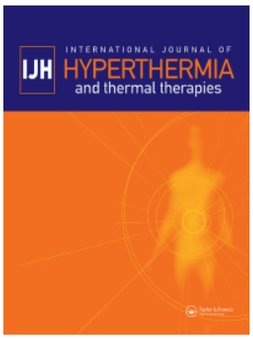

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# The heat shock response: Role in radiation biology and cancer therapy

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# The heat shock response: Role in radiation biology and cancer therapy

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**Abstract**
Since prehistoric times, elevated temperatures have been used to treat cancer in a variety of forms. In modern times (the last 40 years) efforts have concentrated on combining heat with other anti-tumour modalities, principally ionizing radiation and some chemotherapeutic drugs. Despite the emphasis on combined therapy, rodent data relating to heat sensitivity and thermal tolerance development assumed principal importance. These considerations suggested treating at 43°C as a target temperature and fractionation schemes emphasizing thermal tolerance avoidance. Concomitantly crucial data on heat-induced tumour reoxygenation and its temperature dependence were largely ignored. In reality these were unrealistic and undesirable goals. The preponderance of evidence now suggests that lower temperatures (40–42°C) administered more frequently, optimally immediately before and during each administration of ionizing radiation, are likely to yield optimal results. Factoring in trimodality therapy and other combinations of chemotherapeutic drugs will require some modifications of such fractionation schemes.

**Keywords:** *Hyperthermia, radiation, chemotherapeutic drugs, thermal tolerance, fractionation*

## Introduction

In the introduction to this special issue, reference was made to the first International Symposium on Cancer Therapy with Hyperthermia and Radiation held in Washington, DC in 1975. In many ways it is somewhat amazing that as many valid conclusions were reached considering that modern techniques of cellular and molecular biology had been applied for less than 10 years. Unfortunately, over the next 20 years the primary emphasis shifted from combined modality cytotoxicity, heat potentiation of other anti-tumour modalities, to heat killing alone and concerns relating to the avoidance of the protective influence of thermal tolerance. These concerns, when combined with misleading

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information from studies with rodent cell lines, led to the dogma that the optimal target temperature was 43°C and that heat should be delivered no more than once or twice weekly even for clinical protocols combining heat with radiation or chemotherapeutic drugs. Other studies with rodent tumour model systems [1] suggested that heat should be delivered long after radiation in combined modality therapy. This dogma, which has prevailed for the past 30 years, has resulted in sub-optimal clinical protocols with sparse heat fractionation, as few as 3–4 fractions, combined with 25–35 radiation fractions. Most, but not all, such protocols did not demonstrate benefit for the addition of hyperthermia which, when combined with poor quality assurance, has caused a significant reduction of interest for this form of therapy, particularly in North America.

As pointed out in another paper in this special issue by Dewhirst et al. [2], this combination of factors caused some leading scientists in this field to ignore or minimize the importance of other observations with mild hyperthermia [3, 4] and heat-induced tumour cell reoxygenation [5, 6]. Taken together, all of these factors support resetting the target temperature to lower values (41–42°C) and increasing heat dose by shifting to denser (more frequent) fraction schemes. The obvious exception to this latter conclusion is therapy with thermal ablation where temperatures can exceed 70–80°C for short periods of time and where coagulation necrosis is the cytotoxic mechanism. Nevertheless, even in this instance the low temperature edges bordering the ablated tissues are subject to the same biological considerations as for all other forms of hyperthermic therapy.

This workshop presentation was intended to give a historical perspective on the subject of cancer therapy with hyperthermia. Consequently, the references were chosen more to reflect this aspect rather than to cite the latest literature on the various subjects presented. Due to limited space, the references are intended to be representative not exhaustive.

## Heat cytotoxicity, dose and the arrhenius relationship

With the application of modern laboratory techniques it rapidly became clear that elevated temperatures caused cell killing in a well-defined dose-dependant manner both as a function of temperature as well as a function of time of exposure to the elevated temperatures [7, 8]. While the dose–response relationship is linear with respect to time it is non-linear with respect to temperature [9]; a fact which has confounded numerous attempts to define a unit of dose for thermal exposure. The most successful definition of thermal dose to date was developed by Sapareto and Dewey [9] using an Arrhenius analysis of a large body of *in vitro* survival data for CHO cells which when reduced results in the relationship:

$$t_{43} = R^{(T-43)}\Delta t$$

where $t_{43}$ is the equivalent time at 43°C, $R$ is the reciprocal of the slope of the Arrhenius plot and is ~2 for temperatures above 43°C and 4 below 43°C for CHO cells, $T$ is the temperature and $\Delta t$ is the time at $T$. Observed deviations from this relationship have been attributed [9] to the development of chronic thermal tolerance. Thermal dose in this context is obviously not the product of time and temperature and leads to higher temperatures being much more effective than lower temperatures. This relationship also supports the well known approximation that thermal effects double for each degree that temperature increases. It is also the basis for the popular clinical definition $CEM_{43,90}$ which is the equivalent minutes at 43°C for the temperature where 90% of the measured intra-tumoural temperature points equal or exceed. The equivalent minute concept was proposed by Oleson et al. [10] and Dewey [8]. This formulation takes into account the clinical reality that

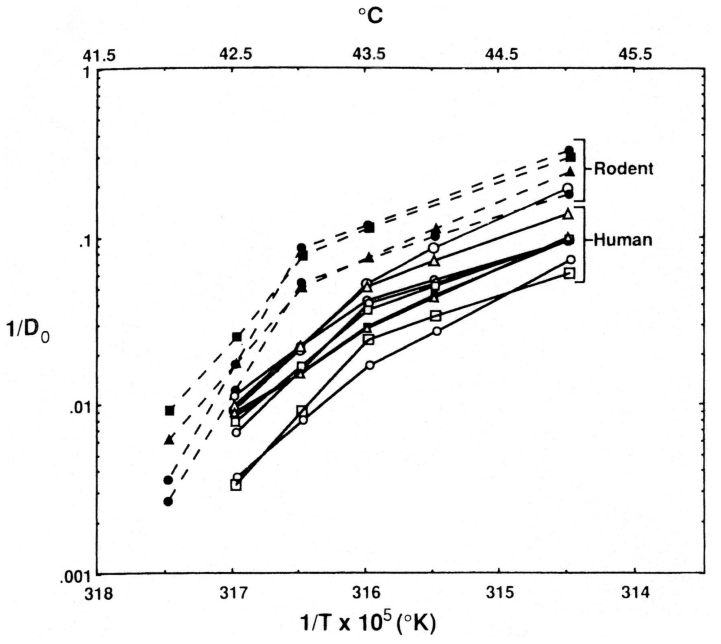


Figure 1. Arrhenius plots for four rodent and eight human cell lines. Human cell lines have a $D_0$ almost an order of magnitude greater that rodent cell lines. Starting from the top of the plot to the bottom the cell lines were: Rodent: CHO, AD-5, AL and 10T-1/2 Human: KB-7, MIA-PACA-2, glioblastoma, WiDR, AG-1522, HTB-66, HTB-72, KB-8 and A549. Of note is the shallow or non-existent break in the curve for human cells at 43.5°C rather than 43°C, usually seen for rodent cell lines. (Figure taken from Roizin-Towle and Pirro [10]. Reproduced with permission.)

intra-tumoural temperatures are non-uniform. Other commonly used equivalents are $CEM_{43,50}$ (for 50% of the measured points) and $CEM_{43,10}$ (for 10% of the measured points).

The Arrhenius plot has proved to be a very valuable and instructive analytical tool. It has been used by other authors in this special issue for addressing mechanistic factors related to thermal inactivation (cell killing) as well thermal enhancement of other anti-tumour modalities such as radiation and chemotherapeutic drugs. It is also useful for comparing the differing effects of heat shock amongst different cell types, as is demonstrated by the data of Roizin-Towle and Pirro [11], which is shown in Figure 1 for four rodent cell lines and eight human cell lines. Examination of this data leads to several important conclusions which directly impact clinical application:

(1) Human cells are, in general, much more resistant to heat shock than their rodent counterparts at temperatures above 42°C.
(2) There is no general pattern which shows that tumour cells are intrinsically more sensitive to heat shock as a result of the transformation to malignancy whether of rodent or human origin. What has been shown to sensitize all cells to heat shock are tumour micro-environmental factors such as nutrient deprivation [12] and low pH [13]. In other words it is cells in tumours rather than tumour cells that are sensitized to heat. This is an important distinction with direct clinical impact and explains early observations that heat (41–50°C) by itself was not an effective cancer treatment exhibiting durable responses [14].

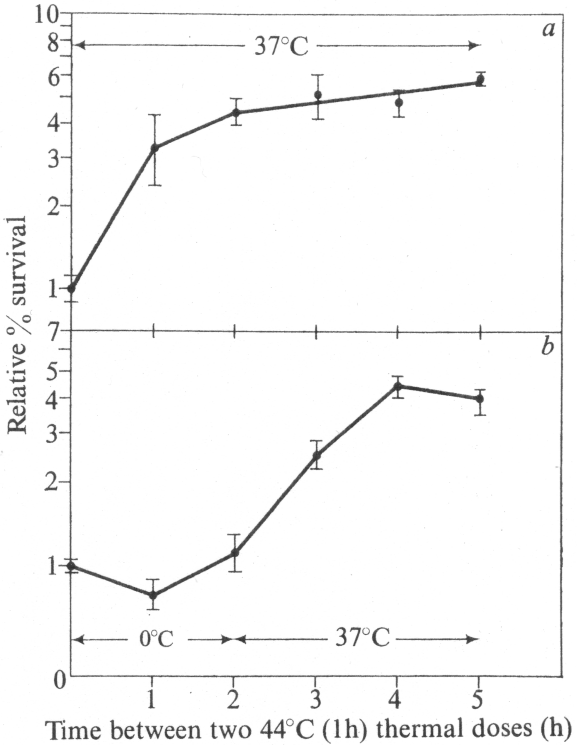


Figure 2. Results of split dose experiments with HeLa cells. Two heat doses of 44°C for 1 h separated by the indicated times 37°C. Note that in panel b, maintaining the cells at 0°C for 2 h before returning the cultures to 43°C resulted in a concomitant delay in the development of tolerance implicating the requirement of active metabolism for the manifestation of the phenomenon. (Data taken from Gerner [15]. Reproduced with permission.)

(3) The pronounced break in the plot for rodent cells at 43°C, which was the basis for the Sapareto Dewey dose formulation, does not occur for human cells. If there is a break it occurs at 43.5°C, but for many human cells there is no substantial break at all. Integrating a larger body of human survival data into account there is a compelling justification for using $R = 2$ as a reasonably accurate approximation over the entire temperature range for estimating thermal dose in a human clinical setting. The non-uniformity of temperature distributions in tumours subjected to hyperthermia and a lack of knowledge as to the cellular sensitivity for any given tumour are added justification for this approximation.

## Thermal tolerance

Thermal tolerance, heat induced resistance to subsequent heat shock, is a fascinating example of biological adaptability to potentially lethal environmental insults. This phenomenon was first described by Crile [15] and quantitated by Gerner [16]. This adaptation has been conserved throughout evolution and for mammalian systems applies not only to single cells but to organs, blood vessels and even whole animals. Figure 2 shows Gerner's original data demonstrating this effect in a quantitative manner for split doses

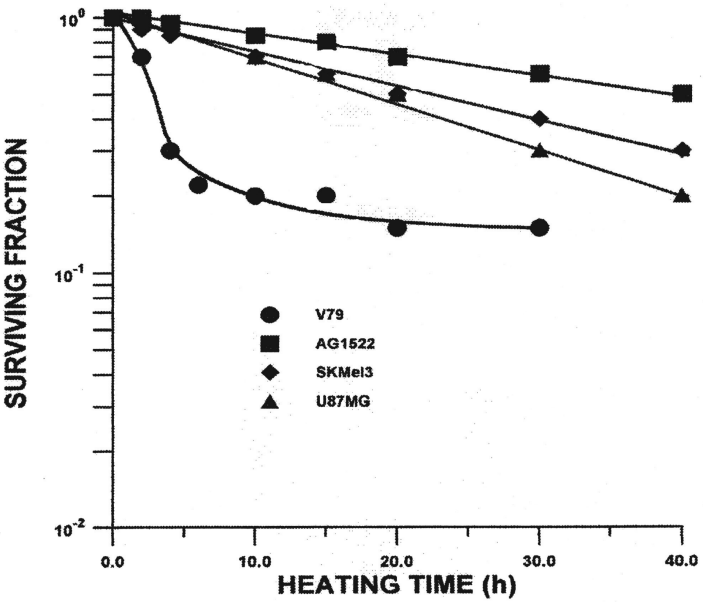


Figure 3. Chinese hamster cells (V79) and human cells (normal fibroblasts, AG1522; melanoma, SkMe13; and glioma, U87MG) were heated at 41°C for up to 40 h. V79 cells developed chronic thermotolerance beginning at 4–5 h of heating and was characterized by a plateau on the survival curve. The three human cell lines were more heat resistant then the V79 rodent cells and showed no chronic thermotolerance plateau. (Data taken from Armour and Raaphorst [16].)

of heat at 44°C in HeLa cells *in vitro* [16]. Over the years, numerous other investigators have shown that this resistance can last for as long as 120 h, usually starting to fall off at about the 72 h time point. Figure 2 also shown that cellular metabolism, protein synthesis, is required for the full development of tolerance to the second dose. Other inhibitors of protein synthesis, such as cycloheximide, have also been demonstrated to abrogate tolerance development. Another form of thermal tolerance, chronic thermotolerance, is illustrated by the data shown in Figure 3. In this case, the tolerance develops during protracted exposure (hours) for temperatures less than 43°C in rodent cell lines. It does not develop to any significant extent in the more heat-resistant human cells nor at temperatures above 43°C in any cell lines.

Clearly, chronic thermal tolerance should have no impact on human clinical cancer treatment. However, the existence of the thermal tolerance phenomenon in general has exhibited a profound influence on the design of human clinical trials with conventional hyperthermia, temperatures in the 40–50°C range. The argument has been that, in order to maximize heat killing, heat doses should be separated by a minimum of 72–96 h. Consequently, many human hyperthermia trials involving combined modality therapy with radiation and/or chemotherapeutic drugs have centred on whether one or two heat treatments per week were more optimal with daily radiation therapy. Some regimens consisted of as few as four heat treatments interspersed with 20–30 radiation fractions.

## Thermal radiosensitization and chemosensitization

For over 40 years it has been known that elevated temperatures supra-additively, perhaps synergistically, enhance the cytotoxic effects of ionizing radiation [17–20] and several

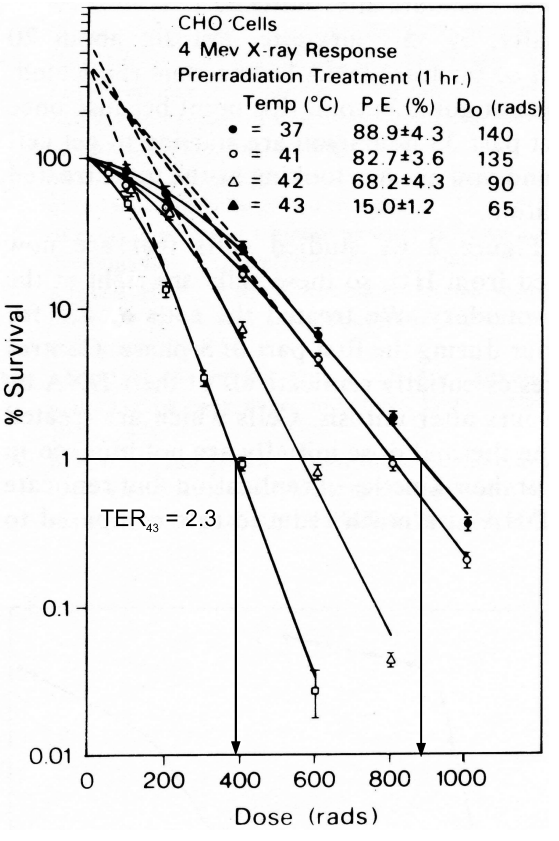


Figure 4. Survival of CHO cells when subjected to a hyperthermic pre-treatment for 1 h at the indicated temperatures. A synergistic interaction is indicated by the continually decreasing values of $D_0$ with increasing temperature. The TER determination is as in the main body of the text. (Data was taken from Gerner [15]. Reproduced with permission.)

common chemotherapeutic drugs [21]. The thermal enhancement ratio (TER) is determined, as demonstrated in Figure 4, by taking the ratio of the dose for a particular cytotoxic isoeffect, usually a survival level of 0.01, at 37°C to the dose for the same isoeffect at the desired temperature after normalizing out the effects of heat killing alone. For the data shown in Figure 4, the $TER_{43}$ is 2.3. The TER for the tri-modality therapy data shown in Figure 5 is ~4 for temperature exposures of only 40°C for protracted periods. For ionizing radiation, hyperthermia causes an inhibition of DNA repair mechanisms [20], particularly double strand breaks, which if left unrepaired are uniformly considered lethal. Robinson et al. [18] showed that the oxygen enhancement ratio (OER) for low linear energy transfer (LET) radiation (e.g. X-rays) can be reduced to values lower that that observed for several high LET particle irradiations where the induction of non-repairable DNA damage is thought to be the principal biological advantage. This observation led Eugene Robinson to coin the term 'poor man's high LET radiation therapy', since the administration of hyperthermia is orders of magnitude less expensive to administer than high LET particle therapy. Other investigators [17] have demonstrated that ionizing radiation's well known dose rate effect can be eliminated entirely by hyperthermia, again pointing to DNA repair inhibition as a mechanistic factor.

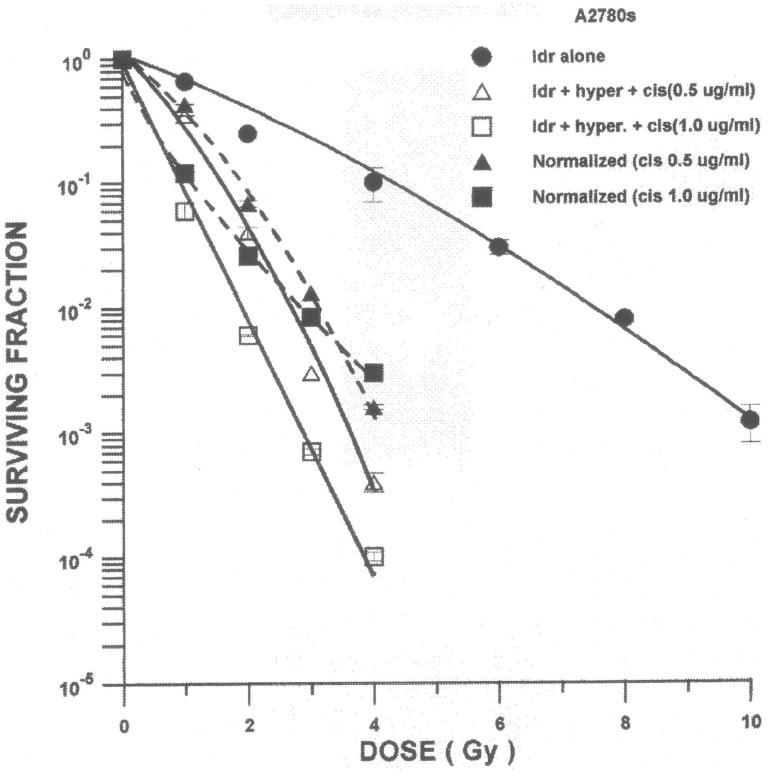


Figure 5. Human ovarian carcinoma cells (A2780s) were given low dose rate radiation (LDR, $0.88\,\mathrm{Gy\,min^{-1}}$) alone or combined with concomitant treatments with cisplatin (0.5 and $1.0\,\mu\mathrm{g\,ml^{-1}}$) and hyperthermia at 40°C. The dashed curves represent survival of the combined treatments with the killing effect of cisplatin alone and hyperthermia alone normalized out. These latter curves fall well below the LDR alone curves, indicating a strong synergistic effect of the combined tri-modality treatment. (Data was taken from Armour and Raaphorst [17].)

A long overlooked effect of hyperthermia *in vivo* is tumour reoxygenation which is discussed in depth in another presentation in this special issue [6]. In this instance there are two factors acting in concert which can enhance the effects of both ionizing radiation and some chemotherapeutic drugs. Hyperthermia causes a marked increase in tumour blood flow at the lower temperatures (40–42°C) as well as suppressing metabolic activity and oxygen consumption for at least 24 h falling off thereafter. Taken together these effects cause a significant decrease in the hypoxic fraction of tumours in both rodent and human tumours [6], long thought to be the culprit for the failure of radiation therapy to control some human tumours. A large hypoxic fraction has also been correlated with poor clinical prognosis. These observations may explain the success of several clinical protocols where measured temperatures are well below the previously considered optimum of 43°C. Hyperthermia is probably the best hypoxic sensitizer, yet discovered and lacks the systemic toxicities that severely limit chemical agents designed for this purpose. This factor alone provides a powerful rational for the application of hyperthermia in cancer treatment.

The situation for the combination of hyperthermia with chemotherapy is less clear, at least relating to optimal fractionation schemes, than with ionizing radiation. While repair inhibition for chemically-induced DNA lesions has been implicated for cisplatin,

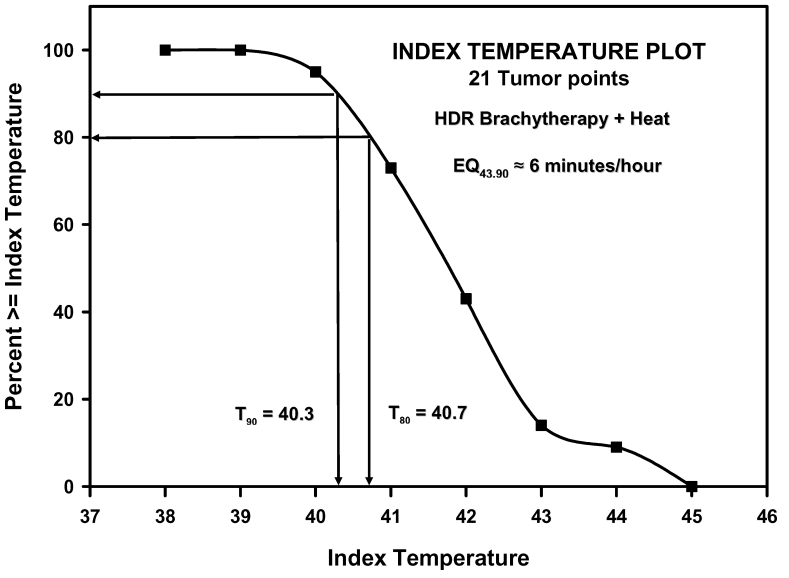


Figure 6. A typical integral temperature distribution from an actual clinical therapy session using the combination of brachytherapy and hyperthermia for a patient with recurrent prostate cancer. A total of 42 h of hyperthermia were administered over a 3 day period combined with five high dose rate (HDR) radiation fractions of 4 Gy each. Hyperthermia was interrupted briefly to administer the HDR radiation. The curve was constructed by taking the total percentage of measured intra-tumoural temperature at or above the indicated temperatures on the abscissa. There were 21 intra-tumoural measured temperature points. $T_{90}$ (40.3°C) is determined by drawing a line horizontally from the 90% point to where it intersects the distribution curve and reading off the corresponding temperature on the abscissa. $T_{80}$, $T_{50}$ and $T_{10}$ are determined in an identical manner. For this distribution the $CEM_{43,90}$ was ~6 min for each hour of exposure, using $R = 2$ in the Sapareto–Dewey formulation. The total heat dose ($CEM_{43,90}$) delivered during treatment was 252 min. This patient exhibited a durable complete biochemical response (>3 years follow-up).

mitomycin-c and bleomycin, this is not the case for many other drugs. Some drugs such as methotrexate and paclitaxel do not show obvious supra-additive interactions *in vitro*. If that is the case *in vivo* there is no advantage to combining them at least in a temporal sense. Membrane permeability to various drugs can be either enhanced or decreased, a factor irrelevant to the case of radiation. This leads to tricky sequencing issues that must be worked out for each individual drug. However, as a general rule the drug should be present before hyperthermia is administered for optimal interaction.

## Discussion

In the past the existence of thermal tolerance has dominated the design of many clinical protocols, but should that continue to be the case? The first issue is whether or not heat killing is important in the human clinical setting outside the application of thermal ablation. Rosner et al. [22] have shown that, for typical non-uniform temperature distributions represented by the data in Figure 6, somewhere between 5–30% of the tumour cells would be killed by heat alone based on CHO cell sensitivity. Conservatively correcting this kill rate for the differences between CHO and human cells the kill rate drops to between 2–10%. Clearly, much higher temperatures or a different strategy are required.

With the exception of ablation therapy, hyperthermia is rarely, if ever, administered as a single agent for reasons discussed above and in the literature [14]. The question is then: is tolerance important in combined modality therapy? Clearly the chronic form of tolerance has little effect, since it does not develop to any significant degree in human cells. The acute form of tolerance (sequential acute doses separated usually by 24 h) does impact the TER with radiation for very high heat doses at higher temperatures. Dewey [8] has shown that, for CHO cells at heat doses >160 $CEM_{43}$, the TER does not increase as quickly as for non-tolerant cells with a conditioning dose of 15 min at 45.5°C 18 h before the test dose. In that instance, the TER reached a plateau at 3.0 as the heat doses increased. What this means is that the temperature distribution shown in Figure 6 would have to be maintained for ~8 h to develop that level of tolerance every day followed by 25–30 h to deliver the total heat dose. This is clearly impossible but even if it were done the TER would be at least 3. For realistic regimens in combined therapy of one or at most 2 h per day the effect of tolerance on the TER will be negligible, perhaps undetectable. An argument against increasing the heat dose by increasing temperature is that at higher temperatures and heat doses tumour reoxygenation disappears due to intra-vascular coagulation within the tumour stopping blood flow [6]. The reoxygenation effect is a profound advantage that one cannot afford to pass up. This concept applies equally well to combined therapy with drugs such as cisplatin or for the tri-modality therapy represented by Figure 5. Here, however, caution is required, since not all drugs behave the same under these conditions.

What does all of this mean for future directions? Clearly, past temperature goals of 43°C (e.g. $CEM_{43} = 60$ each day for a 1 h treatment) is both unrealistic and undesirable. Realistic temperature distributions, such as that in Figure 6, need to be administered more frequently or longer than in the past, possibly before each radiation fraction. This appears to be the only method of optimizing both heat dose and cytotoxic anti-tumour effects. Such distributions can be delivered by several state-of-the-art heat delivery systems and are very compatible with advanced delivery methods for a variety of compounds discussed by Dewhirst et al. [2]. Indeed, these are fortunate and encouraging coincidences.

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