced in these scenarios involve inhibition to DNA repair mechanisms and cytotoxicity to chemoresistant cells.\cite{165,298} Thermotolerance data is similarly understudied here and so further work is required to establish the extent of developed treatment resistance in this case.

### 5.2.3. Hyperthermia and Immunotherapy

There have been plenty of reviews published describing hyperthermia as a key potential enhancer of cancer immunotherapy.\cite{207,256,291,292} Despite this, combining localized hyperthermia with immunotherapies is still to be explored in clinical studies.\cite{239} Since hyperthermia is a known stimulant of innate and adaptive anti-tumor immunity under the right thermal conditions, it provides ample opportunities for enhancing cancer immunotherapies currently in use in the clinic, and also potentially stimulating non-immunogenic tumors in order to make them more receptive to immunotherapies. As hyperthermia has been shown to activate DC’s and facilitate their antigen presentation capacity to elicit cytotoxic T cells response,\cite{237} this makes hyperthermia a prime candidate adjunct therapy for immune checkpoint inhibitors in a variety of cancers.\cite{239} Of course, like radiation and chemotherapy, the same questions exist with regards to what the optimum thermal treatment regime is to achieve immune stimulation. Hence, research into thermal dosing, interval times and thermotolerance are considerations that must be addressed in future studies.\cite{203,239}

### 5.3. Thermotolerance

Although favorable for healthy tissue to prevent off-target effects when treating tumors with local hyperthermia, the development of thermotolerance in response to fractionated heat treatment is a potential hurdle for clinical use.\cite{274,277} As described in Section 5.2, clinical studies have reported no significant benefits to the inclusion of extra hyperthermia cycles on overall treatment efficacy when combined with radiation, suggesting thermotolerance builds with each thermal treatment.\cite{267,268} Although the exact mechanisms are not yet fully defined, the expression of HSPs has been shown to positively correlate with developed thermotolerance.\cite{293–295} In particular, HSP70 has proven to have a considerable role.\cite{294} Interestingly, HSP70 forms a complex with the Bcl2 interacting cell death suppressor (BAG3), which promotes autophagy.\cite{296} Reports also demonstrate that the HSP-BAG3 complex can promote cell survival by modulating the activity of NF\( \kappa \)B, HIF1\( \alpha \), p21, and survivin.\cite{297,298} HSPs can be released actively via extracellular vesicles (EV) or passively through dying cells, communicated with cells naïve to the thermal shock.\cite{299–301} One elegant study confirmed this bystander effect in a variety of other cancer and non-cancer cells.\cite{293–295} Of course, like radiation and chemotherapy, the same questions exist with regards to what the optimum thermal treatment regime is to achieve immune stimulation. Hence, research into thermal dosing, interval times and thermotolerance are considerations that must be addressed in future studies.\cite{203,239}

### Table 3. Notable clinical studies with hyperthermia as an adjunct to chemotherapy.

| Cancer                           | HT treatment                           | CT treatment                                      | Interval                   | Result                                      | Ref. |
|----------------------------------|----------------------------------------|---------------------------------------------------|-----------------------------|---------------------------------------------|------|
| Advanced gastric cancer          | Radiofrequency HT: Bi-weekly during CT cycle, lasting one hour and reaching 42–43 °C (90% of tumor surface) | 3x daily treatments of 80 mg m\(^{-2}\) of S-1 lasting two weeks, alongside 130 mg m\(^{-2}\) of oxaliplatin on day one of the cycle. [Note: 3-week break between each cycle] | HT directly after CT | Disease control rate: 70.9% for HT and CT versus 46% for CT alone (\(p = 0.006\)). Median survival: 23.5 months for HT and CT versus 14 months for CT alone (\(p = 0.01\)). 3-year survival: 11.4% for HT and CT versus 0% for CT alone (\(p = 0.018\)). | [284] |
| Localized soft tissue sarcoma     | Microwave hyperthermia: Applied at day 1 and 4 of CT cycle, lasting one-hour at 42 °C | 4 x 50 mg m\(^{-2}\) of doxorubicin on day one, 1500 mg m\(^{-2}\) of ifosfamide on days one and four and 125 mg m\(^{-2}\) of etoposide also on days one and four | During ifosfamide administration | 10-year survival: 51.3% for HT and CT versus 42.7% for CT alone (\(p = 0.04\)) | [285] |
| Non-muscle-invasive bladder cancer | Microwave HT: Applied during every CT session, lasting ≥40 min at 42 ± 2 °C | Eight weekly sessions of intravesically-administered Mitomycin-C at 20 mg, followed by four monthly sessions at the same dose | During Mitomycin administration | 10-year disease-free survival: 53% for HT and CT versus 15% for CT alone (\(p = 0.001\)) | [286] |

Abbreviations: HT, hyperthermia; CT, chemotherapy; S-1, gimeracil/tegafur/oteracil; Ref, reference.
and in vivo studies. In these cases, both gene silencing and inhibitors of HSP70 function were used to successfully achieve the desired response. Moreover, drug-mediated inhibition of STAT3 has been suggested as a potential target for thermotolerance as phosphorylated STAT3 is a regulator for HSP70 expression. This targeted inhibition resulted in heightened cytotoxic responses in follow-up heat treatments.\[108\] Hence, targeting the molecular mechanisms behind thermotolerance may enable a greater number of hyperthermia cycles to be applied in the future.

6. Conclusion

Localized heat can influence all hallmarks of cancer. Depending on the temperatures generated within a tumor, alternative responses can be induced. Tailoring these affects to maximize the efficacy of chemotherapies, radiation, and immunotherapies is key to enable thermal treatment to reach its full potential in the clinic. Further pre-clinical and clinical studies will determine the optimal protocols (temperatures, duration, and treatment intervals) for each combination therapy and whether they are cancer dependent. Additionally, advancements in the monitoring of temperature parameters and thermal energy delivery will provide improved control over dosimetry in the future. Although much more work is required, it is certain that localized heat treatment still holds promise for the treatment of cancer.

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Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

G.H. conceptualized the review, wrote the first draft, reviewed subsequent drafts, and finalized it for submission. F.L.T. reviewed and edited the manuscript. I.H. reviewed and edited the manuscript. A.P.M. reviewed and edited the manuscript and finalized it for submission.

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Gary Hannon is a postdoctoral researcher with the Nanomedicine and Molecular Imaging Group at Trinity College Dublin, Ireland. He received his B.Sc. from Maynooth University and M.Sc. and Ph.D. from Trinity College Dublin. His research focuses on the application of nanomedicine to the field of oncology, with a key interest in the use of iron oxide nanoparticles to treat cancer through a thermal therapy known as magnetic hyperthermia.

Felista L. Tansi is a research scientist at the Institute of Diagnostic and Interventional Radiology (Experimental Radiology Group), of the Jena University Hospital, Jena, Germany. She received her M.Sc. and Ph.D. from the Free University of Berlin, Germany and recently completed a habilitation in molecular medicine at the Friedrich Schiller University Jena, Germany. Her research focuses on the design and application of nanomedicines for drug delivery, imaging, and thermal therapy in inflammatory disorders and oncology.
Ingrid Hilger is head of the “Experimental Radiology” Department at the University Hospital Jena, Germany. Born in Argentina, she studied biology at the Christian-Albrechts-University in Kiel, Germany, and received her diploma in 1990. She performed several studies in biology in South America and Asia. Later on, she got interested in human biology and biochemistry, received her Ph.D. at the Medical High School Hannover, Germany, in 1996. Since then, she focused her research activities to the areas of thermo-therapeutic or diagnostic nanotechnology and translational molecular imaging. Since 2008, she is full professor at the University Hospital Jena, Germany.

Adriele Prina-Mello is the Ussher Assistant Professor in Translational Nanomedicine at the Department of Clinical Medicine, Principal Investigator of the Nanomedicine and Molecular Imaging group, Director of the Laboratory for Biological Characterisation of Advanced Materials (LBCAM) at the Trinity Translational Medicine Institute (TTMI), and Associate Director of Research in the School of Medicine. He specialized in nanotechnology applied to inflammation, acute and chronic disease and oncology. His research activities are focused on the development, characterization, and translation of innovative nanotechnology-enabled diagnostics, therapeutics and theranostics solutions and tools for inflammation, infection, and cancer. Among the many, magnetic hyperthermia as the use of physically-triggered iron oxide nanoparticles for cancer treatment.