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Review of preclinical data on hyperthermia treatment in lymphomas and its potential for clinical application

Moritz V. Scharr[a,b], Frederik Damm[b,c], Paul Krahl[a,b], Anna Dieper[a,b], Paraskevi D. Veltsista[a,b], Alexander Hansch[a,b], Marcus Beck[a,b], Dominik Gerster[a,b], Anna-Chiara Giovannelli[a,b], Lars Bullinger[b,c], Daniel Zips[a,b] and Pirus Ghadjar[a,b]

[a]Department of Radiation Oncology, Charité – Universitätsmedizin Berlin corporate member of Free University Berlin and Humboldt University Berlin, Berlin, Germany; [b]German Cancer Consortium (DKTK), Partner Site Berlin, and German Cancer Research Center (DKFZ), Heidelberg, Germany; [c]Department of Hematology, Oncology and Cancer Immunology, Charité – Charité – Universitätsmedizin Berlin corporate member of Free University Berlin and Humboldt University Berlin, Berlin, Germany

ABSTRACT

Introduction: Hyperthermia (HT) at temperatures between 39 °C and 44 °C is utilized as an adjunctive cancer therapy, serving as potent radio- and chemosensitizer. Its effectiveness in treating solid malignancies has been well established. This raises the question of whether HT can also benefit patients with nonsolid tumors, such as lymphomas.

Objective: To provide an overview of the current literature on research involving the use of HT in the treatment of lymphomas.

Material and Methods: This systematic literature review was conducted following the PRISMA guidelines. For this purpose, a MeSH-term-defined literature search on MEDLINE (Pubmed) and Embase (Ovid) was conducted from June 25 to June 28, 2024. Included were *in vitro* studies on lymphoma cell lines and preclinical studies on animal models with lymphoma that were both treated with HT as monotherapy or HT in combination with another treatment, and studies on patients with lymphoma. Excluded were studies that used thermal ablation and hyperthermic perfusions.

Results: Thirty-nine studies were included, predominantly *in vitro* studies ($n=32$) or studies on animal models ($n=5$). The *in vitro* studies utilized HT either as monotherapy ($n=6$), with substances that enhance HT efficacy ($n=18$) or as a sensitizer for other treatments ($n=8$). Additionally, two clinical case reports on the treatment of lymphoma patients were included.

Conclusions: *In vitro* results suggest that HT can have anticancer effects on lymphoma cells and may enhance existing treatments. These findings are supported by *in vivo* studies and case reports. However, additional clinical data are needed before translation into the clinic can be implemented.

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Introduction

Hyperthermia (HT) is defined as raising the temperature of tumor-loaded tissue above physiological levels, typically up to between 39 °C and 44 °C. This elevation in temperature is used as a supplementary treatment to enhance conventional cancer therapies such as chemotherapy or radiation therapy [1–3].

In vitro studies demonstrated that HT can directly and indirectly cause DNA damage and inhibit DNA repair, induce immunogenic cell death via the release of damage associate molecular patterns and increase blood flow improving oxygenation in hypoxic tumors, thereby enhancing the efficacy of chemotherapeutic drugs such as PARP-inhibitors, platinum-based agents, gemcitabine, radiation or immunomodulatory therapies [4–8].

Multiple clinical trials have shown that HT can enhance the effects of both radiotherapy and chemotherapy [9]. For instance, in patients with localized high-risk soft tissue sarcoma, combining radiofrequency HT with neoadjuvant chemotherapy resulted in increased progression-free- and overall survival compared to those who only received neoadjuvant chemotherapy. Similarly, in patients with locally advanced cervical cancer, radiofrequency HT in combination with chemoradiotherapy led to increased disease-free survival compared to the control group who only received chemoradiotherapy [10, 11]. Van der Zee et al. demonstrated the radiosensitizing effect of HT, reporting a significantly higher overall survival ($p=0.05$) and complete response ($p<0.001$) in patients with locally advanced cervical carcinoma receiving HT combined with radiotherapy compared to those receiving only radiotherapy [12].

CONTACT Pirus Ghadjar pirus.ghadjar@charite.de Endorsed Professor for Radiation Oncology main focus Electromagnetic Fields, Charité – Universitätsmedizin Berlin, Department of Radiation Oncology, Augustenburger Platz 1, 13353 Berlin, Germany

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A deficit in knowledge exists regarding the use of HT in patients with non-solid tumors, such as lymphomas. Therefore, the goal of this systematic review is to assess the existing data on HT treatment in patients suffering from this disease.

Methods

Inclusion and exclusion criteria

The systematic literature review was conducted following the PRISMA guidelines (https://www.prisma-statement.org/). It incorporates clinical as well as preclinical trials that used any form of HT treatment on patients with lymphoma, lymphoma animal models and human and animal lymphoma cell lines.

Excluded were all studies using HT methods that induce direct necrosis, such as thermal ablation (i.e., through photothermal therapy or high-intensity focused ultrasound therapy) and hyperthermic perfusions

Only English papers published after the year 2000 were included.

Search strategy

A systematic search-term-defined literature review was conducted to find relevant resources reporting on the use HT treatment on lymphomas. The literature search was performed across PubMed (MEDLINE) and Ovid (Embase). Additionally, further literature meeting the inclusion criteria was identified through a manual search of previously gathered papers. A search in the international clinical trial registry www.clinicaltrials.gov was carried out to identify ongoing or completed clinical trials that may not yet be published.

The literature search was conducted from June 25 to June 28, 2024.

To ensure the inclusion of all relevant publications, the MeSH system was used for the search on PubMed, and thesaurus terms were employed for the search on Embase.

The search in MEDLINE was as follows:

(HT[MeSH Terms]) OR (HT, induced[MeSH Terms])) OR (HT, local[MeSH Terms])) OR (HT, therapeutic[MeSH Terms])) OR (heat treatment[MeSH Terms])) OR (thermal therapy[MeSH Terms])) OR (thermotherapy-[MeSH Terms])) OR (thermo chemotherapy[MeSH Terms])

AND

(lymphoma[MeSH Terms]) OR (abnormality, lymphatic[MeSH Terms])) OR (lymphoid neoplasm[MeSH Terms])) OR (b cell lymphoma[MeSH Terms])) OR (lymphoma cell line[MeSH Terms])) OR (cell, u937[MeSH Terms])) OR (RAJI cell[MeSH Terms])) OR (lymphatic cancer[MeSH Terms])

Filters: From 2000–2024
and in Embase (Ovid):

HT/or thermotherapy/or thermotherapy device

AND

Lymphoma/or lymphoma cell/

As an example, a search using the thesaurus term thermotherapy includes the following synonyms: "Artificial HT, dry heat therapy, hyperthermic therapy, hyperthermic treatment, induced HT, infrared therapy, therapy, heat, thermal therapy"

Data extraction

To systematically assess the studies, they were reviewed based on inclusion and exclusion criteria, heating method, duration of heating, applied temperature, study population characteristics and whether another intervention was involved. A risk of bias assessment was not performed as it was deemed unfitting for a preclinical review.

Results

Search results

The systematic search on MEDLINE (Pubmed) and Embase (Ovid) resulted in 498 publications identified on MEDLINE and 54 publications identified on Embase. After screening of title and abstract, 37 publications were sought for retrieval. Another 19 publications that met the inclusion criteria were found manually by screening the references of the identified studies. No ongoing or not yet published studies could be identified. The studies excluded after the screening of titles and abstracts were, for example, removed because they used incorrect heating methods, such as thermal ablation, or because the intervention targeted solid tumors or noncancerous tissue. 51 full-text articles were assessed for eligibility. In the end 39 articles that met the inclusion criteria were included. For an overview of the selection process see Figure 1.

Characteristics of the studies

The identified studies were categorized into three main groups: *in vitro* studies on various lymphoma cell lines ($n=32$), preclinical studies on animal models ($n=5$) and case reports on patients with lymphoma ($n=2$). The *in vitro* studies were further divided into the following subcategories:

1. 'Combination of potential thermosensitizer with HT' ($n=18$):
   - Studies that combined HT with a potential thermosensitizer to enhance the efficacy of HT.
2. 'HT as a Monotherapy' ($n=6$):
   - Studies that explored HT treatment as a monotherapy.
3. 'HT as an additive therapy to improve another treatment' (n=**9**):
   - Studies that used HT to boost the effectiveness of a different primary intervention.

The goal of the review is to highlight key findings from the literature that are relevant for clinical translation in lymphoma treatment. However, studies that combined HT with thermosensitizers were excluded from the main analysis due to their limited clinical relevance and are only summarized in Supplementary Table 1. The most important methodological characteristics of the included studies, as well as features of the study populations, are summarized in Supplementary

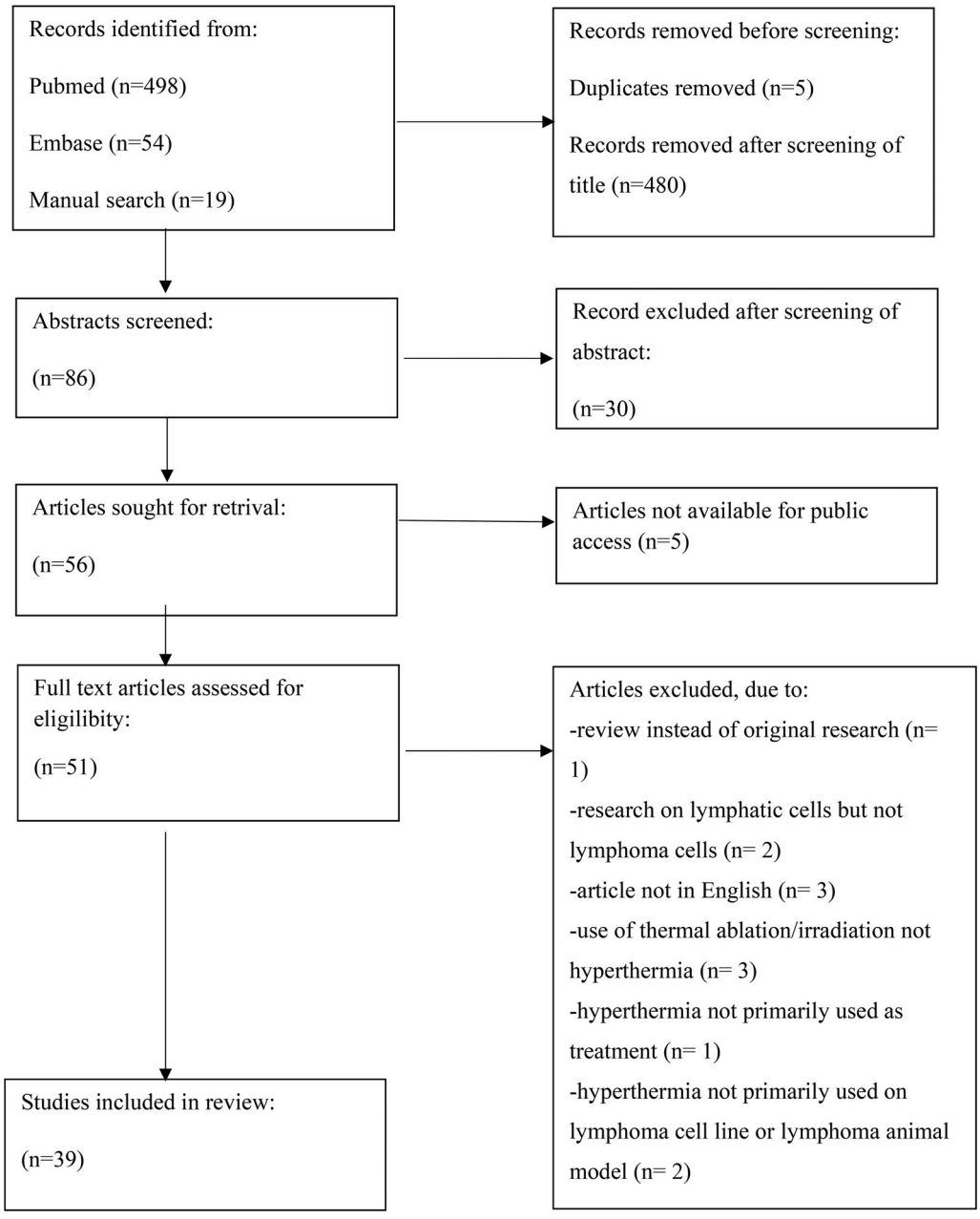

Figure 1. *Publications with titles already revealing a conflict with predetermined inclusion and exclusion criteria (i.e. use of thermal ablation methods such as high intensity focused ultrasound or thermal ablation, or the application on strictly non-cancerous tissue, the application on solid tumors, etc.) were excluded before abstract and full text screening.

Tables 2, 3, 4 and 5 based on their respective categories. One study utilized both *in vitro* cell lines and animal models and is therefore included in both categories.

Effects and molecular mechanisms of HT as a monotherapy on lymphoma cell lines

All studies ($n=6$) from that group are summarized in supplementary Table **2**

There are four studies [13–16] evaluating the effects of HT in the form of a water bath on U937 lymphoma cell lines (Histiocytic lymphoma cells isolated from a 37-year-old male patient) ($n=3$) and chicken B-Lymphoma DT40 cells ($n=1$). The effects of HT were measured by detecting apoptosis and apoptosis-related markers, as well as gene networks responsive to HT.

It was observed that HT-induced apoptosis occurs only at temperatures higher than 42.5 °C, which is described as the inflection point of HT [13]. However, the induction of apoptosis also depends on the duration of heating. In U937, lymphoma cells that were heated via water bath hyperthermia (WHT) at 42 °C, significant DNA fragmentation was only observed after heating for more than 60 min [15].

Tabuchi et al. [16] identified changes in genetic networks associated with the response to mild HT in human U937 lymphoma cells, offering new insights into cellular responses to heat stress. Specifically, it was revealed that mild hyperthermia in U937 cells resulted in increased

expression of heat shock proteins (HSPs) like Hsp27, Hsp40, and Hsp70.

Furusawa et al. [13, 15], further identified HT-specific gene networks and cellular response patterns, for example, the activation of apoptosis-related gene networks in conditions of severe heat stress. They are also emphasizing on the balance between cytoprotective and pro-apoptotic genes

A particular focus was on the expression of HSP in response to HT. HSPs are expressed in response to various cellular stressors, including heat stress. HSPs are often overexpressed in lymphomas and are associated with increased therapy resistance [17, 18]. They function as chaperones for other proteins and have cytoprotective effects by inhibiting apoptosis. However, they also have immunomodulatory effects that may enhance anti-tumor responses. HSPs have also shown to be potent immune system activators by facilitating antigen presentation, inducing cytotoxic T-cell responses, and stimulating cytokine production [19, 20]. It was shown that HSP27, HSP40 and HSP70 are significantly upregulated mediated *via* the heat shock transcription factor (HSF) following HT treatment at 44 °C and even after mild HT at 42 °C and 41 °C. HSPs may be key factor in inhibiting apoptosis in lymphoma cells [4–6].

Two studies compared conventional WHT to modulated electro-HT (mEHT) and presented the molecular differences in cell death mechanisms and cellular response toward HT [21, 22]. mEHT is a form of amplitude-modulated radiofrequency heating that generates heat using capacitive-coupled energy at 13.56 MHz. It was shown that the temperature-dependent cell death in WHT and mEHT were different. A significant increase in cell death was observed at 41 °C in cells treated with mEHT. After peaking at 41 °C, cell death decreased and only started to rise again at 45 °C. In contrast, significant cell death in cells treated with WHT was observed only at temperatures higher than 44 °C. The authors attribute this observation to the stronger activation of the extrinsic apoptotic pathway and reduced expression of HSPs in cells treated with mEHT compared to those treated with WHT. In summary, mEHT and WHT result in different biological responses. At lower temperatures, apoptotic-inducing pathways were dominant in cells treated with mEHT, while in cells treated with WHT cell-protective mechanisms prevailed.

These studies identified pathways and proteins that play important roles in HT-induced apoptosis and heat tolerance. This could be used as a steppingstone for further research assessing the sensitivity of lymphoma cells to HT. Moreover, understanding the mechanisms behind HT-induced apoptosis as well as the signaling pathways upregulated in response to heat is important for determining which cytostatic drugs present enhanced efficacy when combined with HT. Despite offering valuable insights into basic mechanisms of HT, these studies are not relevant for potential clinical translation. It is also uncertain to what degree these findings can be applied to other lymphoma cell lines or if they are specific to lymphoma cells.

HT as an additive treatment to cytostatic drugs and radiotherapy in lymphoma cell lines

All studies considering HT as an additive treatment to cytostatic drugs or radiotherapy in lymphoma cell lines are summarized in Supplementary Table 3.

Seven studies using different chemotherapeutic drugs in combination with HT on lymphoma cell lines were found [23–29].

Istomin et al. investigated the effect of HT on the cytostatic activity of different chemotherapeutic drugs (carboplatin, cisplatin, oxaliplatin, carmustine, etoposide and gemcitabine) on RAJI human lymphoma cells. The cells were treated with these drugs at either 37 °C or 42 °C for 15 or 30 min in combination with HT. Treatment efficacy was measured using the IC50 dose (drug concentration resulting in growth inhibition by 50% vs. the control) and the thermal enhancement ratio (TER) (IC50 dose for drug alone divided by IC50 dose for drug combined with HT). They demonstrated a synergistic enhancement in the efficacy of carboplatin (TER = 1.8–2.4), cisplatin (TER = 2.0), and oxaliplatin (TER = 3.0) when combined with HT, as well as additive effects when combined with carmustine and etoposide. HT for 15 min in combination with gemcitabine resulted in a slight increase in cell growth inhibition (TER = 1.4), whereas hyperthermia for 30 min led to a decrease in cell growth inhibition (TER = 0.8). The strongest synergistic effect was observed when platinum-based drugs were combined with gemcitabine [26]. However, Fang et al. reported that HT at 42 °C for an hour resulted in increased resistance of RAJI lymphoma cells to adriamycin and cisplatin. A possible explanation for that is the induction of HSPs by heat that have a cytoprotective effect [25]. Milani et al. and Saliev et al. described an addition of HT-induced and bortezomib-induced effects when treating U937 lymphoma cells and different mantle cell lymphoma cell lines [23, 24]. Milani et al. also demonstrated that increased levels of HSPs induced by heat do not interfere with the sensitivity of mantle cell lymphoma cells to bortezomib [23]

These findings indicate that HT has chemo and radiosensitizing [30] effects on lymphoma cells. However, the mixed results, varying study designs, which do not allow for direct comparison, and the limited number of studies, emphasize the need for further research.

In vivo *studies on animal models with lymphoma*

There are five studies that are using a form of HT on animal models with lymphoma [31–35], (Supplementary Table 4).

Two of them are investigating the immunogenic effect of HT on EL-4 lymphoma cells injected in C57L/6 and E.G7-OVA mice ($n = 8$ per group) [31, 32].

Ando et al. evaluated whether local HT can induce a tumor-specific immune response mediated through cytotoxic t-cells. C57BL/6J mice were injected with the lymphoma cell line E.G7-OVA (2.0×10^6 cells/mouse) in the right femur. The mice were then divided into four groups: a control group that received no treatment (control), a group that received only local HT, a group treated with only immunomodulatory antibodies (anti-mouse CTLA4 mAb/anti-mouse CD8 mAb), and a group that received a combination of immunomodulatory antibodies and local HT. One week after tumor inoculation, the mice underwent local HT in the form of a water bath for 60 min at 42 °C. Treatment efficacy was evaluated by measuring tumor size and tumor growth duration (defined as the number of days taken until the tumor volume reaches 1000 mm^3 following local HT treatment). T-cell activation was assessed by measuring the production of interferon-gamma. The results showed that local HT significantly suppressed tumor growth duration ($p = 0.01$) and clearly induced E.G7-OVA-specific interferon-gamma-producing splenocytes, suggesting a systemic antitumor response. Additionally, treatment with

anti-mouse CD8 mAb significantly ($p = 0.02$) reduced the therapeutic efficacy of local HT, but had no effect on the control group, indicating that the CD8+ T-cell immune response is a component of the local HT-induced antitumor immunity. However, anti-CTLA4 mAbs did not significantly enhance the therapeutic efficacy of local HT.

Tanaka et al. utilize magnetic nanoparticles to create heat at the tumor site as a method of localized HT. When an external alternating magnetic field is applied, these nanoparticles generate heat specifically within the tumor. To investigate HT-induced immunogenic effects mouse T-lymphoid EL4 cells were injected into the right flank of C57BL/6 mice. The mice were divided into four groups, either receiving no treatment (control), HT treatment for 30 min at 45°, an injection of immature dendritic cells or HT and dendritic cells combined. In the mice receiving the combined treatment complete regression was observed in 75% compared to 12,5% in the mice that only received HT. In the mice that did not receive any treatment or only DCs no complete regression was observed. Both studies showed that HT can have an immunomodulatory effect probably through helping antigen presentation to antigen-presenting cells such as DCs leading to enhanced activation of cytotoxic T-cells (CTL). A potential mechanism for this involves apoptotic tumor cells induced by HT releasing intracellular antigens, which are then taken up by DCs. These antigens are subsequently presented on MHC class I or II molecules to T cells [31]

Dai et al. were using magnetic nanoparticles for thermochemotherapy in mice that were injected with the diffuse large B-cell lymphoma cell line OCI-LY18 [33].

The study confirmed that magnetic hyperthermia synergizes with chemotherapy by inducing intrinsic apoptosis and targeting tumor cells effectively, enhancing overall treatment efficacy while reducing chemotherapy drug usage. In five different treatment groups, the mice either received only methotrexate (MTX), only Fe_3O_4 nanoparticles, nanoparticles loaded with MTX, only nanoparticles that were heated using an electromagnetic field for 60 min or nanoparticles loaded with MTX in combination with heat treatment for 60 min. According to MRI and pathological analyses tumor volume in the Fe_3O_4 + HT group was significantly lower than in the Fe_3O_4 only group ($p < 0.05$) suggesting that HT can significantly inhibit tumor progression. The same interventions performed *in vitro* showed synergistic effects between chemotherapy and thermotherapy as evidenced by the upregulation of pro-apoptotic genes (Caspase-3 and Bax) and downregulation of the antiapoptotic gene Bcl-2. Masanuga et al. demonstrated a recovery inhibiting effect of HT in EL4 lymphoma cells injected into mice following radiotherapy [36].

These results suggest the efficacy of HT on animal models with lymphoma and suggest an immunomodulatory effect of HT that may be used in treatment in combination with other immunotherapies. The low number of *in vivo* studies and the heterogenous study design underscore the need for further research.

Case reports

Two case reports describing a HT treatment on patients with lymphoma were found [37,38], (Supplementary Table 5).

Barni et al. describe the treatment of a 63-year-old patient with non-Hodgkin lymphoma, primarily localized in the axilla [38]. The patient underwent two sessions of capacitive RF HT with a frequency of 13.56 MHz for 90 min each, with a 72-h interval between treatments, during a relapse while receiving chemotherapy. The temperature was kept between 41–43 °C. A biopsy was taken after each treatment to assess treatment efficacy by measuring apoptosis and mitotic activity of the tumor cells, comparing these results with biopsies taken before HT treatment. The biopsy showed significant ($p < 0.01$) increase in apoptosis, and significant inhibition of cell proliferation in cells treated with HT compared to cells that were taken before treatment. Incidence of apoptotic cell death almost tripled after the second treatment. Indicating a significant reduction of the tumor growth.

Honma et al. reported almost complete regression of a primary cutaneous anaplastic large-cell lymphoma in a 89-year-old patient treated with local thermotherapy using pocket hand warmers [37]. The tumor, which was localized on the chest, recurred after resection. Local HT in the form of pocket hand warmers was applied for 2 h daily for the first seven days and then every other day. The skin temperature can approximately reach 42–43 °C using the pocket hand warmers. Treatment efficacy was assessed by measuring tumor growth and levels of blood thimidine kinase (normal range 0–5 U/L). After 10 weeks of treatment the tumor almost completely regressed and levels of blood thimidine kinase decreased from 19 U/L to 10 U/L. However, the results can hardly be considered significant because HT was used as monotherapy, and the patient died from pneumonia four months after the treatment began.

In both cases, the lymphoma was primarily localized, which is not representative of the majority of lymphoma patients. Furthermore, case reports lack statistical significance and, therefore, only suggest that HT might be beneficial in the treatment of lymphoma.

Discussion

The results of our systematic literature review show mostly preclinical evidence suggesting anticancer effectiveness of HT in lymphomas [13]. In addition, enhancement of HT was observed using substances such as isofraxidin, cordycepin or cold atmospheric helium plasma [39–41]. However, these substances, along with the other thermosensitizers, are not relevant for potential clinical translation. Furthermore, HT synergistically enhanced the efficacy of chemotherapeutic drugs, that is, oxaliplatin, cisplatin or carboplatin as well as enhancing the effect of radiotherapy [26, 30]. *In vivo* studies, where diffuse large B-cell or T-cell lymphoma cells were injected in mice, supported those findings while also reporting on the immune modulatory effects of HT [31, 33].

The main modes of action of HT in lymphomas were summarized in Figure 2.

However, only two clinical case reports were identified; in addition, a search of the clinicaltrials.gov database showed no apparent ongoing clinical trial activity studying HT in hematological malignancies.

Lymphomas are malign neoplasms originating from lymphatic cells accounting for about 4% of all cancers. They are divided into Hodgkin (HL) and non-Hodgkin lymphomas (NHL). NHL can be further divided into B-cell and T-cell lymphomas, with approximately 85% being B-cell lymphomas.

First-line therapy usually consists of a combination of chemotherapy regimes, radiotherapy and targeted therapy. In

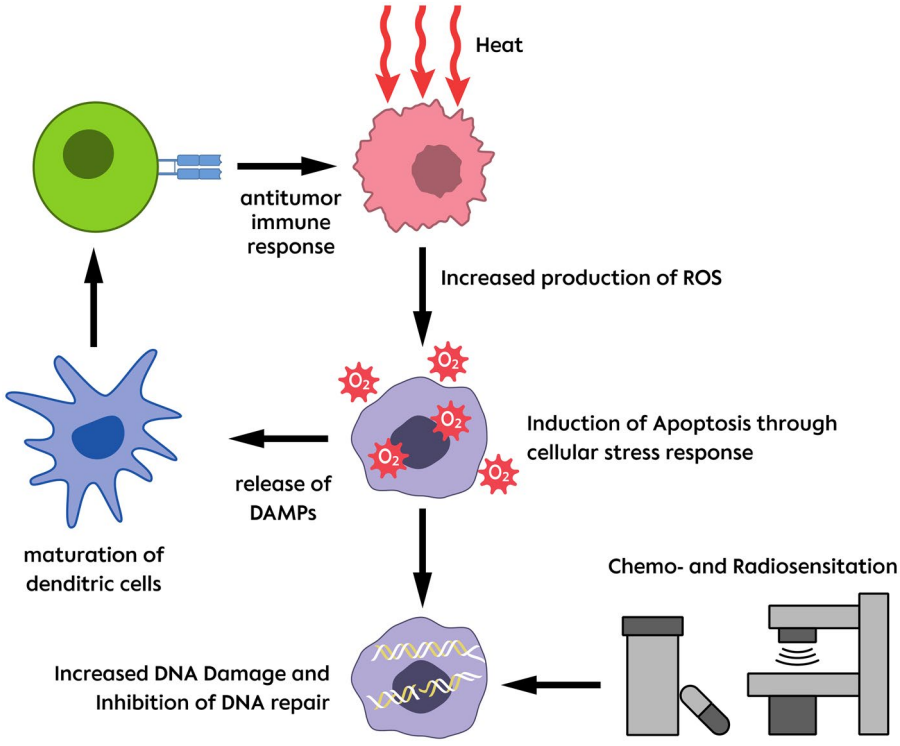

Figure 2. Heat exposure increases intracellular ROS levels, which can trigger apoptosis through a cellular stress response. Elevated ROS levels can also lead to more DNA damage and inhibit the activity of DNA repair enzymes. The DNA damage caused by HT has chemo- and radiosensitizing effects. Apoptosis induced by HT results in the release of DAMPs, which function as antigens and promote the maturation of dendritic cells. These cells, in turn, activate T-cells, enhancing the antitumor immune response.
Abbreviations: ROS: Reactive oxygen species; DAMPs: Damage associated molecular patterns.

relapsed or refractory disease, high-dose chemotherapy, immunotherapy or stem cell transplantation are used [42].

Based on the results of this literature review, several findings suggest potential benefits of HT in lymphoma treatment. Platinum-based chemotherapy, which is synergistically enhanced by HT, is currently part of the high-dose chemotherapy regime for patients with HL experiencing their first relapse, and for patients with diffuse large B-cell lymphoma (DLBCL) as part of salvage therapy combined with stem cell transplantation [26, 43, 44].

Radiotherapy, frequently used in lymphoma treatment, for example, in patients with follicular lymphoma in stages I and II or as part of first-line therapy for HL, has also been shown to be enhanced by HT in lymphoma cell lines and animal models [30, 36, 45]. The abscopal effect of radiotherapy may be enhanced when combined with HT, considering the immune modulatory effects of HT [31, 32].

Additionally, results indicate a synergistic enhancement of MTX by HT in DLBCL cells [33]. MTX is commonly used as first-line treatment in patients with primary central nervous system lymphoma [46]. Furthermore, two studies reported enhanced lymphoma cell killing when HT was combined with bortezomib, a treatment commonly used for multiple myeloma [23, 24, 47].

Lastly, considering the immune-modulatory effects of HT, there is a potential for enhancing CAR T-cell therapy as well as bispecific antibodies, a treatment option for patients with DLBCL experiencing a second relapse, when combined with HT [43].

Andocs et al. [21]: suggest that anticancer effects beyond pure temperature elevation/heating exist. This is a controversial topic. We have also found *in vitro* and *in vivo* evidence that nontemperature induced/nonthermal anticancer effects exist, in a colorectal cancer model [48]. Combined thermal and nonthermal effects of radiofrequency-based hyperthermia (for instance the mEHT approach) might be beneficial for patients. More research is required to further explore the potential of this treatment method.

The review has several notable limitations. First, it relies heavily on preclinical studies, with only two papers involving clinical trials, both of which are case reports. Consequently, there is insufficient clinical evidence to demonstrate the efficacy of HT in lymphoma patients. Furthermore, most *in vitro* studies explored HT either as a monotherapy or in combination with a thermosensitizer. However, these forms of application are not relevant to clinical practice. Additionally, it is unclear whether the results indicate a lymphoma-specific effect distinct from previously published findings on cancer cells.

Many studies used the U937 lymphoma cell line due to its rapid and robust response to HT-induced apoptosis, making it practical for laboratory use. However, this cell line was often employed in studies of cancer treatments more broadly, rather than being specifically targeted for lymphoma. Similarly, Masunaga et al. utilized the EL4 lymphoma cell line primarily for its strong apoptotic response. As a result, the generalizability of these findings to other lymphoma cell lines remains uncertain.

The heterogeneity among the studies further complicates evaluation. Variations in heating techniques (e.g., WHT, mEHT, nanoparticles), duration of treatment, and the cell lines used make it difficult to draw definitive conclusions. Moreover, the combination of HT with cytostatic drugs such as gemcitabine does not consistently produce synergistic effects in lymphoma cell lines, suggesting that HT may only be effective when paired with specific drugs, such as platinum-based cytostatics.

Animal studies also exhibited variability in their heating methods. A significant experimental difference was whether HT was applied to lymphoma cells before they were injected into animals or after tumors had developed. This distinction complicates the translation of results to clinical practice, as lymphomas are generally systemic diseases, while the studies focused on localized tumors. Therefore, a key challenge lies in adapting locoregional treatments like HT for the broader, systemic nature of lymphomas.

HSPs such as hsp27, hsp70 or hsp90 can have a cytoprotective effect by inhibiting apoptosis [25]. These are commonly overexpressed in various type of lymphoma [17] and are associated with a resistance to certain chemotherapeutic drugs, for instance bortezomib [18]. A concern with HT treatment is that it may further increase the expression of heat shock proteins in lymphomas, leading to enhanced resistance to therapy.

However, the studies evaluating the role of HSPs only used HT on cell lines. Thus neglecting the immunological functions of these proteins. In other cancer cell lines, HSPs have been shown to bind antigens and facilitating their presentation to the immune system, leading to activation and maturation of dendritic cells which enhances antitumor immune responses [49, 50].

A potential approach to integrating HT into lymphoma treatment is to use its potential immunogenic effects to enhance lymphoma immunotherapy. HT can kill malignant cells in an apoptotic way that leads to release of damage-associated molecular patterns (DAMPs) such as HSPs, known as immunogenic cell death (ICD) [20]. The most important factors triggering ICD are ROS and endoplasmatic reticulum stress [6]. Additionally, DNA damage caused under HT can lead to the formation of tumor-specific neoantigens. Therefore, ICD can trigger an increased immune response via dendritic cell maturation, T-cell activation and increase of natural killer cell activity against tumor cells [51]. Through activation of cytotoxic T-cells and T-helper cells ICD can even stimulate an antitumor immune response all over the body. By amplifying an immune response HT can make the tumor microenvironment more accessible for therapies and can possibly be incorporated in lymphoma immunotherapy to enhance the effectiveness of treatments such as CAR-T-cell therapy or natural killer cell-based therapies [6, 19, 20, 51]. These immunomodulatory therapies are increasingly significant in the treatment of lymphoma. An emerging form of targeted therapy for lymphoma patients are bispecific antibodies (BsAbs) [52]. BsAbs possess two different antigen-binding sites, enabling them, for example, to simultaneously bind the CD3 antigen on CTLs and a tumor-specific antigen. This dual binding can induce MHC-independent activation of T-cells against tumor cells [53]. HT has only been utilized in combination with conventional chemotherapeutic agents on lymphoma cell lines, thereby overlooking the immunomodulatory effects of HT. Two *in vivo* studies on lymphoma mouse models have demonstrated that HT may have the potential to induce a CTL-mediated antitumor immune response [31, 32]. Further investigation into the combination of immunotherapy with HT would therefore be interesting to determine whether HT can provide therapeutic benefits in the treatment of lymphoma.

Conclusions

Based on the predominantly preclinical data, evidence suggests that HT treatment may have a potential anticancer effect on lymphoma. However, due to the limited number of studies and the fact that all but two concern *in vitro* or animal work, additional data are needed to prove the effects of HT in lymphoma. Only then can treatment schedules on how to best implement HT in current treatment strategies for lymphoma be developed. Therefore, designing clinical studies that utilize locoregional HT in lymphoma patients is necessary to determine the efficacy of HT and identify combined HT treatment regimens that would benefit most patients.

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Data sharing

Data sharing does not apply to this article as no new data were created or analyzed in this study.

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ORCID

Pirus Ghadjar http://orcid.org/0000-0002-2747-165X

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