

Review

From Localized Mild Hyperthermia to Improved Tumor Oxygenation: Physiological Mechanisms Critically Involved in Oncologic Thermo-Radio-Immunotherapy

Peter Vaupel ^{1,2,*}, Helmut Piazena ³, Markus Notter ⁴, Andreas R. Thomsen ^{1,2}, Anca-L. Grosu ^{1,2}, Felix Scholkmann ^{5,6}, Alan Graham Pockley ⁷ and Gabriele Multhoff ^{8,9}

- ¹ Department of Radiation Oncology, University Medical Center, University of Freiburg, 79106 Freiburg, Germany
 - ² German Cancer Consortium (DKTK), Partner Site Freiburg, German Cancer Research Center (DKFZ), 69120 Heidelberg, Germany
 - ³ Department of Anaesthesiology and Intensive Care Medicine, Charité-University Medicine, Cooperative Member of Freie Universität Berlin and Humboldt Universität zu Berlin, 10117 Berlin, Germany
 - ⁴ Department of Radiation Oncology, Lindenhofspital Bern, 3012 Bern, Switzerland
 - ⁵ Biomedical Optics Research Laboratory, Department of Neonatology, University Hospital Zurich, University of Zurich, 8091 Zurich, Switzerland
 - ⁶ Institute of Complementary and Integrative Medicine, University of Bern, 3012 Bern, Switzerland
 - ⁷ John van Geest Cancer Research Centre, Nottingham Trent University, Nottingham NG11 8NS, UK
 - ⁸ TranslaTUM—Central Institute for Translational Cancer Research, Technische Universität München (TUM), 81675 Munich, Germany
 - ⁹ Department of Radiation Oncology, Klinikum Rechts der Isar (TUM), 81675 Munich, Germany
- * Correspondence: profvaupel@t-online.de or vaupel@uni-mainz.de; Tel.: +49-171-1240073

Simple Summary: Mild hyperthermia (mHT, 39–42 °C) is a potent modality when combined with existing radio-, chemo-, or immunotherapy, leading to enhanced microcirculatory blood flow and improved tumor oxygenation. Currently, the mechanisms responsible for these mHT-related effects are not fully understood, and the extent and kinetics of therapy-promoting changes are not yet completely clarified. Herein, we review and discuss mHT-induced biological mechanisms that are relevant to radio-oncology and immunotherapy. The short-term increase in tumor perfusion induced by mHT may be caused by vasodilation of co-opted vessels and upstream normal tissue vasculature, as well as decreases in viscous resistance to flow. More sustained effects seem to result from a reduction in interstitial fluid pressure and VEGF-triggered angiogenesis. Increased microcirculatory tumor blood flow after mHT seems to be the prime driver for the enhanced tumor oxygenation, and this is supported by increasing O₂ diffusivities and O₂ extraction and facilitated O₂ unloading from oxyhemoglobin due to right-shifts of the HbO₂-dissociation curve by hyperthermia per se and intensified tumor acidosis.

Abstract: (1) Background: Mild hyperthermia (mHT, 39–42 °C) is a potent cancer treatment modality when delivered in conjunction with radiotherapy. mHT triggers a series of therapeutically relevant biological mechanisms, e.g., it can act as a radiosensitizer by improving tumor oxygenation, the latter generally believed to be the commensurate result of increased blood flow, and it can positively modulate protective anticancer immune responses. However, the extent and kinetics of tumor blood flow (TBF) changes and tumor oxygenation are variable during and after the application of mHT. The interpretation of these spatiotemporal heterogeneities is currently not yet fully clarified. (2) Aim and methods: We have undertaken a systematic literature review and herein provide a comprehensive insight into the potential impact of mHT on the clinical benefits of therapeutic modalities such as radio- and immuno-therapy. (3) Results: mHT-induced increases in TBF are multifactorial and differ both spatially and with time. In the *short term*, changes are preferentially caused by vasodilation of co-opted vessels and of upstream normal tissue vessels as well as by improved hemorheology. *Sustained* TBF increases are thought to result from a drastic reduction of interstitial pressure, thus restoring adequate perfusion pressures and/or HIF-1 α - and VEGF-mediated activation of angiogenesis. The enhanced



Citation: Vaupel, P.; Piazena, H.; Notter, M.; Thomsen, A.R.; Grosu, A.-L.; Scholkmann, F.; Pockley, A.G.; Multhoff, G. From Localized Mild Hyperthermia to Improved Tumor Oxygenation: Physiological Mechanisms Critically Involved in Oncologic Thermo-Radio-Immunotherapy. *Cancers* **2023**, *15*, 1394. <https://doi.org/10.3390/cancers15051394>

Academic Editor: David Wong

Received: 27 December 2022

Revised: 14 February 2023

Accepted: 16 February 2023

Published: 22 February 2023



Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

oxygenation is not only the result of mHT-increased TBF and, thus, oxygen availability but also of heat-induced higher O₂ diffusivities, acidosis- and heat-related enhanced O₂ unloading from red blood cells. (4) Conclusions: Enhancement of tumor oxygenation achieved by mHT cannot be fully explained by TBF changes alone. Instead, a series of additional, complexly linked physiological mechanisms are crucial for enhancing tumor oxygenation, almost doubling the initial O₂ tensions in tumors.

Keywords: radio-oncology; mild hyperthermia; immuno-oncology; enhanced tumor blood flow; improved tumor oxygenation; physiological responses; transient changes; sustained effects; pleiotropic hyperthermia effects

1. Introduction

Physiological responses of malignant tumors upon mild hyperthermia (mHT, i.e., therapeutically heating malignant tumors at a (preferred) temperature range of 39–42 °C for 30–60 min [1–5]) have been reviewed during the last two decades with different focuses and perspectives (e.g., [6–13]). Preclinical hyperthermia (HT) studies published since the late 1970s and into the early 1980s are unfortunately limited in terms of their relevance for the clinical setting, e.g., due to the (i) use of impractical heating techniques, (ii) tissue temperature levels > 43 °C (combined with long exposure times) which are cytotoxic per se and not achievable in clinical settings, (iii) bulky, fast-growing rodent tumors exhibiting very low blood flow rates do not reflect the clinical situation, (iv) incorrect and misguided conclusions from *in vitro* experiments, and (v) incorrect and misguided interpretation of biological responses upon mHT [14].

As a multifaceted adjuvant cancer treatment modality, mHT triggers a series of therapeutically relevant biological mechanisms at this temperature level without direct cytotoxicity. Localized mHT is usually administered as an adjuvant modality to primarily improve the therapeutic efficacy of “concurrent” radio- and/or chemotherapy and in heavily pre-treated patients with recurrent cancers [15,16]. Delivered within a close time frame before external radiation (RT) and/or chemotherapy (CT), mHT has unequivocally shown distinct beneficial effects in numerous clinical studies (e.g., [17–19]).

mHT is a proven potent radio- and chemosensitizer exerting pleiotropic effects in solid tumors. mHT can be considered as a “general sensitizer” to therapeutic modalities and is a strong modulator of the anticancer immune system:

- **Hyperthermic radio-sensitization:** This condition is generally believed to be the result of a “physiological vasodilation”, which increases tumor blood flow (“reperfusion”), and oxygen levels (“reoxygenation”), occasionally lasting up to 24–48 h post-HT [12].
- **Hyperthermia enhances cytotoxicity of anticancer drugs:** Besides direct sensitization to a series of anticancer agents (e.g., Cisplatin, Carboplatin, Oxaliplatin, Bleomycin, Doxorubicin), HT can improve the blood-borne delivery (via an increase in heat-induced tumor perfusion and/or a homogenization of blood flow), and enhanced extravasation in the leaky microvasculature of malignant tumors or a temperature-triggered drug release from thermo-sensitive liposomes for localized thermo-chemotherapy.
- **Hyperthermia inhibits DNA repair enzymes:** In the upper range of mHT (41–43 °C), several DNA damage repair enzymes responsible for the repair of potentially lethal or sublethal DNA damage have been reported, thus increasing the efficacy of radiotherapy and some chemotherapeutic drugs [1,3,13].
- **Hyperthermia affects radio-immuno-oncology:** It is known that hypoxia compromises anticancer immune responses such as reducing the survival, cytolytic, and migratory activity of key effector cells such as natural killer (NK) cells and NK-like T cells, as well as CD4⁺ helper and CD8⁺ cytotoxic T cells, reduces the production of essential “effector” cytokines, as well as fostering an immunosuppressive environment by supporting immunoregulatory Treg cells, myeloid-derived suppressor cells (MDSCs)

and inducing the expression of immune checkpoint inhibitors (reviewed in [20]). HT-induced improvements of tumor oxygenation status (“reversal of tumor hypoxia”) and the increased perfusion triggered by mild HT enhances the trafficking of immune cells, and intra-tumoral access to crucial immune regulators such as antibodies and cytokines, all of which are needed to generate effective antitumor immune responses. Hyperthermia is also known to be an effective immune modulator that has multiple effects on the innate and adaptive immune systems (reviewed in [21]).

- **Hyperthermia and the innate immune system:** With respect to the *innate immune system*, hyperthermia increases the expression of activation receptors such as NKG2D and MHC class I-related chain A (MICA) on the surface of natural killer (NK) cells, thereby enhancing their antitumor potential [21]. This is confirmed by findings that NK cells are important mediators of antitumor immunity after radiotherapy and hyperthermia [22] and that cells of the innate immune system in patients recover faster when hyperthermia and radio-chemotherapy are combined [23].
- **Hyperthermia and the adaptive immune system:** With regards to the *adaptive immune system*, hyperthermia influences all aspects of adaptive antitumor immunity, from the function and antigen presentation capacity of antigen-presenting cells (APCs) to the responsiveness of CD4⁺ and CD8⁺ T-cell populations [23]. Combining hyperthermia with radiotherapy promotes the infiltration of dendritic cells—crucial antigen-presenting cells and initiators of adaptive immune cells—into solid tumors [23], as well as inducing the maturation of DCs and the release of pro-inflammatory cytokines from DCs and macrophages [24,25]. In addition to direct effects on cellular immunity, combining hyperthermia and radiotherapy has also been shown to mediate immune effects via multiple mechanisms, including the release of Danger Associated Molecular Pattern (DAMP) signals such as heat shock proteins (HSPs) and HMGB1 [24,25].

Taken together, these findings and observations demonstrate and support the concept that combining hyperthermia, radiotherapy and immunotherapy with next-generation cellular and antibody-based approaches such as checkpoint inhibition has great therapeutic potential. However, before this approach can be fully exploited, it is crucial to fully understand the physiological consequences of hyperthermia on the tumor microenvironment and identify the optimal doses for achieving the optimal therapeutic conditions.

Based on experimental preclinical and clinical data, it is often concluded that heat-induced increases in TBF are the principal (or even exclusive) drivers of concurrent (“secondary”) changes in the oxygenation status [26] and are principally involved in most of the heat-induced sensitizing mechanisms described above. However, when analyzing the heat-induced changes of tumor blood flow in more detail, it is evident that there are some enigmas (or even deficits) in the interpretation of the results, especially with respect to (i) the genesis of HT-induced flow increases, and (ii) the interpretation of “exclusively flow-related enhancements” of tumor oxygenation status. A more detailed analysis of the functional background clearly shows that mHT-induced secondary effects cannot be fully explained by blood flow improvements alone. Instead, these effects are the result of complex interactions of a multitude of processes. Furthermore, partly dependent on hyperthermia levels and durations, inconsistent directions, as well as highly variable individual extents and kinetics of blood flow changes upon mHT, have also to be considered. In addition, taking into account the pathogenesis-related classification and different timeframes of tumor hypoxia [27], it is rather unlikely that enhancements of the oxygenation status upon mHT closely reflect the improvements in TBF.

2. Methods, Search Strategies and Sources of Information

A systematic literature search of relevant research articles and reviews published between 1 January 1980 and 15 November 2022 provided an updated, comprehensive data review (PubMed, Web of Science). Values presented in this condensed article are averaged means. Search items were as follows: tumor hyperthermia, tumor blood flow, tumor perfusion, tumor oxygenation status, tumor oxygen supply, physiological mechanisms,

oxygen-dependent radio-sensitization, HT-dependent radio-sensitization, HT-induced immune modulation (and combinations of these terms). This review is also based on one author's expertise in this research area since the late 1970s (PV).

Exclusion criteria were as follows: (i) preclinical data obtained in fast-growing, bulky rodent tumors (> 1% of body weight!), (ii) heating above 43 °C for more than 60 min because information obtained is clinically not translatable or can only be translated to a limited extent, and (iii) immersion of tumors into heated water baths, because the latter can be accompanied by severe edema formation due to osmotic water shifts upon treatment, thus probably concealing HT-related effects [9]. This osmotic water shift adds to the HT-induced edema, thus further expanding the extracellular space [28]. In addition, the whole leg is at elevated temperatures, a condition which might impact blood flow and the temperature distribution within the tumor to be treated (i.e., through "steal phenomena").

3. Results: Assessment of Reliable Experimental and Clinical Data

3.1. Transiently Improved Tumor Blood Flow upon Localized Mild Hyperthermia: Potential Mechanisms Involved

3.1.1. Tumor Vascularization and Blood Flow Are Decisive Parameters Critically Affecting Efficacy of Localized Hyperthermia

The vascular physiology of tumors is uniquely different from that of the corresponding normal tissues [29]. When considering the continuous and indiscriminate formation of a vascular network in a growing tumor, five different pathophysiological mechanisms have to be taken into consideration: (i) *co-option* (*accessing or "hijacking"*) of (*pre-existing vessels*) at the site of tumor growth, (ii) *angiogenesis* by endothelial sprouting from existing venules, (iii) *vasculo-genesis*, i.e., *de novo* vessel formation through the incorporation of circulating endothelial precursor cells, (iv) *intussusception* (i.e., splitting of the lumen of an existing vessel into two), and (v) *formation of pseudo-vascular channels*, lined by tumor cells rather than endothelial cells ("vascular mimicry") [29].

The tumor (micro-)vasculature is characterized by vigorous proliferation, which leads to immature, structurally defective, and in terms of perfusion, ineffective microcirculation [29]. This occurs partly due to missing functional innervation and a lack of pharmacological receptors of contractile elements (smooth muscle cells, pericytes). Relevant thermoregulatory vascular responsiveness and flow regulation within malignant tumors can, therefore, only take place in/via co-opted vessels.

Blood flow rates in malignant tumors vary considerably, ranging from 0.01 to 1.0 mL g⁻¹ min⁻¹. In squamous cell carcinomas of the head and neck, as well as in colorectal cancers, flow rates up to ≈ 2 mL g⁻¹ min⁻¹ have been found, whereas, in prostate cancers, flow rates even up to 3 mL g⁻¹ min⁻¹ have been described [4,29,30]. In addition, variability of TBF flow *within* an individual tumor (i.e., within different tumor sub-volumes, "intra-tumor heterogeneity") can be in the same flow range as described above for different tumor histologies, stages, and growth sites ("inter-tumor heterogeneity") [31].

3.1.2. Prime Role of Tumor Blood Flow in Hyperthermia Treatments

Considering the major role of TBF in HT treatments, since the early 1980s, it has mistakenly been postulated that malignant tumors generally have lower blood flow rates than the adjacent normal tissue, thus, favoring quasi-selective heating of solid malignancies due to (i) compromised heat dissipation via convective heat transfer and (ii) the ability for "selective" heat deposition. Using electromagnetic irradiation, this latter notion completely disregards the role of tumor hyperhydration responsible for higher specific heat capacities ("heat storage capacities") and thermal conductivities [4].

Besides its seminal impact on the heating properties of tumors, the efficacy of TBF can greatly impact parameters of the tumor microenvironment (TME), e.g., the tumor hypoxia and depletion of the bioenergetic status as well as tumor acidity (pH-distribution), i.e., parameters that substantially enhance the cytotoxic effect of HT at 43–45 °C.

As shown in Figure 1A–E, which summarizes own preclinical data published since 1980 [32–35] (using various heating techniques and detection methods, subtle control of key parameters of the cardiovascular and respiratory system, and of body core temperature), temperature-controlled, local mHT at 39.5–40.5 °C for 30–60 min, regardless of the heating technique (immersion into heated water not included), leads, on average, to transient (during heating and shortly after terminating mHT) and significant increases in TBF with concurrent significant improvements in the oxygenation status during the heating period (Figure 1A). Usually, both enhancements are no longer evident 1 h after heating [32]. At tumor tissue temperatures ≥ 43 °C for 60 min, a shutdown of TBF is often observed upon heating due to a wide range of mechanisms, including a series of intravascular events, vessel wall alterations, extravascular events, and opposing host tissue/tumor mechanisms (extensively summarized in [33,34]) (Figure 1B,C).

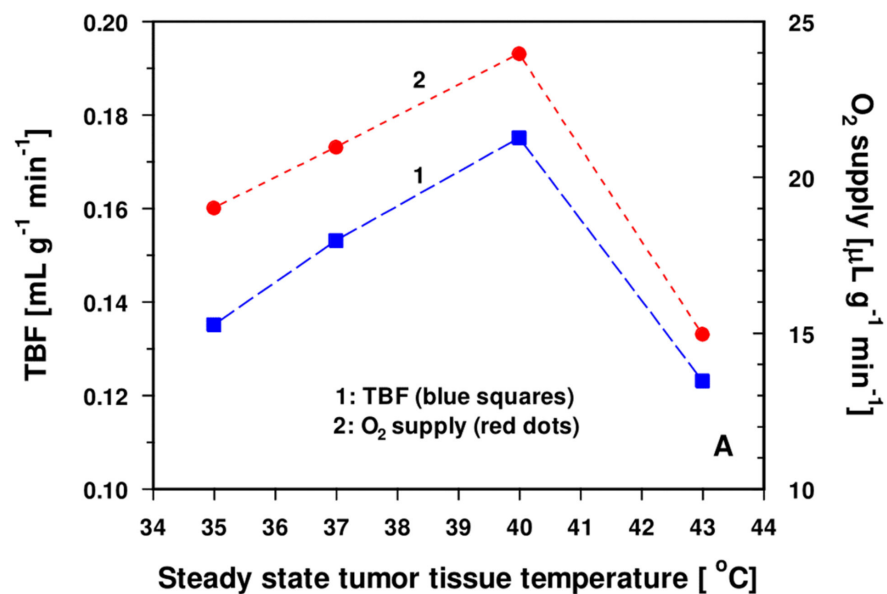


Figure 1. Cont.

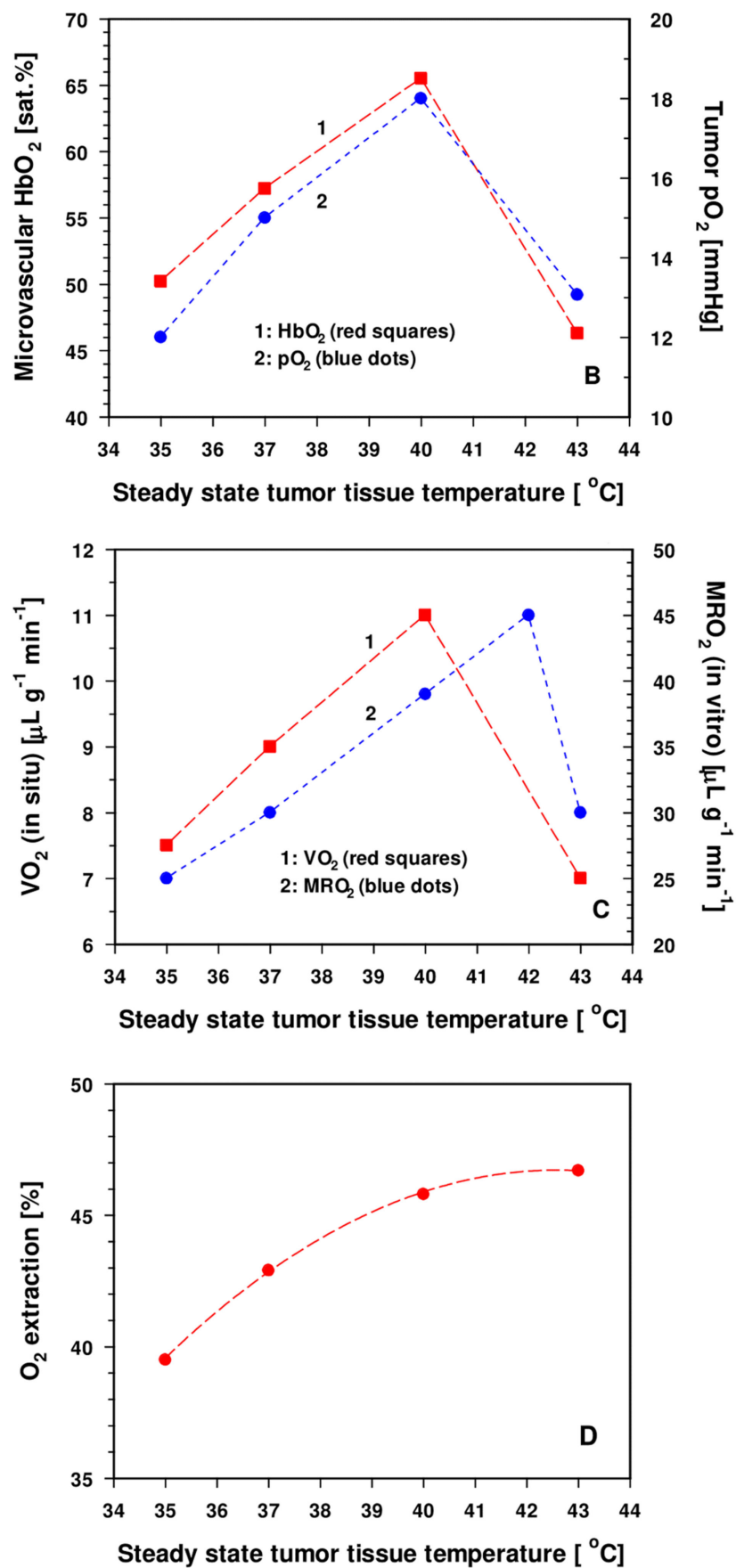


Figure 1. Cont.

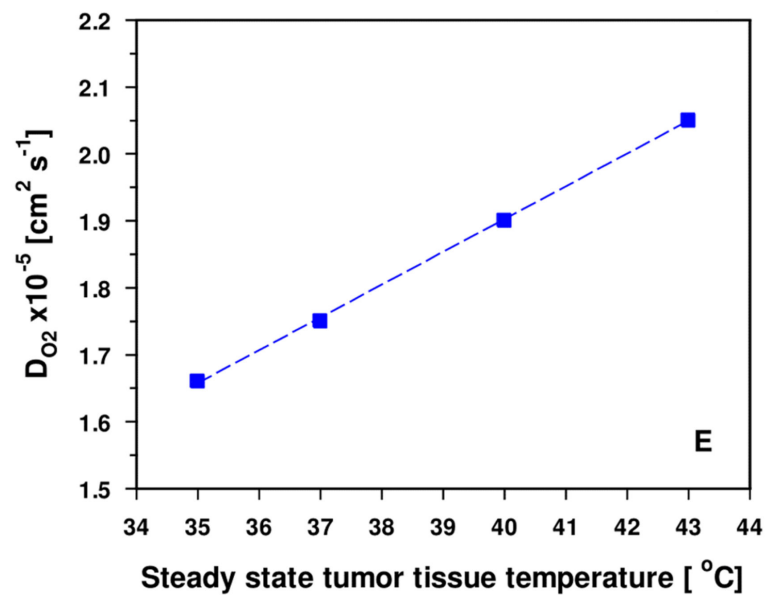


Figure 1. Changes of relevant physiological parameters upon localized mHT as a function of tumor temperature. Results show tumor blood flow (TBF) and oxygen (O_2) supply (=TBF \times arterial O_2 concentration, panel (A)), microvascular oxyhemoglobin (HbO_2) saturation, and tumor tissue O_2 partial pressure (pO_2 , panel (B)), in situ O_2 consumption (V_{O_2}) and—completely independent of blood flow—the O_2 consumption rate of isolated tumor cells *in vitro* (MRO_2), panel (C)). There is clear evidence that TBF and the directly flow-dependent parameters have a maximum between 39.5 °C and 40.5 °C, whereas—at maintained, unrestricted O_2 supply under *in vitro* conditions— MRO_2 values have a distinct maximum at 42 °C with a decline at temperatures ≥ 43 °C (at maintained, adequate O_2 supply under *in vitro* conditions). The oxygen extraction rate (= O_2 uptake/ O_2 supply), a measure of the adequacy of the O_2 supply to the tissue, increases with rising temperature (panel (D)). Oxygen diffusivity (D_{O_2}) increases linearly with temperature (panel (E)). Based on data provided in [32–35]. Values are averaged means.

Using water-filtered infrared-A- (wIRA)-heating for 60 min, mild hyperthermia significantly increased TBF by 30–80% above baseline throughout the observation period, with elevations remaining until the end of the treatment period of the tumors (with wet weights $\leq 0.5\%$ of body weight). Upon mHT treatment, pO_2 values increased by 50% on average. These favorable conditions were no longer evident ≈ 1 h post-heat [9,35,36]. HT-induced changes of TBF are often equivocal in that they can change in non-predictable directions and to different extents and durations. In addition, localized divergent changes of blood flow in neighboring tissue sub-volumes have been observed [37], again indicating great variability (Figure 1B).

Note: The preclinical data presented in Figure 1D contradict the notion proposed earlier that the improvement of the tumor oxygenation (pO_2) upon mHT might be due to a decreased O_2 consumption rate (V_{O_2}) [12]. Compared to the 35 °C data, TBF at 40 °C increased by 30%, the O_2 supply by 26%, the O_2 consumption V_{O_2} by 47%, the O_2 extraction rate by 17%, and the tumor tissue pO_2 by 50%. Our own data are indicative of a significantly reduced O_2 consumption rate of tumors in situ only at temperatures ≥ 43.5 °C for > 30 min (Figure 1B,C). Due to equivalent (“proportionate”) increases in microcirculatory blood flow (red blood cell flux) and in O_2 -consumption rates during mHT, no changes in the mitochondrial redox status were reported [38].

Mechanisms most probably involved in *transient blood flow improvements* during mHT (and ≤ 1 h post-heat) are shown in Figure 2. In this flowchart, mHT-induced (patho-)physiological mechanisms contributing to a short-term increase in TBF and, thereby, to a more efficient convective (i.e., blood-borne) transport are summarized.

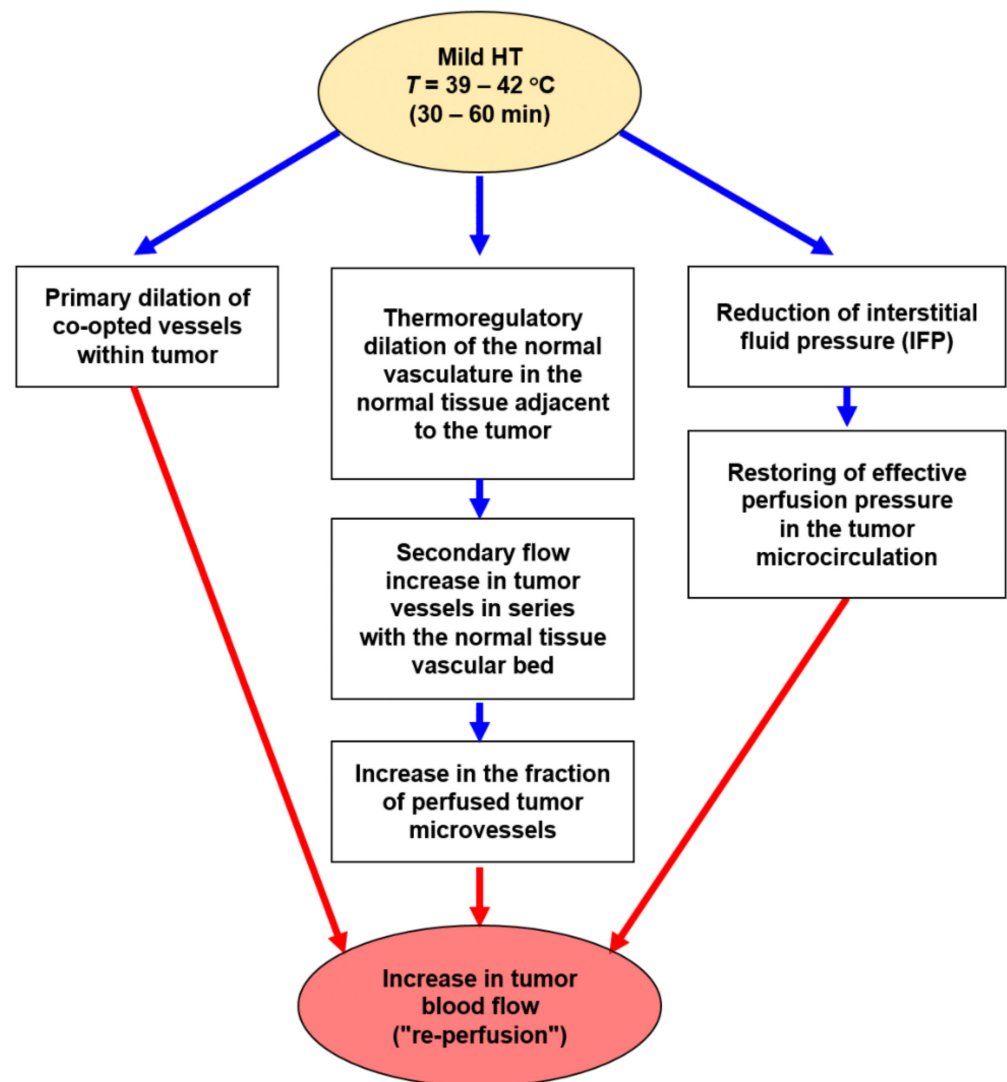


Figure 2. Mild hyperthermia leads to transient increases in tumor blood flow through primary dilation of co-opted pre-existing blood vessels (left pathway) and secondary flow increase (“re-perfusion”) through tumor blood vessels following thermoregulatory “upstream-vasodilation” in the normal tissue adjacent to the “downstream” tumor vessels (central pathway). More sustained improvements may be due to “resumptions” of blood flow due to drastic reduction in interstitial hypertension (i.e., lowering of interstitial fluid pressure, right pathway) and activation of molecular pathways that stimulate VEGF-secretion and thus forced tumor angiogenesis (see Figure 4, left pathway).

Short-term mechanisms include:

- Primary dilation of co-opted vessels within tumors;
- Thermoregulatory dilation of upstream blood vessels in the normal tissue adjacent to the growing tumor, a regulation that leads to secondary flow increases (“re-perfusion”) through downstream tumor vessels in series with the normal tissue vascular bed [39];
- Distinct reduction of viscous resistance to flow due to significant improvements in the key rheological parameters that determine blood flow behavior [40]. *In vitro*, a temperature rise of 1 K significantly decreases the blood viscosity by $\approx 3.5\%$ and the plasma viscosity by $\approx 2.5\%$ [41–43]. The relative kinematic viscosity of blood (at different hematocrit values) and of plasma as a function of temperature is shown in Figure 3 [40]. Taken together, these mHT-induced changes in key parameters clearly improve the blood flow behaviour.

Note: Together with the fall in the geometric resistance due to vasodilation observed in mHT, it may be expected that the above-mentioned reduction in viscous resistance may also result in a drop of total resistance to flow ($\text{mmHg}\cdot\text{min}\cdot\text{mL}^{-1}$) in tumors *in vivo*. During hyperthermic perfusion of tumors *in situ*, a rise in tissue temperature from 37 °C up to 39.5 °C caused a drop of total resistance to the flow of 16%, i.e., $\approx 6.5\%/K$ [44]. This result may—at least partly—be explained by a significant loss of red blood cell deformability in severely acidic tumors *in vivo* [45].

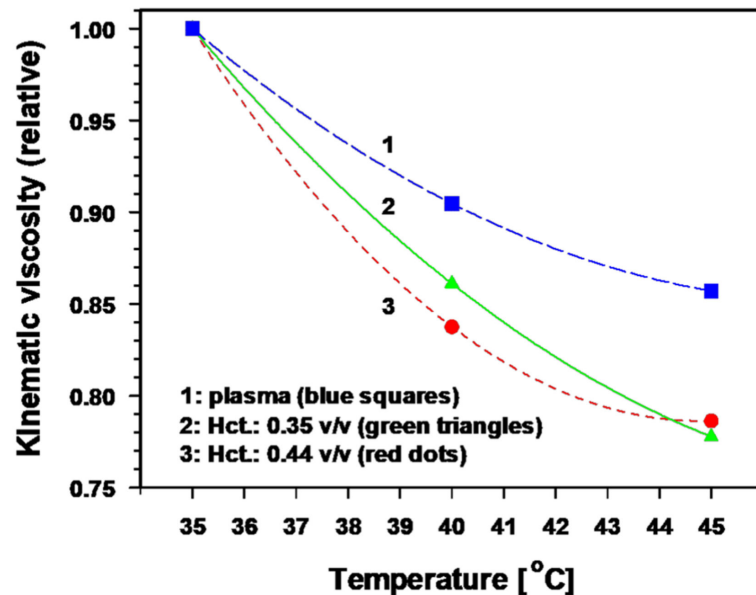


Figure 3. Relative kinematic blood viscosity and plasma viscosity as a function of temperature at physiological pH (based on data provided in [45]). Additional information: At 37 °C, plasma viscosity is ≈ 1.3 cPoise (cP), and apparent viscosity of whole blood is ≈ 3.5 cP (shear rate: 150 s^{-1}).

Sustained “re-perfusion” up to 24–48 h after heating has been attributed to a strong ($\approx 80\%$) reduction of interstitial fluid pressure (IFP in normal tissues: -2 to 0 mmHg vs. IFP in tumors: up to $+10$ – 30 mmHg, “interstitial hypertension”) [31,46–48]. Increased blood flow in the latter case is the result of restoring effective perfusion pressures in the tumor microcirculation [49].

The therapeutically relevant increases in TBF upon mHT, which are sustained up to 48 h post-HT, most probably result from a series of (plausible and/or experimentally proven) mechanisms, as shown in Figure 2. Besides (i) mHT-induced lowering of IFP, followed by subsequent restoration of adequate perfusion pressure (see above), another mechanism for sustained blood flow increases for 24–48 h is based on (ii) the mHT-induced activation of ERK-/Akt-pathways [50,51] followed by HIF-1 α activation with subsequent increased VEGF-secretion, the latter leading to robust tumor angiogenesis and rise in TBF (see Figure 4).

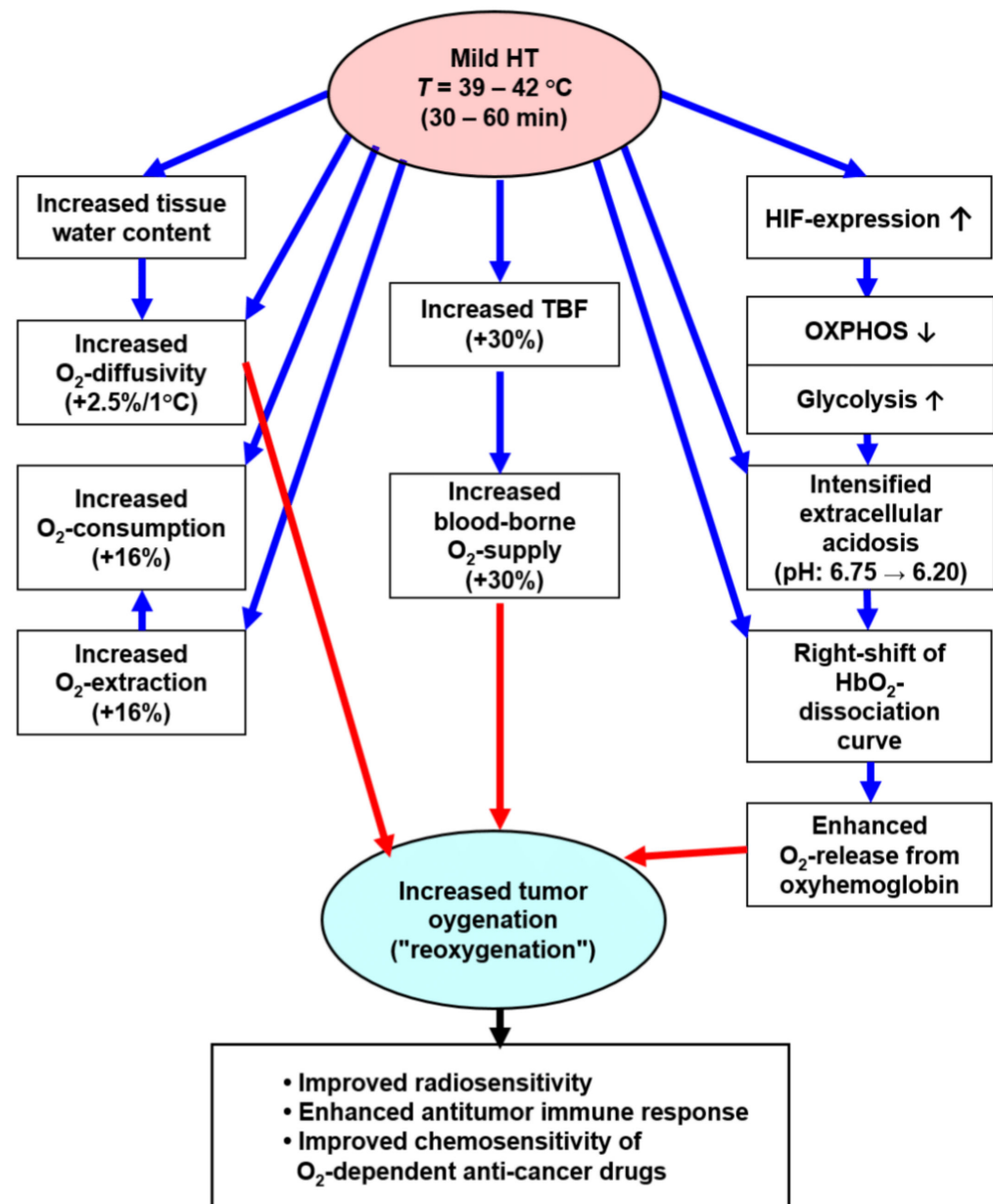


Figure 4. Mechanisms responsible for the improvement of the O₂ status upon localized mHT through enhancement of the O₂ availability (central pathway), increased O₂ diffusivity due to hyperhydration of tumor tissues and direct mHT-impact (left pathway), and intensified tumor acidosis leading to a right-shift of the HbO₂-dissociation curve, thus facilitating the O₂ release from the blood into the tissue (right pathway). Most probably, the mHT-induced increment in the O₂ consumption is compensated by a comparable increase in the O₂-extraction (left pathway).

3.2. Enhanced Tumor Oxygenation Status upon Localized Mild Hyperthermia

3.2.1. A Multifactorial, Complex Scenario Is Involved in the Transient Improvement of Tumor Oxygenation upon Mild Hyperthermia

In general, an adequate O₂ supply in malignant tumors (primaries and metastases) is spatially and temporally restricted by (i) inadequate perfusion (convective transport) and (ii) limited diffusion (diffusive transport), both leading to heterogeneously distributed hypoxia (i.e., pO₂ < 10 mmHg; severe hypoxia: pO₂ < 1 mmHg). Perfusion- and diffusion-limited hypoxia is detectable even in early growth stages. A major goal of localized mHT in the combined treatment setting is the improvement of the tumor oxygenation status, which critically impacts inter alia radio-sensitivity, the efficacy of a series of anticancer drugs,

protective antitumor immune responses, tumor progression (tumor aggressiveness), and finally patient outcome.

As shown in Figure 4, localized mHT can improve the tumor oxygenation status via several mechanisms:

Role of oxygen availability: As outlined above, mHT can lead to transient improvements of TBF. In most normal tissues, increases in nutritive blood flow usually result in concurrent increases in the O_2 availability (O_2 supply = blood flow \times arterial O_2 concentration) to a comparable extent, assuming a constant O_2 concentration in the arterial blood (≈ 20 mL O_2 /dL blood).

In patient tumors, a significant fraction of arterial blood (up to 25–30% [31,52,53]) can be shunted to the venous side without participating in the microvascular exchange processes. This fraction may significantly increase during mHT, thus excluding the assumption of a straightforward proportionality between TBF and O_2 availability (at constant arterial O_2 content).

Note: During the HT treatment of anemic cancer patients ($cHb < 12$ g/dL), a special situation regarding the O_2 supply conditions has to be taken into consideration. In this situation, two counteracting mechanisms determining the O_2 supply of tumors have to be discussed. On the one hand, the O_2 availability is reduced due to the lower O_2 concentration in the arterial blood; on the other hand, the oxygen release from oxyhemoglobin (HbO_2) into the surrounding tissue is enhanced in anemic patients due to increased concentrations of hemoglobin-bound 2,3-bisphosphoglycerate (2,3-BPG), which leads to a right-shift of the HbO_2 dissociation curve (see below). However, the latter adaptation can only partially compensate for anemic hypoxemia.

Role of oxygen diffusivity: On average, in situ tumors have a $\approx 15\%$ higher water content than their tissues of origin (“tumor hyperhydration”) [4]. As a consequence of this *hyperhydration*, heat deposition upon electromagnetic irradiation is higher in tumors than in their corresponding normal tissues. Additionally, hyperhydration causes a distinctly higher O_2 diffusivity (Fick’s oxygen diffusion coefficient, D_{O_2}).

- D_{O_2} values (O_2 diffusivities) for tumors are increased by a factor of ≈ 1.9 compared to the tissues of origin [54]. Additional edema formation is often seen upon HT [9], thus increasing hyperhydration and—as a result—further improving O_2 diffusivity, finally supporting re-oxygenation of the tumor upon mHT.
- O_2 diffusivity is also increased by mHT per se. Heating a tissue/tumor to 43 °C increases D_{O_2} by 2.1–4.6%/K [55,56].

Role of intensified tumor acidosis: Tissue exposure to mHT triggers a series of events that aggravate tumor tissue acidosis ($pH \downarrow$), finally reaching extracellular pH values of ≈ 6.20 :

- Besides anaerobic glycolysis (because of tumor hypoxia), cancer cells rely on aerobic glycolysis (due to metabolic reprogramming, a core hallmark of cancer [57]). Both pathways produce high amounts of lactate and protons H^+ (“lactic acid”), which are exported into the extracellular space, leading to extracellular acidosis (mean $pH_e \approx 6.75$). Aerobic glycolysis is stimulated by mHT-induced activation of HIF-1 α , leading to an intensified Warburg effect for 24–48 h (schematically shown in Figure 5).
- In addition, tissue heating intensifies ATP hydrolysis with proton production as well as inhibiting the Na^+/H^+ antiport of the cell membrane [10].
- mHT per se intensifies tissue acidosis due to changes in chemical equilibria of the intra- and extracellular buffer systems: $\Delta pH/\Delta T = -0.016$ pH units/K [10].

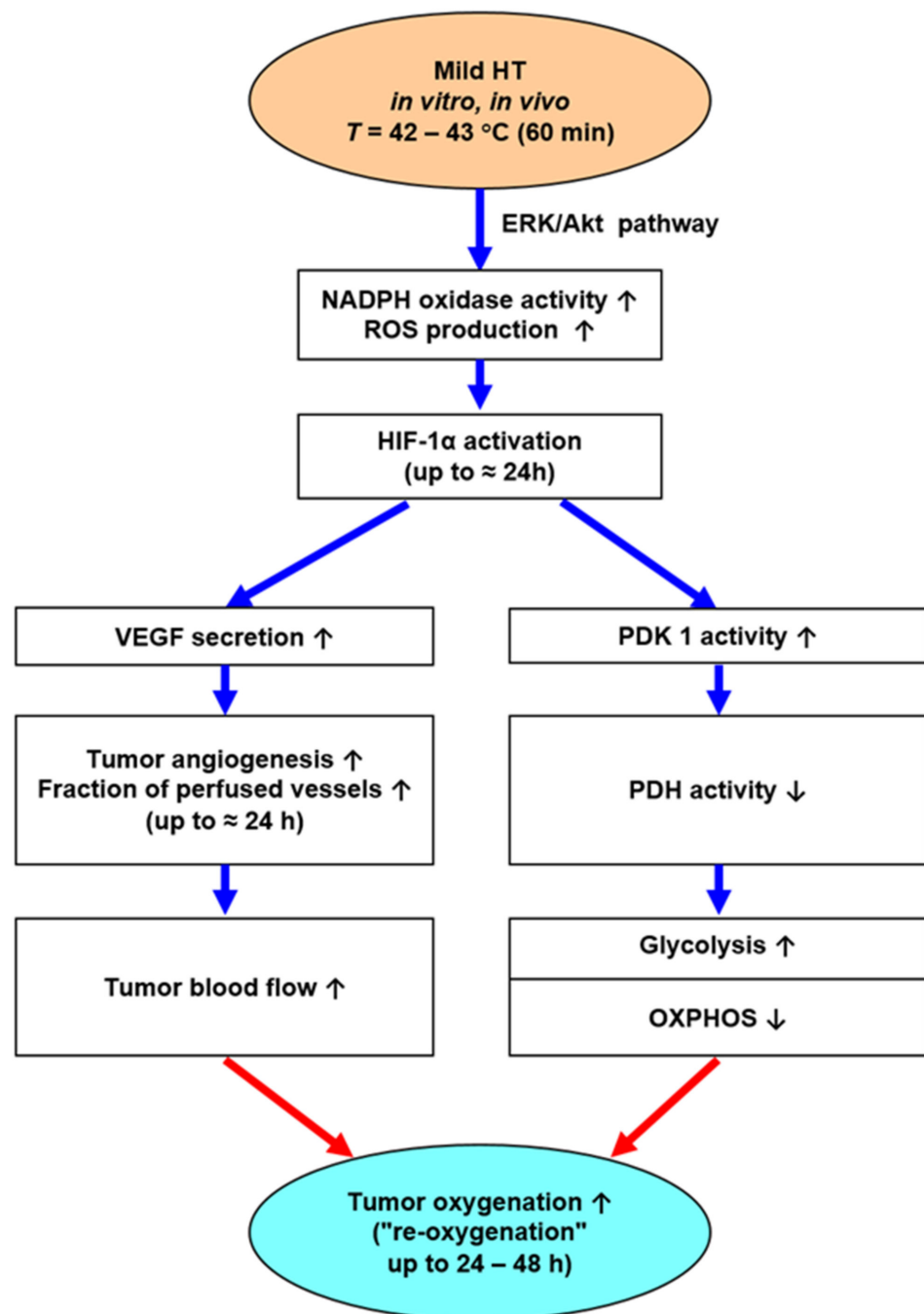


Figure 5. Heat-sensitive ERK/Akt-pathways (central pathway), metabolic reprogramming (right pathway), and VEGF-triggered robust angiogenesis (left pathway) and their putative role in sustained (up to 24–48 h) improvement of the tumor oxygenation status.

Upon mHT, pH_e values steadily decrease, finally reaching ≈ 6.20 [58–63]. This effect is temporary, i.e., it is observed only during mHT-application and until ≤ 6 h post-heat, leading to parallel effects only in this period.

Intensified acidosis per se leads to a right shift of the HbO_2 -dissociation curve, regardless of the mechanisms causing acidosis (pH-Bohr effect) [63–65]. As shown in Figure 6A, the respective difference in the pO_2 values is ≈ 9 mmHg at 50% HbO_2 saturation, which is comparable to the right shift when considering the fetal (HbF) and the maternal (HbA) blood at term.

mHT per se also leads to a temperature-dependent right shift of the HbO_2 -dissociation curve ($\Delta pO_2 \approx 13$ mmHg at 50% HbO_2 saturation, see Figure 6B) [63–65]. Both mechanisms

(i.e., intensified acidosis and temperature rise) result in an enhanced O_2 release from oxyhemoglobin into the surrounding tissue, thus supporting tumor tissue “reoxygenation”.

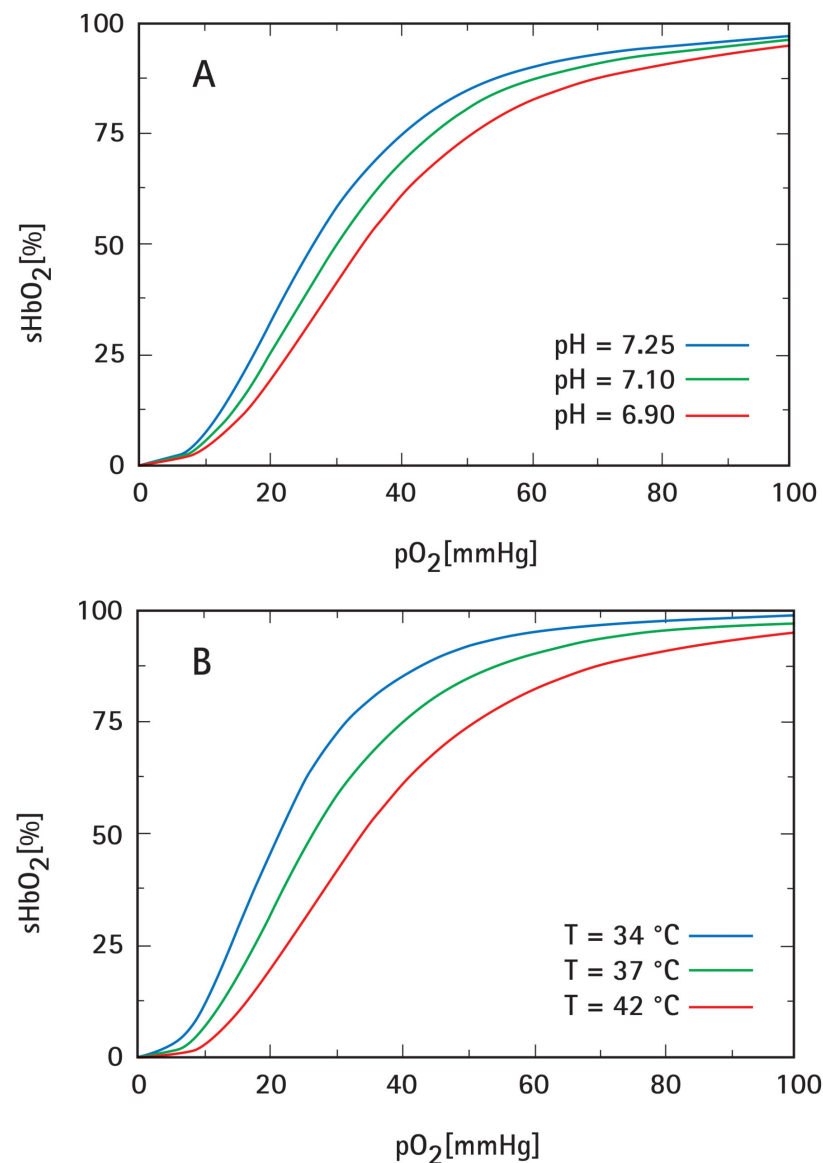


Figure 6. Right-shift of the HbO_2 -dissociation curve resulting from intensified tumor acidosis (A) and hyperthermic tumor tissue temperatures (B), both facilitating the O_2 release from oxyhemoglobin (HbO_2), i.e., enhancing O_2 unloading from red blood cells into the surrounding tissue.

During mHT, a significant rise (+30%) of the CO_2 partial pressures in the tumor-venous blood was found, paralleled by the pH drop mentioned above, thus amplifying the right-shift of the HbO_2 -dissociation curve (CO_2 -Bohr effect) [44,63–65].

Role of hyperthermia-activated ERK/Akt-signaling pathways: In clinical settings, improvements in oxygenation status have occasionally been observed up to 24–48 h post-heating, most probably accompanying longer-lasting increases in TBF upon VEGF-driven forced angiogenesis [50], and—less likely—by HIF-1 α -mediated inhibition of mitochondrial functions (see Figure 4). As already mentioned above, changes in the mitochondrial redox status were not detected shortly after finishing mHT [38]. As shown in Figure 1D, the O_2 extraction steadily increases with temperature, clearly speaking against the notion of impaired OXPHOS upon mHT. This latter finding agrees with the basic results

of Gullino et al. [66], who investigated the relationship between temperature and blood supply, and consumption of oxygen in rat mammary carcinomas.

3.2.2. Experimental and Clinical Evidence for Improved Tumor Oxygenation upon Localized Mild Hyperthermia: Updated Data Analysis

Considering relevant data describing the tumor oxygenation status upon clinical mHT, the relationship between mean baseline (pre-HT) tissue pO_2 (NT- pO_2) and post-HT pO_2 values (HT- pO_2) can be linearly fitted for experimental and patient tumors upon mHT (see Figure 7A). These data suggest an equivalency of the HT techniques used in preclinical and clinical experiments to achieve this effect. Considering additional measurements of subcutaneous pO_2 values in the clinical setting (red dot [67]), steady state mHT leads to a 1.64-fold increase in the mean pO_2 values. Considering only experimental rodent tumors [6,7,35,61,62,68–75], primary canine [76,77], and human tumors (breast cancers, soft tissue sarcomas [78–80] with baseline values between 1 and 10 mmHg (i.e., hypoxic tumors), mHT for 30–60 min almost doubles the pre-heat pO_2 values (see Figure 7B), again irrespective of the heating technique (e.g., hyperthermic perfusion of tumors in situ, microwaves, radiofrequencies, water-filtered Infrared A), even if data of reasonably reliable experiments with water bath immersion at 38.5–41.5 °C were included [75]. In these experiments, the mean pO_2 before mHT was 7 mmHg (hypoxic fraction HF < 10 mmHg: 67%); during mHT, the average pO_2 was 17 mmHg (HF: 46%), with a rapid return (within \approx 10 min) to the pre-HT oxygenation status.

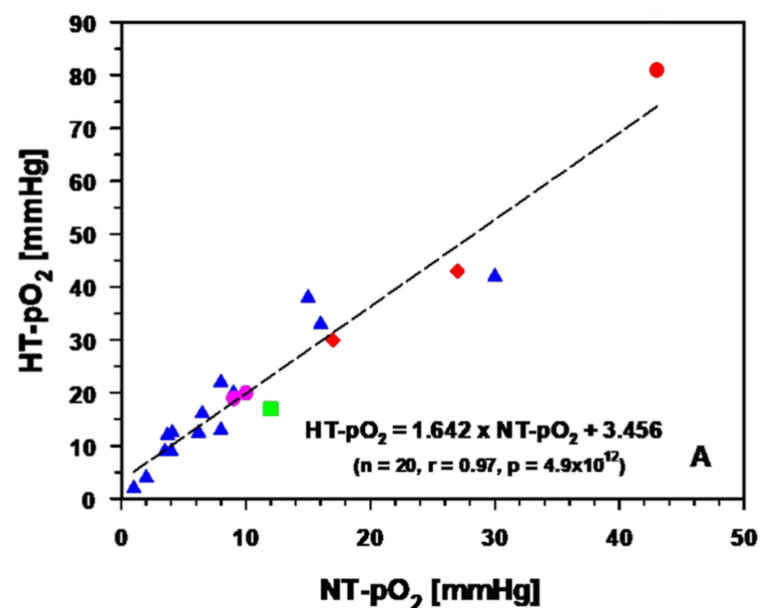


Figure 7. Cont.

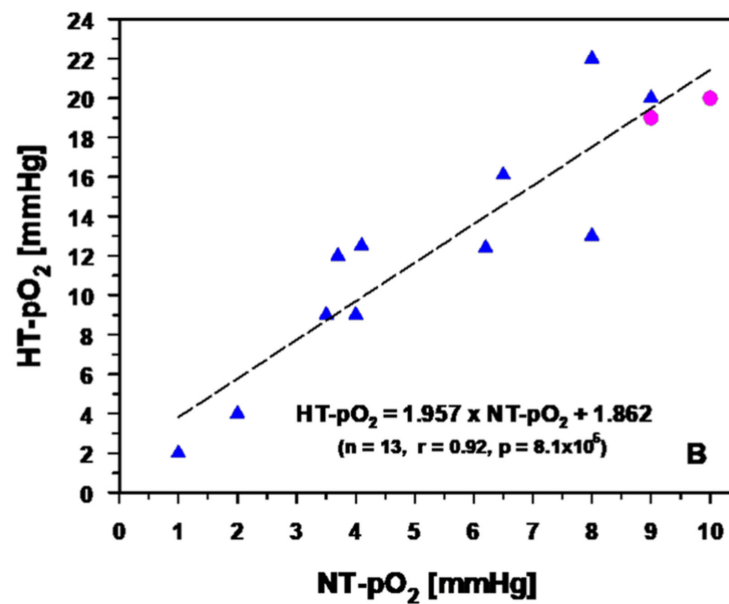


Figure 7. Linear relationship between mean baseline (pre-HT) tumor tissue pO_2 (NT- pO_2) and mean post-HT pO_2 values (HT- pO_2) in experimental and patient tumors using various heating techniques. In (A), the relationship includes normal tissue (dermis) measurements upon heating with water-filtered infrared-A irradiation (wIRA), red dot [67]. Data clearly demonstrate the equivalence of the various heating techniques chosen (blue triangles: microwave heating of rodent tumors; purple dots: microwave heating of patient tumors; red diamonds: wIRA-induced heating of experimental tumors; green square: mean pO_2 value of all data presented). In general, the use of mHT for 30–60 min leads to a 1.6-fold increase in mean pO_2 . In (B) only data of hypoxic tumors (mean $pO_2 < 10$ mmHg) are shown. Here, post-mHT pO_2 values almost doubled.

Significant improvements in the tumor oxygenation status described above are fully comparable with data from preclinical experiments upon short-term spontaneous breathing pure oxygen (100% O_2). In these experiments, steady state hyperoxic pO_2 values of 400–450 mmHg within the arterial blood were reached. In contrast to mHT, an immediate, rapid drop of tumor pO_2 to baseline values was observed upon return to room air breathing (≤ 1 –2 min) [81].

Note: As mentioned above, tumor oxygenation is considered as the key factor to substantially increase radiosensitivity. Therefore, mHT should be applied immediately/as close as possible before radiotherapy (RT). Using this schedule for recurrent breast cancers, re-RT doses could be significantly reduced compared to RT doses reported earlier [82].

Exposure of severely hypoxic tumors (average NT- $pO_2 \approx 1$ mmHg) to mHT for 30–60 min increases the HT- pO_2 to a mean of ≈ 3 mmHg. As shown in Figure 8, this small improvement in the oxygenation status increases the relative radiosensitivity (oxygen enhancement ratio, OER) from 1.32 to 1.84 ($\Delta_{OER} = 0.52$) due to the relatively steep slope of this section of the OER curve (blue range, A). In hypoxic tumors (e.g., locally advanced or recurrent breast cancers) with mean baseline pO_2 values of ≈ 5 mmHg, mHT can cause a rise up to ≈ 10 mmHg, which is accompanied by an increase in OER from 2.10 to 2.39 ($\Delta_{OER} = 0.29$), caused by a less steep course of the OER curve (green range, B). In less hypoxic tumors (NT- $pO_2 \approx 17$ mmHg), mHT leads to an increment up to a mean HT- pO_2 of ≈ 32 mmHg, yielding an increase in OER from 2.53 to 2.79 ($\Delta_{OER} = 0.26$; red range, C). From these data, it may be concluded that the mHT-related gain in relative OER is greatest in the very hypoxic tumors.

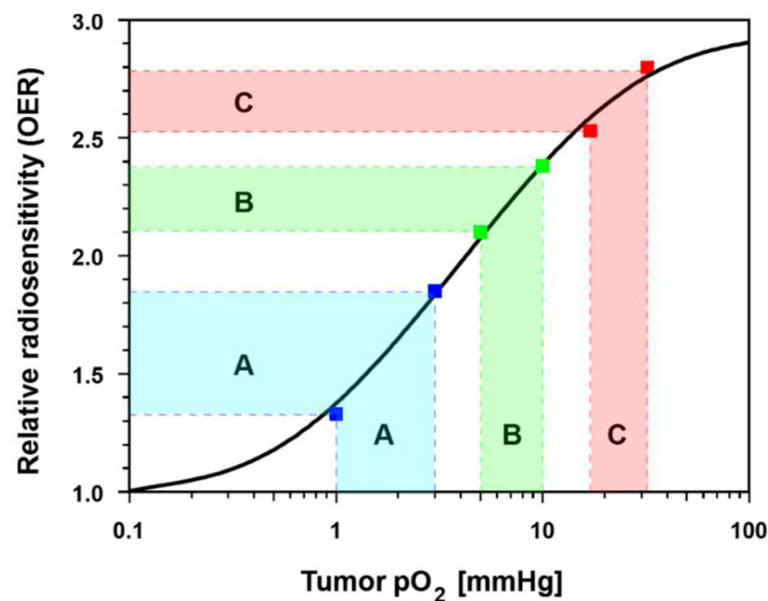


Figure 8. In severely hypoxic tumors (mean pretreatment $pO_2 \approx 1$ mmHg) exposed to mHT for 30–60 min, the mean post-HT pO_2 was ≈ 3 mmHg, resulting in a rise in the Oxygen Enhancement Ratio (OER, relative radiosensitivity) from 1.32 to 1.84 (blue range, A). In hypoxic tumors (mean pre-HT $pO_2 = 5$ mmHg), HT caused a rise to 10 mmHg, which was accompanied by an increase in OER from 2.10 to 2.39 (green range, B). In less hypoxic tumors (mean pre-HT $pO_2 = 17$ mmHg), mHT led to a pO_2 increment up to 32 mmHg, resulting in an increase in OER from 2.53 to 2.79 (red range, C).

4. Conclusions and Outlook

Localized mHT can lead to transient (and occasionally sustained) improvements in TBF, both conditions being caused by a variety of different mechanisms. Increased O_2 levels upon mHT are roughly equivalent to changes in TBF and O_2 availabilities. However, a series of mechanisms, in addition to the improved TBF, significantly contribute to the increased oxygenation observed upon mHT, such as (i) increased diffusivities and (ii) intensified tumor acidosis and mHT per se, both leading to a right-shift of the HbO_2 dissociation curve, thus enhancing O_2 release from HbO_2 , and contingently (iii) HIF1- α -mediated inhibition of mitochondrial functions, a condition sometimes proposed. Whether mHT-induced suppression of cell proliferation and apoptosis of “aerobic” tumor cells (e.g., [50]), eventually reducing oxygen consumption and thus improving the oxygenation status, can be considered valid is the subject of current investigations, especially when translating this observation into clinical settings. Summarizing the result of mHT application, it can be stated that the transient reduction of tumor tissue hypoxia is—most probably—the result of a reduction of both perfusion-limited and diffusion-limited hypoxia.

Localized mHT (39–42 °C for 30–60 min) leads to an almost doubling of initial O_2 levels in tumors, independent of the heating technique used. In essence, we recommend considering the provided data in treatment protocols comprising mHT, particularly when sequences and time intervals in combined treatment modalities (e.g., thermo-radio-immunology) are discussed.

Author Contributions: Conceptualization, original draft preparation, and draft of figures: P.V. Finalizing of figures: P.V., H.P., and M.N. Review and editing: P.V., A.G.P., G.M., F.S., H.P., M.N., A.R.T., and A.-L.G. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Acknowledgments: The authors thank Anett Lange for her technical support in the preparation of this review article.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. van Rhoon, G.C.; Franckena, M.; ten Hagen, T.L.M. A moderate thermal dose is sufficient for effective free and TSL based thermochemotherapy. *Adv. Drug Deliv. Rev.* **2020**, *163–164*, 145–156. [\[CrossRef\]](#)
2. Crezee, J.; Franken, N.A.P.; Oei, A.L. Hyperthermia-based anti-cancer treatments. *Cancers* **2021**, *13*, 1240. [\[CrossRef\]](#)
3. Kok, H.P.; van Rhoon, G.C.; Herrera, T.D.; Overgaard, J.; Crezee, J. Biological modeling in thermoradiotherapy: Present status and ongoing developments toward routine clinical use. *Int. J. Hyperth.* **2022**, *39*, 1126–1140. [\[CrossRef\]](#)
4. Vaupel, P.; Piazena, H. Strong correlation between specific heat capacity and water content in human tissues suggests preferred heat deposition in malignant tumors upon electromagnetic irradiation. *Int. J. Hyperth.* **2022**, *39*, 987–997. [\[CrossRef\]](#)
5. Hannon, G.; Tansi, F.L.; Hilger, I.; Prina-Mello, A. The effects of localized heat on the hallmarks of cancer. *Adv. Therap.* **2021**, *4*, 2000267. [\[CrossRef\]](#)
6. Vujaskovic, Z.; Song, C.W. Physiological mechanisms underlying heat-induced radiosensitization. *Int. J. Hyperth.* **2004**, *20*, 163–174. [\[CrossRef\]](#)
7. Song, C.W.; Park, H.J.; Lee, C.K.; Griffin, R. Implications of increased tumor blood flow and oxygenation caused by mild temperature hyperthermia in tumor treatment. *Int. J. Hyperth.* **2005**, *21*, 761–767. [\[CrossRef\]](#)
8. Horsman, M.R. Tissue physiology and the response to heat. *Int. J. Hyperth.* **2006**, *22*, 197–203. [\[CrossRef\]](#)
9. Vaupel, P.W.; Kelleher, D.K. Pathophysiological and vascular characteristics of tumours and their importance for hyperthermia: Heterogeneity is the key issue. *Int. J. Hyperth.* **2010**, *26*, 211–223. [\[CrossRef\]](#)
10. Vaupel, P.W.; Kelleher, D.K. Blood flow and associated pathophysiology of uterine cervix cancers: Characterisation and relevance for localised hyperthermia. *Int. J. Hyperth.* **2012**, *28*, 518–527. [\[CrossRef\]](#)
11. Dewhirst, M.W.; Lee, C.T.; Ashcraft, K.A. The future of biology in driving the field of hyperthermia. *Int. J. Hyperth.* **2016**, *32*, 4–13. [\[CrossRef\]](#)
12. Dewhirst, M.W.; Oleson, J.R.; Kirkpatrick, J.; Secomb, T.W. Accurate three-dimensional thermal dosimetry and assessment of physiologic response are essential for optimizing thermoradiotherapy. *Cancers* **2022**, *14*, 1701. [\[CrossRef\]](#)
13. Oei, A.L.; Kok, H.P.; Oei, S.B.; Horsman, M.R.; Stalpers, L.J.A.; Franken, N.A.P.; Crezee, J. Molecular and biological rationale of hyperthermia as radio- and chemosensitizer. *Adv. Drug. Deliv. Rev.* **2020**, *163–164*, 84–97. [\[CrossRef\]](#)
14. Lee, S.Y.; Fiorentini, G.; Szasz, A.M.; Szigeti, G.; Szasz, A.; Minnaar, C.A. Quo vadis oncological hyperthermia (2020)? *Front. Oncol.* **2020**, *10*, 1690. [\[CrossRef\]](#)
15. Notter, M.; Thomsen, A.R.; Nitsche, M.; Hermann, R.M.; Wolff, H.A.; Habl, G.; Munch, K.; Grosu, A.L.; Vaupel, P. Combined wIRA-hyperthermia and hypofractionated re-irradiation in the treatment of locally recurrent breast cancer: Evaluation of therapeutic outcome based on a novel size classification. *Cancers* **2020**, *12*, 606. [\[CrossRef\]](#)
16. Notter, M.; Stutz, E.; Thomsen, A.R.; Vaupel, P. Radiation-associated angiosarcoma of the breast and chest wall treated with thermography-controlled, contactless wIRA-hyperthermia and hypofractionated re-irradiation. *Cancers* **2021**, *13*, 3911. [\[CrossRef\]](#)
17. Datta, N.R.; Rogers, S.; Ordonez, S.G.; Puric, E.; Bodis, S. Hyperthermia and radiotherapy in the management of head and neck cancers: A systematic review and meta-analysis. *Int. J. Hyperth.* **2016**, *32*, 31–40. [\[CrossRef\]](#)
18. Datta, N.R.; Puric, E.; Klingbiel, D.; Gomez, S.; Bodis, S. Hyperthermia and radiation therapy in locoregional recurrent breast cancers: A systematic review and meta-analysis. *Int. J. Radiat. Oncol. Biol. Phys.* **2016**, *94*, 1073–1087. [\[CrossRef\]](#)
19. Peeken, J.C.; Vaupel, P.; Combs, S.E. Integrating hyperthermia into modern radiation oncology: What evidence is necessary? *Front. Oncol.* **2017**, *7*, 132. [\[CrossRef\]](#)
20. Multhoff, G.; Vaupel, P. Hypoxia compromises anti-cancer immune responses. *Adv. Exp. Med. Biol.* **2020**, *1232*, 131–143. [\[CrossRef\]](#)
21. Muthana, M.; Multhoff, G.; Pockley, A.G. Tumour infiltrating host cells and their significance for hyperthermia. *Int. J. Hyperth.* **2010**, *26*, 247–255. [\[CrossRef\]](#)
22. Finkel, P.; Frey, B.; Mayer, F.; Bosl, K.; Werthmoller, N.; Mackensen, A.; Gaipf, U.S.; Ullrich, E. The dual role of NK cells in antitumor reactions triggered by ionizing radiation in combination with hyperthermia. *Oncoimmunology* **2016**, *5*, e1101206. [\[CrossRef\]](#)
23. Hader, M.; Frey, B.; Fietkau, R.; Hecht, M.; Gaipf, U.S. Immune biological rationales for the design of combined radio- and immunotherapies. *Cancer Immunol. Immunother.* **2020**, *69*, 293–306. [\[CrossRef\]](#)
24. Werthmoller, N.; Frey, B.; Ruckert, M.; Lotter, M.; Fietkau, R.; Gaipf, U.S. Combination of ionising radiation with hyperthermia increases the immunogenic potential of b16-f10 melanoma cells in vitro and in vivo. *Int. J. Hyperth.* **2016**, *32*, 23–30. [\[CrossRef\]](#)
25. Schildkopf, P.; Frey, B.; Ott, O.J.; Rubner, Y.; Multhoff, G.; Sauer, R.; Fietkau, R.; Gaipf, U.S. Radiation combined with hyperthermia induces HSP 70-dependent maturation of dendritic cells and release of pro-inflammatory cytokines by dendritic cells and macrophages. *Radiother. Oncol.* **2011**, *101*, 109–115. [\[CrossRef\]](#)
26. Dunne, M.; Regenold, M.; Allen, C. Hyperthermia can alter tumor physiology and improve chemo- and radio-therapy efficacy. *Adv. Drug. Deliv. Rev.* **2020**, *163–164*, 98–124. [\[CrossRef\]](#)
27. Vaupel, P.; Flood, A.B.; Swartz, H.M. Oxygenation status of malignant tumors vs. normal tissues: Critical evaluation and updated data source based on direct measurements with pO₂ microsensors. *Appl. Magn. Reson.* **2021**, *52*, 1451–1479. [\[CrossRef\]](#)
28. Gersing, E.; Kelleher, D.K.; Vaupel, P. Tumour tissue monitoring during photodynamic and hyperthermic treatment using bioimpedance spectroscopy. *Physiol. Meas.* **2003**, *24*, 625–637. [\[CrossRef\]](#)
29. Vaupel, P. Tumor microenvironmental physiology and its implications for radiation oncology. *Semin. Radiat. Oncol.* **2004**, *14*, 198–206.

30. Rich, L.J.; Winslow, T.B.; Alberico, R.A.; Repasky, E.A.; Seshadri, M.; Singh, A.K. Enhanced tumour perfusion following treatment with water-filtered IR-A radiation to the thorax in a patient with head and neck cancer. *Int. J. Hyperth.* **2016**, *32*, 539–542. [[CrossRef](#)]
31. Vaupel, P. *Pathophysiology of solid tumors: In The Impact of Tumor Biology on Cancer Treatment and Multidisciplinary Strategies*; Molls, M., Vaupel, P., Nieder, C., Anscher, M.S., Eds.; Springer: Berlin/Heidelberg, Germany, 2009; pp. 51–92.
32. Horsman, M.R.; Vaupel, P. Tumor hypoxia and the response to radiotherapy. In *Tumor Hypoxia in the Clinical Setting*; Osinsky, S., Friess, H., Vaupel, P., Eds.; Adacemperiodyka: Kyiv, Ukraine, 2011; pp. 69–110.
33. Vaupel, P. Pathophysiological mechanisms of hyperthermia in cancer therapy. In *Biological Basis of Oncologic Thermo-therapy*; Gautherie, M., Ed.; Springer: Berlin/Heidelberg, Germany; New York, NY, USA, 1990; pp. 73–134.
34. Kelleher, D.K.; Vaupel, P. Vascular effects of localized hyperthermia. In *Hyperthermia in Cancer Treatment: A Primer*; Baronzio, G.F., Hager, E.D., Eds.; Springer Science: New York, NY, USA, 2006; pp. 99–109.
35. Kelleher, D.K.; Vaupel, P. No sustained improvement in tumor oxygenation after localized mild hyperthermia. *Adv. Exp. Med. Biol.* **2010**, *662*, 393–398. [[CrossRef](#)]
36. Sun, X.; Xing, L.; Ling, C.C.; Li, G.C. The effect of mild temperature hyperthermia on tumour hypoxia and blood perfusion: Relevance for radiotherapy, vascular targeting and imaging. *Int. J. Hyperth.* **2010**, *26*, 224–231. [[CrossRef](#)]
37. Rhee, J.G.; Eddy, H.A.; Hong, J.J.; Suntharalingam, M.; Vaupel, P.W. Divergent changes of flow through individual blood vessels upon localized heating. *Int. J. Hyperth.* **1996**, *12*, 757–769. [[CrossRef](#)]
38. Thews, O.; Li, Y.; Kelleher, D.K.; Chance, B.; Vaupel, P. Microcirculatory function, tissue oxygenation, microregional redox status and ATP distribution in tumors upon localized infrared-A -hyperthermia at 42 °C. *Adv. Exp. Med. Biol.* **2003**, *530*, 237–247. [[CrossRef](#)]
39. Vaupel, P. Oxygenation of solid tumors. In *Drug Resistance in Oncology*; Teicher, B.A., Ed.; Marcel Dekker: New York, NY, USA; Basel, Switzerland; Hongkong, China, 1993; pp. 53–85.
40. Kelleher, D.K.; Nauth, C.; Thews, O.; Krueger, W.; Vaupel, P. Localized hypothermia: Impact on oxygenation, microregional perfusion, metabolic and bioenergetic status of subcutaneous rat tumours. *Br. J. Cancer.* **1998**, *78*, 56–61. [[CrossRef](#)]
41. Leonhardt, H.; Stelter, I. Investigations on the influence of thrombocytes and temperature on the whole blood- and plasma-viscosity as comparative measurements between a capillary- and a plate-cone-viscosimeter (author's transl). *Experientia* **1975**, *31*, 1179–1181. [[CrossRef](#)]
42. Buono, M.J.; Krippes, T.; Kolkhorst, F.W.; Williams, A.T.; Cabrales, P. Increases in core temperature counterbalance effects of haemoconcentration on blood viscosity during prolonged exercise in the heat. *Exp. Physiol.* **2016**, *101*, 332–342. [[CrossRef](#)]
43. Khnouf, R.; Karasneh, D.; Abdulhay, E.; Abdelhay, A.; Sheng, W.; Fan, Z.H. Microfluidics-based device for the measurement of blood viscosity and its modeling based on shear rate, temperature, and heparin concentration. *Biomed. Microdevices* **2019**, *21*, 80. [[CrossRef](#)]
44. Vaupel, P.; Ostheimer, K.; Müller-Klieser, W. Circulatory and metabolic responses of malignant tumors during localized hyperthermia. *J. Cancer. Res. Clin. Oncol.* **1980**, *98*, 15–29. [[CrossRef](#)]
45. Kuzman, D.; Znidarcic, T.; Gros, M.; Vrhovec, S.; Svetina, S.; Zeks, B. Effect of pH on red blood cell deformability. *Pflügers Arch.* **2000**, *440*, R193–R194. [[CrossRef](#)]
46. Sen, A.; Capitano, M.L.; Sperryak, J.A.; Schueckler, J.T.; Thomas, S.; Singh, A.K.; Evans, S.S.; Hylander, B.L.; Repasky, E.A. Mild elevation of body temperature reduces tumor interstitial fluid pressure and hypoxia and enhances efficacy of radiotherapy in murine tumor models. *Cancer Res.* **2011**, *71*, 3872–3880. [[CrossRef](#)]
47. Ariffin, A.B.; Forde, P.F.; Jahangeer, S.; Soden, D.M.; Hinchion, J. Releasing pressure in tumors: What do we know so far and where do we go from here? A review. *Cancer Res.* **2014**, *74*, 2655–2662. [[CrossRef](#)]
48. Baronzio, G.; Parmar, G.; Baronzio, M. Overview of methods for overcoming hindrance to drug delivery to tumors, with special attention to tumor interstitial fluid. *Front. Oncol.* **2015**, *5*, 165. [[CrossRef](#)]
49. Bayer, C.; Shi, K.; Astner, S.T.; Maftai, C.A.; Vaupel, P. Acute versus chronic hypoxia: Why a simplified classification is simply not enough. *Int. J. Radiat. Oncol. Biol. Phys.* **2011**, *80*, 965–968. [[CrossRef](#)]
50. Moon, E.J.; Sonveaux, P.; Porporato, P.E.; Danhier, P.; Gallez, B.; Batinic-Haberle, I.; Nien, Y.C.; Schroeder, T.; Dewhirst, M.W. NADPH oxidase-mediated reactive oxygen species production activates hypoxia-inducible factor-1 (HIF-1) via the ERK pathway after hyperthermia treatment. *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 20477–20482. [[CrossRef](#)]
51. Wan, J.; Wu, W. Hyperthermia induced HIF-1 α expression of lung cancer through akt and erk signaling pathways. *J. Exp. Clin. Cancer Res.* **2016**, *35*, 119. [[CrossRef](#)]
52. Vaupel, P. Pathophysiology of human tumors. In *Tumor Hypoxia in the Clinical Setting*; Osinsky, S., Friess, H., Vaupel, P., Eds.; Adacemperiodyka: Kyiv, Ukraine, 2011; pp. 20–66.
53. Vaupel, P.; Kallinowski, F.; Okunieff, P. Blood flow, oxygen and nutrient supply, and metabolic microenvironment of human tumors: A review. *Cancer Res.* **1989**, *49*, 6449–6465.
54. Vaupel, P.; Piazena, H. Hyperhydration of cancers: A characteristic biophysical trait strongly increasing O₂, CO₂, glucose and lactate diffusivities, and improving thermophysical properties of solid malignancies. *Adv. Exp. Med. Biol.* **2023**, in press.
55. Grote, J.; Süsskind, R.; Vaupel, P. Oxygen diffusivity in tumor tissue (DS-carcinosarcoma) under temperature conditions within the range of 20–40 °C. *Pflügers Arch.* **1977**, *372*, 37–42. [[CrossRef](#)]

56. Bentley, T.B.; Meng, H.; Pittman, R.N. Temperature dependence of oxygen diffusion and consumption in mammalian striated muscle. *Am. J. Physiol.* **1993**, *264*, H1825–H1830. [[CrossRef](#)]
57. Vaupel, P.; Multhoff, G. Revisiting the Warburg effect: Historical dogma versus current understanding. *J. Physiol.* **2021**, *599*, 1745–1757. [[CrossRef](#)]
58. Bicher, H.I.; Hetzel, F.W.; Vaupel, P.; Sandhu, T.S. Microcirculation modifications by localized microwave hyperthermia and hematoporphyrin phototherapy. *Bibl. Anat.* **1981**, *20*, 628–632.
59. Vaupel, P. Impact of localized microwave hyperthermia on the pH-distribution in malignant tumors (author's transl). *Strahlentherapie* **1982**, *158*, 168–173.
60. Vaupel, P.; Müller-Klieser, W.; Otte, J.; Manz, R.; Kallinowski, F. Blood flow, tissue oxygenation, and pH-distribution in malignant tumors upon localized hyperthermia. Basic pathophysiological aspects and the role of various thermal doses. *Strahlentherapie* **1983**, *159*, 73–81.
61. Vaupel, P.; Kallinowski, F. Physiological effects of hyperthermia. *Recent Results Cancer Res.* **1987**, *104*, 71–109. [[CrossRef](#)]
62. Bicher, H.I.; Sandhu, T.S.; Vaupel, P.; Hetzel, F.W. Effect of localized microwave hyperthermia on physiological responses. *J. Natl. Cancer Inst.* **1980**, *61*, 217–219.
63. Severinghaus, J.W. Blood oxygen dissociation line charts: Man. In *Biological Data Book*, 2nd ed.; Altman, P.L., Dittmar, D.S., Eds.; Federation of American Societies for Experimental Biology: Bethesda, MD, USA, 1971; pp. 1871–1872.
64. Dash, R.K.; Bassingthwaite, J.B. Blood HbO₂ and HbCO₂ dissociation curves at varied O₂, CO₂, pH, 2,3-DPG and temperature levels. *Ann. Biomed. Eng.* **2010**, *38*, 1683–1701. [[CrossRef](#)]
65. Woyke, S.; Brugger, H.; Strohle, M.; Haller, T.; Gatterer, H.; Dal Cappello, T.; Strapazon, G. Effects of carbon dioxide and temperature on the oxygen-hemoglobin dissociation curve of human blood: Implications for avalanche victims. *Front. Med.* **2021**, *8*, 808025. [[CrossRef](#)]
66. Gullino, P.M.; Yi, P.N.; Grantham, F.H. Relationship between temperature and blood supply or consumption of oxygen and glucose by rat mammary carcinomas. *J. Natl. Cancer Inst.* **1978**, *60*, 835–847. [[CrossRef](#)]
67. Thomsen, A.R.; Saalman, M.R.; Nicolay, N.H.; Grosu, A.L.; Vaupel, P. Temperature profiles and oxygenation status in human skin and subcutis upon thermography-controlled wIRA-hyperthermia. In *Water-Filtered Infrared A (wIRA) Irradiation*; Vaupel, P., Ed.; Springer Nature: Cham, Switzerland, 2022; pp. 69–80.
68. Vaupel, P.; Frinak, S.; Mueller-Klieser, W.; Bicher, H.I. Impact of localized hyperthermia on the cellular microenvironment in solid tumors. *Natl. Cancer Inst. Monogr.* **1982**, *61*, 207–209.
69. Tanaka, Y.; Hasegawa, A.; Murata, T. Effect of irradiation and hyperthermia on vascular function in normal and tumor tissue. In *Hyperthermic Oncology*; Overgaard, J., Ed.; Springer: Berlin/Heidelberg, Germany, 1984; pp. 145–148.
70. Iwata, K.; Shakil, A.; Hur, W.J.; Makepeace, C.M.; Griffin, R.J.; Song, C.W. Tumour pO₂ can be increased markedly by mild hyperthermia. *Br. J. Cancer Suppl.* **1996**, *27*, S217–S221.
71. Shakil, A.; Osborn, J.L.; Song, C.W. Changes in oxygenation status and blood flow in a rat tumor model by mild temperature hyperthermia. *Int. J. Radiat. Oncol. Biol. Phys.* **1999**, *43*, 859–865. [[CrossRef](#)]
72. Song, C.W.; Park, H.; Griffin, R.J. Improvement of tumor oxygenation by mild hyperthermia. *Radiat. Res.* **2001**, *155*, 515–528. [[CrossRef](#)]
73. Kelleher, D.K.; Thews, O.; Scherz, A.; Salomon, Y.; Vaupel, P. Combined hyperthermia and chlorophyll-based photodynamic therapy: Tumour growth and metabolic microenvironment. *Br. J. Cancer* **2003**, *89*, 2333–2339. [[CrossRef](#)]
74. Griffin, R.J.; Dings, R.P.; Jamshidi-Parsian, A.; Song, C.W. Mild temperature hyperthermia and radiation therapy: Role of tumour vascular thermotolerance and relevant physiological factors. *Int. J. Hyperth.* **2010**, *26*, 256–263. [[CrossRef](#)]
75. Horsman, M.R.; Overgaard, J. Can mild hyperthermia improve tumour oxygenation? *Int. J. Hyperth.* **1997**, *13*, 141–147. [[CrossRef](#)]
76. Vujaskovic, Z.; Poulson, J.M.; Gaskin, A.A.; Thrall, D.E.; Page, R.L.; Charles, H.C.; Macfall, J.R.; Brizel, D.M.; Meyer, R.E.; Prescott, D.M.; et al. Temperature-dependent changes in physiologic parameters of spontaneous canine soft tissue sarcomas after combined radiotherapy and hyperthermia treatment. *Int. J. Radiat. Oncol. Biol. Phys.* **2000**, *46*, 179–185. [[CrossRef](#)]
77. Thrall, D.E.; Larue, S.M.; Pruitt, A.F.; Case, B.; Dewhirst, M.W. Changes in tumour oxygenation during fractionated hyperthermia and radiation therapy in spontaneous canine sarcomas. *Int. J. Hyperth.* **2006**, *22*, 365–373. [[CrossRef](#)]
78. Brizel, D.M.; Scully, S.P.; Harrelson, J.M.; Layfield, L.J.; Dodge, R.K.; Charles, H.C.; Samulski, T.V.; Prosnitz, L.R.; Dewhirst, M.W. Radiation therapy and hyperthermia improve the oxygenation of human soft tissue sarcomas. *Cancer Res.* **1996**, *56*, 5347–5350.
79. Vujaskovic, Z.; Rosen, E.L.; Blackwell, K.L.; Jones, E.L.; Brizel, D.M.; Prosnitz, L.R.; Samulski, T.V.; Dewhirst, M.W. Ultrasound guided pO₂ measurement of breast cancer reoxygenation after neoadjuvant chemotherapy and hyperthermia treatment. *Int. J. Hyperth.* **2003**, *19*, 498–506. [[CrossRef](#)]
80. Jones, E.L.; Prosnitz, L.R.; Dewhirst, M.W.; Marcom, P.K.; Hardenbergh, P.H.; Marks, L.B.; Brizel, D.M.; Vujaskovic, Z. Thermochemoradiotherapy improves oxygenation in locally advanced breast cancer. *Clin. Cancer Res.* **2004**, *10*, 4287–4293. [[CrossRef](#)]

81. Vaupel, P.; Thews, G. pO₂ distribution in tumor tissue of DS-carcinosarcoma. *Oncology* **1974**, *30*, 475–484. [[CrossRef](#)]
82. Notter, M.; Piazena, H.; Vaupel, P. Hypofractionated re-irradiation of large-sized recurrent breast cancer with thermography-controlled, contact-free water-filtered infra-red-A hyperthermia: A retrospective study of 73 patients. *Int. J. Hyperth.* **2017**, *33*, 227–236. [[CrossRef](#)]

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.