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IMPRINT

EDITORIAL

DEAR READER, DEAR COLLEAGUES, DEAR HYPERTHERMIA EXPERTS

The 34th issue of the Oncothermia Journal presents the most updated information about modulated electrohyperthermia (mEHT) published in various high-impact journals. In the first three articles, the volume presents the oncothermia presentations of the recent conference of the European Society of Hyperthermic Oncology (ESHO) held in Cologne, Germany. The mEHT presentations focused on the most problematic tumor: advanced cervix with HIV, glioblastoma multiforme, and pancreas carcinoma. Dr. Minnaar presented the final results of the phase III cervix study in which she found a significant prolongation of survival time compared to conventional radio-chemotherapy treatments. Prof. van Gool presented the same significant improvement for advanced glioblastoma multiform treatment with oncothermia combined with multimodal immunotherapy. Prof. Dr. Fiorentini showed a definite and significant benefit for advanced, second, and third-line treated pancreatic cancer patients, including 217 individuals in the study.

Many of the presentations at the conference showed an unmistakable shift from only thermal effects to the importance of the nonthermal electromagnetic components and, importantly, using fever range temperatures in local treatments. This trend clearly shows the right path for Oncotherm development, which has been following this path for 35 years now.

Besides the three conference presentations, the present volume contains five newly published articles that have been published in renowned international journals. Dr. Kleef† and his group published integrative immunomodulatory treatment for gastrointestinal patients, reporting survival benefits by oncothermia combination. Prof. Fiorentini published his pancreatic results in extended form as well. Dr. Marcel Szasz and his group's results also drew attention to the relationship between thermal and nonthermal molecular effects in the use of oncothermia therapy. Prof. Dank and her group discussed the results achieved by pancreatic cancer patients using bioelectromagnetism in their treatment. Finally, Prof. Andras Szasz published a new hypothesis about the development of the charge distribution during malignant tumor growth. This hypothesis can explain some specialties of malignant development and the cause of cancer from the electromagnetic point of view.

I am convinced that expert readers will find their expectations in the current issue of Oncothermia Journal and will be able to use this information in their daily medical practice. I am pleased to recognize the growing activity and extensive publications in the mEHT topic, validating and verifying the method's promised advantages. I am thankful for the cooperation of research scientists and practicing oncologists, and their valuable results are highly appreciated.

Dr. Andras Szasz
Professor, Chair, Biotechnics Department of St. Istvan University

LIEBE LESER, LIEBE KOLLEGEN, LIEBE HYPERTHERMIE-EXPERTEN,

Die 34. Ausgabe des Oncothermia Journals präsentiert die aktuellsten Informationen über modulierte Elektrohyperthermie (mEHT), die in verschiedenen hochrangigen Fachzeitschriften veröffentlicht wurden. In den ersten drei Artikeln werden die Oncothermie-Vorträge der jüngsten Konferenz der European Society of Hyperthermic Oncology (ESHO) in Köln, Deutschland, vorgestellt. Die mEHT-Vorträge konzentrierten sich auf die problematischsten Tumore: fortgeschrittener Gebärmutterhals mit HIV, Glioblastoma multiforme und Pankreaskarzinom. Dr. Minnaar stellte die Endergebnisse der Phase-III-Studie am Gebärmutterhals vor, in der sie eine signifikante Verlängerung der Überlebenszeit im Vergleich zu herkömmlichen Radiochemotherapien feststellte. Prof. van Gool präsentierte die gleiche signifikante Verbesserung für die Behandlung des fortgeschrittenen Glioblastoma multiform mit Oncothermie in Kombination mit multimodaler Immuntherapie. Prof. Dr. Fiorentini zeigte einen eindeutigen und signifikanten Nutzen für Patienten mit fortgeschrittenem Bauchspeicheldrüsenkrebs in der zweiten und dritten Behandlungslinie, darunter 217 Personen in der Studie.

Viele der Präsentationen auf der Konferenz zeigten eine unverkennbare Verlagerung von den rein thermischen Effekten hin zur Bedeutung der nichtthermischen elektromagnetischen Komponenten und vor allem zur Verwendung von Temperaturen im Fieberbereich bei lokalen Behandlungen. Dieser Trend zeigt eindeutig den richtigen Weg für die Oncotherm-Entwicklung, die diesen Weg nun schon seit 35 Jahren beschreitet.

Neben den drei Konferenzbeiträgen enthält der vorliegende Band fünf neu veröffentlichte Artikel, die in renommierten internationalen Fachzeitschriften erschienen sind. Dr. Kleef† und seine Gruppe veröffentlichten eine integrative immunmodulatorische Behandlung für Magen-Darm-Patienten und berichteten über Überlebensvorteile durch die Kombination mit Oncothermie. Prof. Fiorentini veröffentlichte seine Ergebnisse zur Bauchspeicheldrüse ebenfalls in erweiterter Form. Die Ergebnisse von Dr. Marcel Szasz und seiner Gruppe lenkten die Aufmerksamkeit auch auf die Beziehung zwischen thermischen und nichtthermischen molekularen Effekten bei der Anwendung der Oncothermietherapie. Prof. Dank und ihre Gruppe diskutierten die Ergebnisse, die bei der Behandlung von Bauchspeicheldrüsenkrebs-Patienten mit Bioelektromagnetismus erzielt wurden. Schließlich veröffentlichte Prof. Andras Szasz eine neue Hypothese über die Entwicklung der Ladungsverteilung während des Wachstums von bösartigen Tumoren. Diese Hypothese kann einige Besonderheiten der bösartigen Entwicklung und die Ursache von Krebs aus elektromagnetischer Sicht erklären.

Ich bin überzeugt, ass fachkundige Leserinnen und Leser in der aktuellen Ausgabe des Oncothermia Journals das finden, was sie erwarten, und diese Informationen in ihrer täglichen medizinischen Praxis nutzen können. Ich freue mich über die wachsende Aktivität und die umfangreichen Veröffentlichungen zum Thema mEHT, die die versprochenen Vorteile der Methode bestätigen und verifizieren. Ich bin dankbar für die Zusammenarbeit von Forschern und praktizierenden Onkologen, und ihre wertvollen Ergebnisse werden sehr geschätzt.

RULES OF SUBMISSION

As the editorial team we are committed to a firm and coherent editorial line and the highest possible printing standards. But it is mainly you, the author, who makes sure that the Oncothermia Journal is an interesting and diversified magazine. We want to thank every one of you who supports us in exchanging professional views and experiences. To help you and to make it easier for both of us, we prepared the following rules and guidelines for abstract submission.

Als redaktionelles Team vertreten wir eine stringente Linie und versuchen, unserer Publikation den höchst möglichen Standard zu verleihen. Es sind aber hauptsächlich Sie als Autor, der dafür Sorge trägt, dass das Oncothermia Journal zu einem interessanten und abwechslungsreichen Magazin wird. Wir möchten allen danken, die uns im Austausch professioneller Betrachtungen und Erfahrungen unterstützen. Um beiden Seiten die Arbeit zu erleichtern, haben wir die folgenden Richtlinien für die Texterstellung entworfen.

I. AIMS AND SCOPE

The Oncothermia Journal is an official journal of the Oncotherm Group, devoted to supporting those who would like to publish their results for general use. Additionally, it provides a collection of different publications and results. The Oncothermia Journal is open towards new and different contents but it should particularly contain complete study-papers, case-reports, reviews, hypotheses, opinions and all the informative materials which could be helpful for the international Oncothermia community. Advertisement connected to the topic is also welcome.

- Clinical studies: regional or local or multilocal Oncothermia or electro cancer therapy (ECT) treatments, case-reports, practical considerations in complex therapies, clinical trials, physiological effects, Oncothermia in combination with other modalities and treatment optimization

- Biological studies: mechanisms of Oncothermia, thermal- or non-temperature dependent effects, response to electric fields, bioelectromagnetic applications for tumors, Oncothermia treatment combination with other modalities, effects on normal and malignant cells and tissues, immunological effects, physiological effects, etc.

- Techniques of Oncothermia: technical development, new technical solutions, proposals

- Hypotheses, suggestions and opinions to improve Oncothermia and electro-cancer-therapy methods, intending the development of the treatments

Further information about the journal, including links to the online sample copies and content pages can be found on the website of the journal: www.oncothermia-journal.com

UMFANG UND ZIELE

Das Oncothermia Journal ist das offizielle Magazin der Oncotherm Gruppe und soll diejenigen unterstützen, die ihre Ergebnisse der Allgemeinheit zur Verfügung stellen möchten. Das Oncothermia Journal ist neuen Inhalten gegenüber offen, sollte aber vor allem Studienarbeiten, Fallstudien, Hypothesen, Meinungen und alle weiteren informativen Materialien, die für die internationale Oncothermie-Gemeinschaft hilfreich sein könnten, enthalten. Werbung mit Bezug zum Thema ist ebenfalls willkommen.

- Klinische Studien: regionale, lokale oder multilokale Oncothermie oder Electro Cancer Therapy (ECT) Behandlungen, Fallstudien, praktische Erfahrungen in komplexen Behandlungen, klinische Versuche, physiologische Effekte, Oncothermie in Kombination mit anderen Modalitäten und Behandlungsoptimierungen

- Biologische Studien: Mechanismen der Oncothermie, thermale oder temperaturunabhängige Effekte, Ansprechen auf ein elektrischesFeld, bioelektromagnetische Anwendungen bei Tumoren, Kombination von Oncothermie und anderen Modalitäten, Effekte auf normale und maligne Zellen und Gewebe, immunologische Effekte, physiologische Effekte etc.

- Oncothermie-Techniken: technische Entwicklungen, neue technische Lösungen

- Hypothesen undMeinungen, wie die Oncothermie- und ECT-Methoden verbessert werden können, um die Behandlung zu unterstützen

Weitere Informationen zum Journal sowie Links zu Online-Beispielen und Inhaltsbeschreibung sind auf der Website zu finden: www.oncothermia-journal.com

2. SUBMISSION OF MANUSCRIPTS

All submissions should be made online via email: info@oncotherm.org

MANUSKRIPTE EINREICHEN

Manuskripte können online eingereicht werden: info@oncotherm.org

3. PREPARATION OF MANUSCRIPTS

Manuscripts must be written in English, but other languages can be accepted for special reasons, if an English abstract is provided.

Texts should be submitted in a format compatible with Microsoft Word for Windows (PC). Charts and tables are considered textual and should also be submitted in a format compatible with Word. All figures (illustrations, diagrams, photographs) should be provided in JPG format.

Manuscripts may be any length, but must include:

- Title Page: title of the paper, authors and their affiliations, 1-5 keywords, at least one corresponding author should be listed, email address and full contact information must be provided

- Abstracts: Abstracts should include the purpose, materials, methods, results and conclusions.

- Test: unlimited volume

- Tables and Figures: Tables and figures should be referred to in the text (numbered figures and tables). Each table and/or figure must have a legend that explains its purpose without a reference to the text. Figure files will ideally be submitted as a jpg-file (300dpi for photos).

- References: Oncothermia Journal uses the Vancouver (Author-Number) system to indicate references in the text, tables and legends, e.g. [1], [1-3]. The full references should be listed numerically in order of appearance and presented following the text of the manuscript.

MANUSKRIPTE VORBEREITEN

Manuskripte müssen in englischer Sprache vorliegen. Andere Sprachen können in Ausnahmefällen akzeptiert werden, wenn ein englisches Abstract vorliegt.

Texte sollten in einem mit Microsoft Word für Windows (PC) kompatiblen Format eingereicht werden. Tabellen sollten in einem Word-kompatiblen Format eingefügt werden. Alle Graphiken (Illustrationen, Diagramme, Photographien) sollten im jpg Format vorliegen.

Manuskripte können jede Länge haben, müssen aber die folgenden Punkte erfüllen:

- Titelseite: Titel der Arbeit, Autor, Klinikzugehörigkeit, 1-5 Schlüsselworte, mindestens ein Autor muss genannt werden, E-Mail-Adresse und Kontaktdetails des Autors

- Abstracts: Abstracts müssen Zielsetzung, Material und Methoden, Ergebnisse und Fazit enthalten.

- Text: beliebige Länge

- Abbildungen und Tabellen: Abbildungen und Tabellen sollten im Text erläutert werden (nummeriert). Jede Abbildung / Tabelle muss eine erklärende Bildunterschrift haben. Bilder sollten als jpg eingereicht werden (300 dpi).
- Zitate: Das Oncothermia Journal verwendet die Vancouver Methode (Autornummer), um Zitate auszuweisen, z.B. [1], [1-3]. Die Bibliographie erfolgt numerisch in Reihenfolge der Erwähnung im Text.

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Das Oncothermia Journal akzeptiert Werbeanzeigen in allen Sprachen, bevorzugt, aber die zumindest teilweise Gestaltung in englischer Sprache. Die Werbung muss eine Beziehung zu den Themen des Oncothermia Journals haben und der Wahrheit entsprechende Inhalte aufweisen.

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Authors of any publications in the Oncothermia Journal are fully responsible for the material which is published. The Oncothermia Journal has no responsibility for legal conflicts due to any publications. The editorial board has the right to reject any publication if its validity has not been verified enough or the board is not convinced by the authors.

HAFTUNG

Die Autoren aller im Oncothermia Journal veröffentlichten Artikel sind in vollem Umfang für ihre Texte verantwortlich. Das Oncothermia Journal übernimmt keinerlei Haftung für die Artikel der Autoren. Die Redaktion hat das Recht Artikel abzulehnen.

9. REVIEWING

The Oncothermia Journal has a special peer-reviewing process, represented by the editorial board members and specialists, to whom they are connected. To avoid personal conflicts the opinion of the reviewer will not be released and her/his name will be handled confidentially. Papers which are not connected to the topics of the journal could be rejected without reviewing.

BEWERTUNG

Die Texte für das Oncothermia Journal werden durch die Redaktion kontrolliert. Um Konflikte zu vermeiden, werden die Namen des jeweiligen Korrektors nicht öffentlich genannt. Artikel, die nicht zu den Themen des Journals passen, können abgelehnt werden

CONTENT

Modulated electro-hyperthermia added to chemoradiotherapy improves five-year survival: final results of a phase III randomised controlled trial - ESHO 2023 presentation

Carrie Anne Minnaar [1], Jeffrey Allan Kotzen [2]

[1] Radiation Sciences, University of the Witwatersrand, Johannesburg, South Africa
[2] Radiation Oncology, Wits Donald Gordon Academic Hospital, Johannesburg, South Africa

Modulated electro-hyperthermia added to chemoradiotherapy improves five-year survival: final results of a phase III randomised controlled trial

Minnaar CA[1,2], Kotzen JA[1,2]

[1]Radiation Sciences, University of the Witwatersrand, Johannesburg, South Africa;
[2] Radiation Oncology, Wits Donald Gordon Academic Hospital, Johannesburg, South Africa;

Wits University Donald Gordon Medical Centre
Patient-centred. Independent. Academic.

MEDICLINIC

ESHO
35th Annual Meeting
European Society for Hyperthermic Oncology

INTRODUCTION

Trial Protocols developed in 2013:

Modulated electro-hyperthermia (mEHT):

- **Mild**, capacitive-coupled heating technology

- **Amplitude modulation** enhance the cell-killing effects

Simple to use and affordable

Immune-modulating effects

> Ethics approval: M190295
> National Clinical Trials Register ID:3012
> ClincialTrials.gov ID: NCT03332069

Therefore used to investigate the radiosensitising effects in out HIV-positive and –negative patients in a resource constricted environment

METHODOLOGY

- **210 participants** randomized to receive CRT +/- mEHT
  - Stratum: HIV status, stage and age

- **HIV-positive** participants (CD4>200 / on ART> 6 months)

- **FIGO Stage IIB-IIIB** (staged clinically)

- **PET/CT** pre- and 6/12 post-RT for disease response

CRT	mEHT
- 50Gy EBRT in 25# **- 3 x 8Gy** HDR BT **- 80mg/m^2 Cisplatin 21 days apart**	- 2/wk immediately before EBRT **- 60 minutes at 130W**

RESULTS

Table 2. Participant characteristics.

Participant Characteristic		mEHT 106 (50.5%)		Control 104 (49.5%)		p-Value
HIV Status	Positive	52	(49.1%)	55	(52.9%)	p = 0.579
	Negative	54	(50.9%)	49	(47.1%)	
Age Group	<50 years	52	(49.1%)	46	(44.2%)	p = 0.483
	≥50 years	54	(50.9%)	58	(55.8%)	
ECOG	0	3	(2.8%)	7	(6.7%)	p = 0.184
	1	103	(97.2%)	97	(93.3%)	
Race	African	98	(92.5%)	97	(93.3%)	
	Caucasian	4	(3.8%)	1	(1.0%)	
	Indian	0	(0.0%)	0	(0.0%)	p = 0.335
	Asian	0	(0.0%)	0	(0.0%)	
	Mixed Race	4	(3.8%)	6	(5.8%)	
Education	Primary	45	(43.3%)	50	(49.0%)	
	Secondary	55	(52.9%)	51	(50.0%)	p = 0.334
	Tertiary	4	(3.8%)	1	(1.0%)	
Employment	Unemployed	83	(78.3%)	82	(78.8%)	p = 0.923
	Employed	23	(21.7%)	22	(21.2%)	
FIGO Staging	IIB	40	(37.7%)	36	(34.6%)	p = 0.895
	IIIA	1	(0.9%)	1	(1.0%)	
	IIIB	65	(61.3%)	67	(64.4%)	
Histological Grade	1	7	(6.9%)	4	(4.1%)	
	2	70	(69.3%)	67	(69.1%)	p = 0.759
	3	24	(23.8%)	26	(26.8%)	
Tumour Dimensions (cm)	Median	7		7.1		
	Min	2.7		1.8		p = 0.1429
	Max	11.7		14.87		
Tumour SUV	Median	18.07		19.26		
	Min	7.01		6.07		p = 0.7769
	Max	63.25		97		
HB (g/dL)	Median	10.9		11		
	Min	5.7		5.2		p = 0.9424
	Max	16.2		16.2		
Age	Median	49.2		50.6		
	Min	27.3		29.2		p = 0.3665
	Max	70.8		74.8		
BMI	Median	27		26.5		
	Min	15		15		p = 0.3883
	Max	49		41.7		

Abbreviations: BMI: Body Mass Index; ECOG: Eastern Cooperative Oncology Group; FIGO: Fédération Internationale de Gynécologie et d'Obstétrique; HB: Haemoglobin; HIV: Human Immunodeficiency Virus; mEHT: Modulated Electro-Hyperthermia; SUV: Standard Uptake Value.

Table 3. Treatment characteristics.

Treatment Characteristics		mEHT 106 (50.5%)		Control 104 (49.5%)		p-Value
No of HDR BT doses	0	0	(0.0%)	0	(0.0%)	
	1	0	(0.0%)	2	(2.0%)	p = 0.223
	2	3	(2.9%)	1	(1.0%)	
	3	101	(97.1%)	99	(97.1%)	
No of Cisplatin Doses	0	14	(13.6%)	11	(10.7%)	
	1	42	(40.8%)	47	(45.6%)	p = 0.727
	2	47	(45.6%)	45	(43.7%)	
Total RT Dose	Median	74		74		
	Min	20		2		p = 0.6133
	Max	74		74		
Days between enrolment and Treatment	Median	37		37		
	Min	18		21		p = 0.2241
	Max	79		104		
No of mEHT doses	Median	10				
	Min	1				
	Max	10				

Abbreviations: HDR BT: High Dose Rate Brachytherapy; HIV: Human Immunodeficiency Virus; mEHT: Modulated Electro-Hyperthermia; RT: Radiotherapy.

PLOS ONE

🔓 OPEN ACCESS 🔓 PEER-REVIEWED

RESEARCH ARTICLE

The effect of modulated electro-hyperthermia on local disease control in HIV-positive and -negative cervical cancer women in South Africa: Early results from a phase III randomised controlled trial

Carrie Anne Minnaar, Jeffrey Allan Kotzen, Olusegun Akinwale Ayeni, Thanushree Naidoo, Mariza Tunmer, Vinay Sharma, Mboyo-Di-Tamba Vangu, Ans Baeyens

Published: June 19, 2019 • https://doi.org/10.1371/journal.pone.0217894

SAFETY AND TOXICITY

- No dose-limiting toxicities
- High Compliance (97% completed ≥8 of 10 treatments)
- No sig. differences in CRT-related toxicity between groups

mEHT Toxicity:
    grade 1–2 adipose burns: 9.5%
    grade 1 surface burns: 2%
    pain during mEHT: 8.6%

> Significant improvement in QoL at 3 and 6 months post-RT in mEHT group

Analysis of the effects of mEHT on the treatment-related toxicity and quality of life of HIV-positive cervical cancer patients

Carrie Anne Minnaar[a], Jeffrey Allan Kotzen[b], Thanushree Naidoo[c], Mariza Tunmer[a,b], Vinay Sharma[a,d], Mboyo-Di-Tamba Vangu[e,f] and Ans Baeyens[a,g]

LOCAL DISEASE CONTROL

210 Randomised Participants	Control		mEHT		Chi Squared
	n	%	n	%	
LDC achieved at 6 months	20	24.1%	40	45.5%	*p = 0.003*
LDFS at six months	20	19.8%	39	38.6%	*p = 0.003*

THREE YEAR SURVIVAL

Disease recurrence at 2 and 3 years was
significantly reduced by 25% with mEHT

KM:3yr Disease Free Survival

mEHT: 35/99   **[35.4%]**
Control: 14/102   **[13.7%]**

OR: 3.4, 95%CI:1.71–6.91, *p=0.001*
HR:0.70, 95%CI:0.51–0.98, *p=0.035*
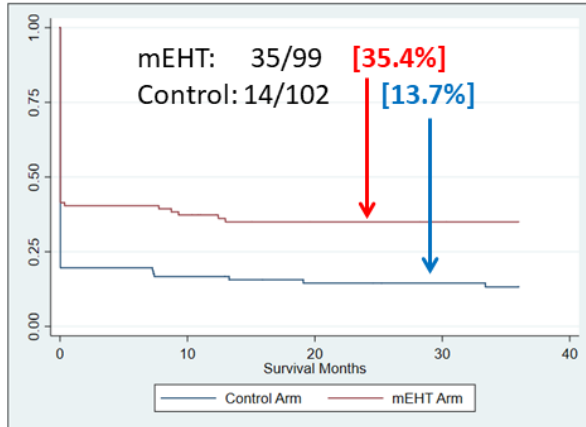
3yr DFS doubled by mEHT

QoL at 2 yrs significantly higher in mEHT group

There were no significant differences in late toxicity between the groups.

cancers MDPI
Article
Effects of Modulated Electro-Hyperthermia (mEHT) on Two and Three Year Survival of Locally Advanced Cervical Cancer Patients
Carrie Anne Minnaar, Innocent Maposa, Jeffrey Allan Kotzen and Ans Baeyens

COST EFFECTIVENESS ANALYSIS

Clinical and Cost benefit to the addition of mEHT to CRT

Probability of 78% and 82% in private and government facilities

mEHT+CRT Dominated the Markov model

FIVE YEAR SURVIVAL

Overall 5year Survival

■ mEHT

All participants: 33% (mEHT), 26%
Stage III disease: 34% ** (mEHT), 23%

HR:0.74; 95%CI: 0.53-1.03; p=0.083

HR:0.65; 95%CI: 0.43-0.99; **p=0.046**

FIVE YEAR SURVIVAL

5 year DFS

mEHT: 32%** 32/99
Control: 14%** 14/102
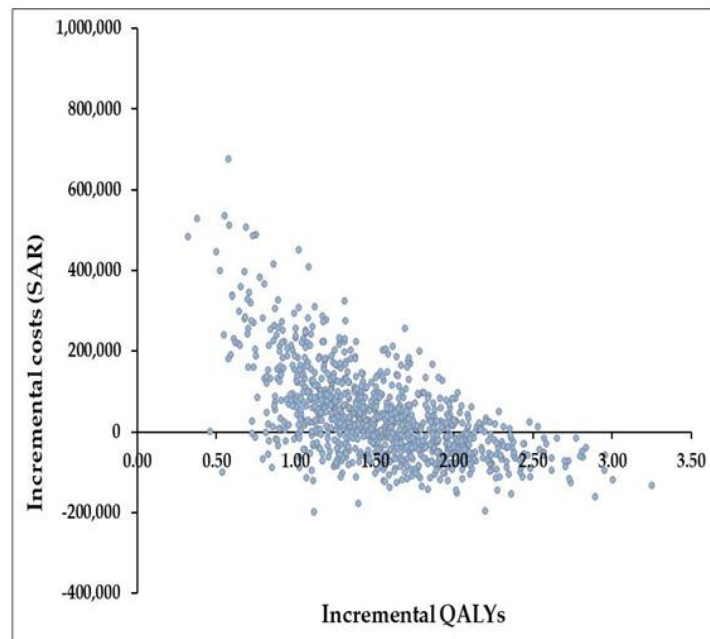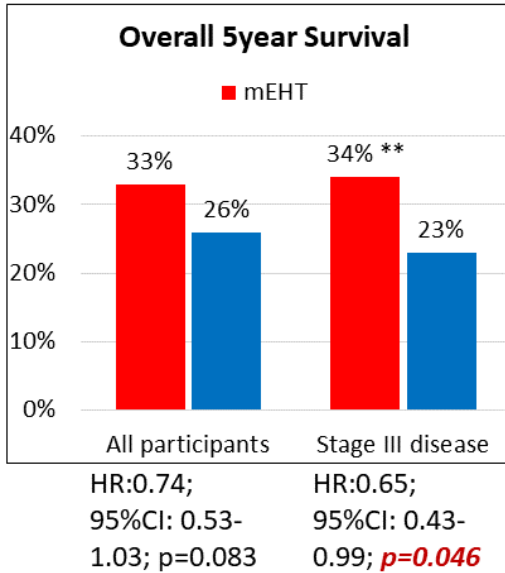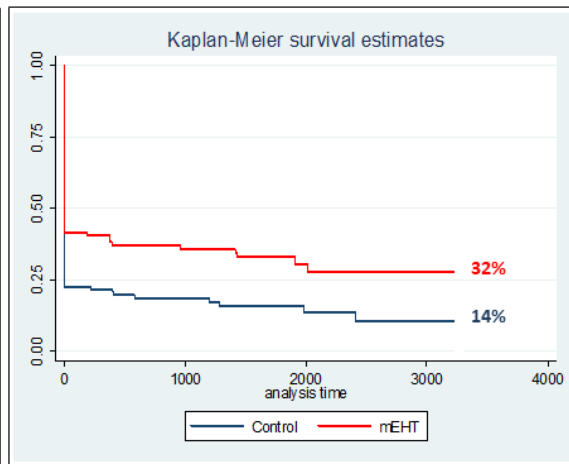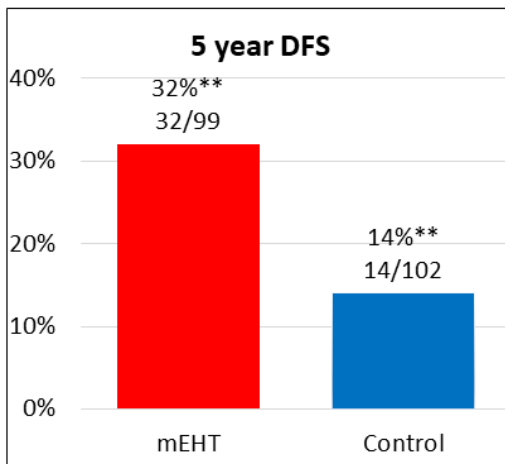
Chi-squared: *p=0.002*
OR:3.00; 95%CI:1.49-6.07; *p=0.002*

Kaplan-Meier survival estimates

HR:0.73; 95%CI:0.53-1.00; *p=0.049*

There were no significant differences in late toxicity between the groups.

ABSCOPAL EFFECT

54 participants in each group had extra-pelvic disease pre-treatment

CMR of all disease at 6 months:
mEHT: 24.1%
Control: 5.6%
Chi-squared *p=0.013*

CMR of all disease, extra-pelvic and pelvic
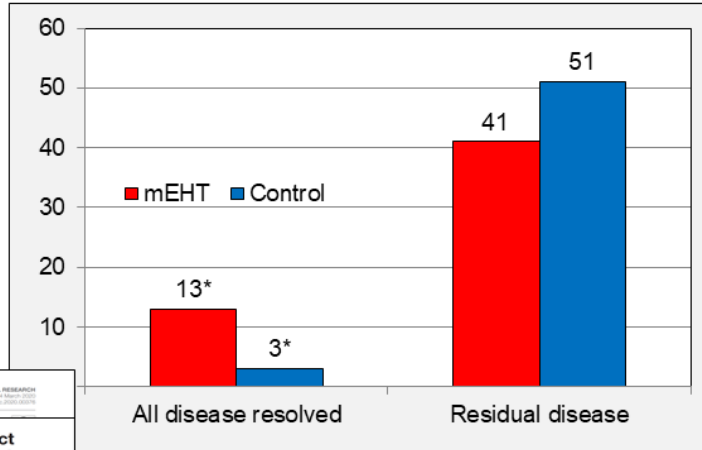
ORIGINAL RESEARCH

Potentiation of the Abscopal Effect by Modulated Electro-Hyperthermia in Locally Advanced Cervical Cancer Patients

Carrie Anne Minnaar, Jeffrey Allan Kotzen, Olusegun Akinwale Ayeni, Mboyo-Di-Tamba Vangu and Ans Baeyens

ABSCOPAL EFFECT

Systemic Control – using the ABSCOPAL effect

The abscopal effect was not associated with:

- HIV status
- No. of cisplatin Doses
- Disease Stage
- Age

**85% Remained alive and DF at 5 years
2/13 died of non-disease related causes**

Abscopal effect and HIV status
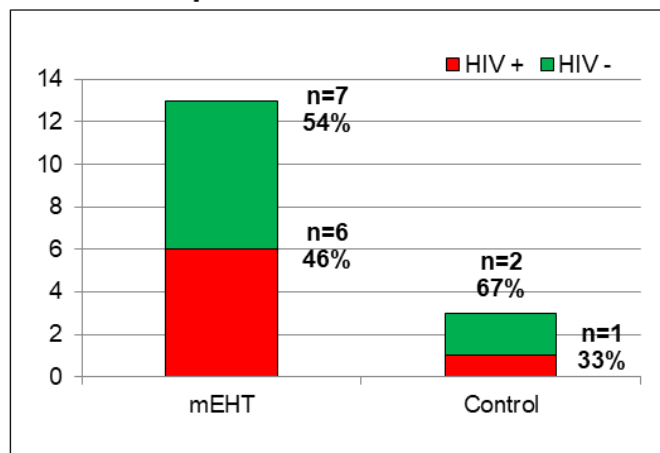
mEHT Group: 13 out of 54 [24.1%]
Control Group: 3 out of 54 [5.6%]
(p=0.013)

IMMUNE RESPONSE TRIGGERED BY MEHT
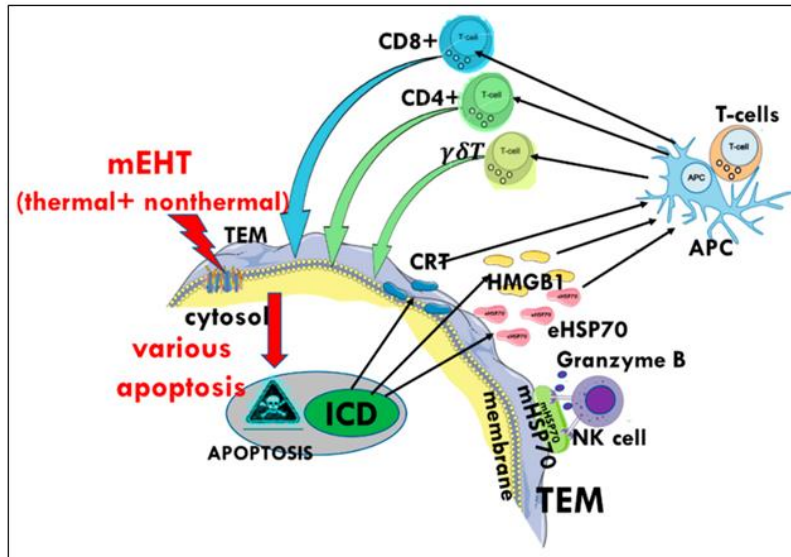
mEHT associated apoptosis = apoptotic bodies

→ release of mHSPs
→ activate NK cells
→ ICD and DAMP
= maturation of DCs into APCs
→ triggers T-cells

Potential for adaptive immune response

Potentiates the abscopal effect: Immune mediated response to RT resulting in resolution of lesions outside the treatment field

Minnaar CA, Szasz A.. Cells. 2022 Jun 4;11(11):1838. doi: 10.3390/cells11111838. PMID: 35681533;

CONCLUSION

mEHT + CRT for the management of LACC:

-Safe

-Improves QoL

-Improves LDC

-mEHT **improves 5 year DFS**

-**SYSTEMIC EFFECTS** – abscopal

-*Lowers treatment costs, without increasing toxicity* in LACC patients, even in resource-constrained settings.

FUTURE PERSPECTIVES

Combining mEHT with immunotherapy

Phase I/II paediatric brainstem glioma study

A larger phase III trial on adult GBM tumours managed with radiotherapy combined with mEHT

ACKNOWLEDGMENTS

THANK YOU

Multiphase combined treatment for adults with GBM, including individualized multimodal immunotherapy: Single institute real world medical data in the light of clinical trial research data - ESHO 2023 presentation

Stefaan W. Van Gool, Peter Van de Vliet, Linde Kampers, Jennifer Kosmal, Tobias Sprenger, Volker Schirrmacher, Wilfried Stücker

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Multiphase combined treatment for adults with GBM, including individualized multimodal immunotherapy:

Single institute real world medical data in the light of clinical trial research data

Stefaan W. Van Gool, Peter Van de Vliet, Linde Kampers, Jennifer Kosmal, Tobias Sprenger, Volker Schirrmacher, Wilfried Stücker

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cancers — MDPI

Article

Individualized Multimodal Immunotherapy for Adults with IDH1 Wild-Type GBM: A Single Institute Experience

Stefaan W. Van Gool *, Jennifer Makalowski, Peter Van de Vliet, Stefanie Van Gool, Tobias Sprenger, Volker Schirrmacher and Wilfried Stuecker

biomedicines — MDPI

Viewpoint

The Application of Evidence-Based Medicine in Individualized Medicine

Peter Van de Vliet *, Tobias Sprenger, Linde F. C. Kampers, Jennifer Makalowski, Volker Schirrmacher, Wilfried Stücker and Stefaan W. Van Gool

Editorial Commentary — Translational Cancer Research, 2023

Dendritic cell vaccination for glioblastoma multiforme patients: has a new milestone been reached?

Stefaan W. Van Gool^, Jennifer Makalowski, Linde F. C. Kampers, Peter Van de Vliet, Tobias Sprenger, Volker Schirrmacher, Wilfried Stücker

[Instruction: Revises AQ: Kindly note we have followed US spelling for this series. Hence, the correction "Towards" has not been followed in chapter title and retained the word "Toward" as is. Please check and confirm is this fine.]Methods behind oncolytic virus-based DC vaccines in cancer: Towards a multiphase combined treatment strategy for Glioblastoma (GBM) patients

Stefaan W. Van Gool, vangool@iozk.de, Peter Van de Vliet, Linde F. C. Kampers, Jennifer Makalowski/Kosmal, Tobias Sprenger, Ella Roeh, Volker Schirrmacher, Wilfried Stuecker

IOZK, Cologne, Germany/Immun-onkologisches Zentrum Köln, Cologne, Germany

Methods in Cell Biology, in press

Treatment phase 3:
Maintenance and Expansion of Immune protection

Treatment phase 2:
Immunization

Treatment phase 1:
Anticancer treatment

Towards Control of GBM ?

Immunomodulatory strategies

Active specific immunotherapy with vaccines

Treatment-induced anticancer immunization
Direct anti-cancer activity

(Surgery)

Radiotherapy

Chemotherapy

Targeted therapy
Adoptive immunotherapy
Passive immunotherapy

Immunogenic cell death (ICD)
Immunotherapy
(Biologic / physics Therapy)

Tumor and host and their interaction are dynamic processes

Complementary medicines, Repurposed drugs
Real World Data have their own scientific and clinical value

Nobel Prize Medicine 2018
J. P. Allison, T. Honjo

Nobel Prize Medicine 2011
B. Beutler, J. Hoffmann, R. Steinman

Smart combination

Individualization

1. Molecular biology
2. Tumor antigens
3. Tumor-host interaction
4. Immune system / inflammation
5. Treatment combination
6. Response to treatment

Treatment adaptation

Treatment phase 1:
Anticancer treatment

THE NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Radiotherapy plus Concomitant and Adjuvant Temozolomide for Glioblastoma

Roger Stupp, M.D., Warren P. Mason, M.D., Martin J. van den Bent, M.D., Michael Weller, M.D., Barbara Fisher, M.D., Martin J.B. Taphoorn, M.D., Karl Belanger, M.D., Alba A. Brandes, M.D., Christine Marosi, M.D., Ulrich Bogdahn, M.D., Jürgen Curschmann, M.D., Robert C. Janzer, M.D., Samuel K. Ludwin, M.D., Thierry Gorlia, M.Sc., Anouk Allgeier, Ph.D., Denis Lacombe, M.D., J. Gregory Cairncross, M.D., Elizabeth Eisenhauer, M.D., and René O. Mirimanoff, M.D., for the European Organisation for Research and Treatment of Cancer Brain Tumor and Radiotherapy Groups and the National Cancer Institute of Canada Clinical Trials Group*

Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomised phase III study: 5-year analysis of the EORTC-NCIC trial   Lancet Oncol, 2009

Roger Stupp, Monika E Hegi, Warren P Mason, Martin J van den Bent, Martin J B Taphoorn, Robert C Janzer, Samuel K Ludwin, Anouk Allgeier, Barbara Fisher, Karl Belanger, Peter Hau, Alba A Brandes, Johanna Gijtenbeek, Christine Marosi, Charles J Vecht, Karima Mokhtari, Pieter Wesseling, Salvador Villa, Elizabeth Eisenhauer, Thierry Gorlia, Michael Weller, Denis Lacombe, J Gregory Cairncross, René-Olivier Mirimanoff, on behalf of the European Organisation for Research and Treatment of Cancer Brain Tumour and Radiation Oncology Groups and the National Cancer Institute of Canada Clinical Trials Group

Dose-Dense Temozolomide for Newly Diagnosed Glioblastoma: A Randomized Phase III Clinical Trial

Mark R. Gilbert, Meihua Wang, Kenneth D. Aldape, Roger Stupp, Monika E. Hegi, Kurt A. Jaeckle, Terri S. Armstrong, Jeffrey S. Wefel, Minhee Won, Deborah T. Blumenthal, Anita Mahajan, Christopher J. Schultz, Sara Erridge, Brigitta Baumert, Kristen I. Hopkins, Tzahala Tzuk-Shina, Paul D. Brown, Arnab Chakravarti, Walter J. Curran Jr, and Minesh P. Mehta   JCO, 2013

A Randomized Trial of Bevacizumab for Newly Diagnosed Glioblastoma

Mark R. Gilbert, M.D., James J. Dignam, Ph.D., Terri S. Armstrong, Ph.D., A.N.P.-B.C., Jeffrey S. Wefel, Ph.D., Deborah T. Blumenthal, M.D., Michael A. Vogelbaum, M.D., Ph.D., Howard Colman, M.D., Ph.D., Arnab Chakravarti, M.D., Stephanie Pugh, Ph.D., Minhee Won, M. A., Robert Jeraj, Ph.D., Paul D. Brown, M.D., Kurt A. Jaeckle, M.D., David Schiff, M.D., Volker W. Stieber, M.D., David G. Brachman, M.D., Maria Werner-Wasik, M.D., Ivo W. Tremont-Lukats, M.D., Erik P. Sulman, M.D., Kenneth D. Aldape, M.D., Walter J. Curran, Jr., M.D., and Minesh P. Mehta, M.D.   NEJM, 2014

Preliminary Communication

Maintenance Therapy With Tumor-Treating Fields Plus Temozolomide vs Temozolomide Alone for Glioblastoma
A Randomized Clinical Trial   JAMA, 2017

Roger Stupp, MD; Sophie Taillibert, MD; Andrew A. Kanner, MD, PhD; David M. Steinberg, PhD; Steven A. Toms, MD, FACS, MPH; Lynne P. Taylor, MD, FAAN; Frank Lieberman, MD; Antonio Silvani, MD; Karen L. Fink, MD, PhD; Gene H. Barnett, MD, MBA; Jay-Jiguang Zhu, MD, PhD; John W. Henson, MD, MBA, FAAN; Herbert H. Engelhard, MD, PhD; Thomas C. Chen, MD, PhD; David D. Tran, MD, PhD; Jan Sroubek, MD; Nam D. Tran, MD, PhD; Andreas F. Hottinger, MD, PhD; Joseph Landolfi, DO; Rajiv Desai, MD; Manuela Caroli, MD; Yvonne Kew, MD, PhD; Jerome Honnorat, MD, PhD; Ahmed Idbaih, MD, PhD; Elion D. Kirson, MD, PhD; Uri Weinberg, MD, PhD; Yoram Palti, MD, PhD; Monika E. Hegi, PhD; Zvi Ram, MD

Rindopepimut with temozolomide for patients with newly diagnosed, EGFRvIII-expressing glioblastoma (ACT IV): a randomised, double-blind, international phase 3 trial

Michael Weller, Nicholas Butowski, David D Tran, Lawrence D Recht, Michael Lim, Hal Hirte, Lynn Ashby, Laszlo Mechtler, Samuel A Goldlust, Fabio Iwamoto, Jan Drappatz, Donald M O'Rourke, Mark Wong, Mark G Hamilton, Gaetano Finocchiaro, James Perry, Wolfgang Wick, Jennifer Green, Yi He, Christopher D Turner, Michael J Yellin, Tibor Keler, Thomas A Davis, Roger Stupp, and John H Sampson, for the ACT IV trial investigators*   Lancet Oncol, 2017

ECA

A Randomized Double-Blind Placebo-Controlled Phase II Trial of Dendritic Cell Vaccine ICT-107 in Newly Diagnosed Patients with Glioblastoma

Patrick Y. Wen[1], David A. Reardon[1], Terri S. Armstrong[2], Surasak Phuphanich[3], Robert D. Aiken[4], Joseph C. Landolfi[5], William T. Curry[6], Jay-Jiguang Zhu[7], Michael Glantz[8], David M. Peereboom[9], James M. Markert[10], Renato LaRocca[11], Donald M. O'Rourke[12], Karen Fink[13], Lyndon Kim[14], Michael Gruber[15], Glenn J. Lesser[16], Edward Pan[17], Santosh Kesari[18], Alona Muzikansky[19], Clemencia Pinilla[20], Radleigh G. Santos[20], and John S. Yu[21,22,23]   CCR, 2019

JAMA Oncology | Original Investigation

Association of Autologous Tumor Lysate-Loaded Dendritic Cell Vaccination With Extension of Survival Among Patients With Newly Diagnosed and Recurrent Glioblastoma
A Phase 3 Prospective Externally Controlled Cohort Trial   JAMA Oncol, 2023

Phase III

Linda M. Liau, MD, PhD; Keyoumars Ashkan, MD, FRCP, FRCS; Steven Brem, MD; Jian L. Campian, MD, PhD; John E. Trusheim, MD; Fabio M. Iwamoto, MD; David D. Tran, MD, PhD; George Ansstas, MD; Charles S. Cobbs, MD; Jason A. Heth, MD; Michael E. Salacz, MD; Stacy D'Andre, MD; Robert D. Aiken, MD; Yaron A. Moshel, MD, PhD; Joo Y. Nam, MD; Clement P. Pillainayagam, MD; Stephanie A. Wagner, MD; Kevin A. Walter, MD; Rekha Chaudary, MD; Samuel A. Goldlust, MD; Ian Y. Lee, MD; Daniela A. Bota, MD, PhD; Heinrich Elinzano, MD; Jai Grewal, MD; Kevin Lillehei, MD; Tom Mikkelsen, MD, FRCPC; Tobias Walbert, MD; Steven Abram, MD; Andrew J. Brenner, MD, PhD; Matthew G. Ewend, MD; Simon Khagi, MD; Darren S. Lovick, MD; Jana Portnow, MD; Lyndon Kim, MD; William G. Loudon, MD; Nina L. Martinez, MD; Reid C. Thompson, MD; David E. Avigan, MD; Karen L. Fink, MD, PhD; Francois J. Geoffroy, MD; Pierre Giglio, MD; Oleg Gligich, MD; Dietmar Krex, MD; Scott M. Lindhorst, MD; Jose Lutzky, MD; Hans-Jörg Meisel, MD, PhD; Minou Nadji-Ohl, MD; Lhagva Sanchin, MD; Andrew Sloan, MD; Lynne P. Taylor, MD; Julian K. Wu, MD; Erin M. Dunbar, MD; Arnold B. Etame, MD, PhD; Santosh Kesari, MD, PhD; David Mathieu, MD; David E. Piccioni, MD, PhD; David S. Baskin, MD; Michel Lacroix, MD; Sven-Axel May, MD; Pamela Z. New, MD; Timothy J. Pluard, MD; Steven A. Toms, MD; Victor Tse, MD; Scott Peak, MD; John L. Villano, MD, PhD; James D. Battiste, MD, PhD; Paul J. Mulholland, MD; Michael L. Pearlman, MD; Kevin Petrecca, MD, PhD; Michael Schulder, MD; Robert M. Prins, PhD; Alton L. Boynton, PhD; Marnix L. Bosch, PhD

Treatment phase 1: Anticancer treatment

frontiers in Oncology

PREVIEW
published: 17 June 2770
doi: 10.3389/fonc.2015.00060

Brain tumor immunotherapy: what have we learned so far?

Stefaan Willy Van Gool *

ANTICANCER RESEARCH 39: 2043-2051 (2019)
doi:10.21873/anticanres.13315

Immune Phenotype Correlates With Survival in Patients With GBM Treated With Standard Temozolomide-based Therapy and Immunotherapy

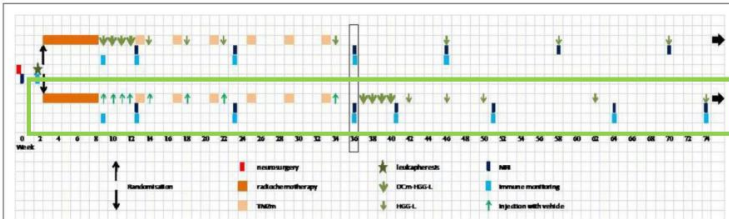MARKOS ANTONOPOULOS[1], STEFAAN W. VAN GOOL[2], DIMITRA DIONYSIOU[1], NORBERT GRAF[3] and GEORGIOS STAMATAKOS[1]
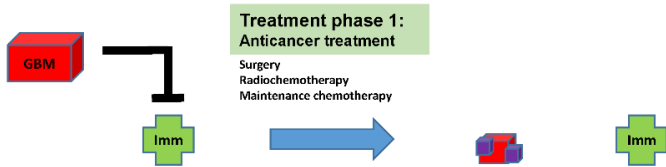
Table I. *Overall survival (OS) data of the total study population and subgroups residual tumor volume (RTV).*

Patient group	No. of patients	Median OS (months)	2-Year OS rate (%)	95%CI
Total group	101	19	33.66	24.66-42.88
Early vaccination, RTV=0	19	22	40.2	18.4-61.2
Late vaccination, RTV=0	29	23	44.8	26.5-61.5
Early vaccination, RTV>0	28	19	25	11-41.7
Late vaccination, RTV>0	25	16	28	12.4-46

FIGURE 5 | Outline of the phase IIb randomized clinical trial HGG-2010.

Neuro-Oncology

240

23(2), 240–250, 2021 | doi:10.1093/neuonc/noaa247 | Advance Access date 1 November 2020

DNA methylation based glioblastoma subclassification is related to tumoral T-cell infiltration and patient survival

Joost Dejaegher*, Lien Solie, Zoé Hunin, Raf Sciot, David Capper, Christin Siewert, Sofie Van Cauter, Guido Wilms, Johan van Loon, Nadine Ectors, Steffen Fieuws, Stefan M. Pfister, Stefaan W. Van Gool, and Steven De Vleeschouwer

GBM

Treatment phase 1: Anticancer treatment

Surgery
Radiochemotherapy
Maintenance chemotherapy

Imm

Imm

Cellular immunity of patients with malignant glioma: prerequisites for dendritic cell vaccination immunotherapy

J Neurosurg, 2006

Marion Rapp *, Zakir Ozcan, Hans-Jakob Steiger, Peter Wernet, Michael C Sabel, Rüdiger V Sorg

Tumor Microenvironment and Immune Escape in the Time Course of Glioblastoma

Mol Neurobiol, 2022

Assunta Virtuoso[1,2] · Ciro De Luca[1] · Giovanni Cirillo[1] · Matteo Riva[3,4] · Gabriele Romano[5] · Angela Bentivegna[2] · Marialuisa Lavitrano[2] · Michele Papa[1,6] · Roberto Giovannoni[7]

Impact of Radiochemotherapy on Immune Cell Subtypes in High-Grade Glioma Patients

Front Oncol, 2020

Valérie Dutoit[1,2]*, Géraldine Philippin[1,2], Valérie Widmer[1,2], Eliana Marinari[1,2], Aurélie Vuilleumier[3], Denis Migliorini[1,2], Karl Schaller[4] and Pierre-Yves Dietrich[1,2,3]

Malignant Gliomas as Second Neoplasms in Pediatric Cancer Survivors: Neuropathological Study

BioMed Res Int, 2018

Ewa Izycka-Swieszewska[1], Ewa Bien[2], Joanna Stefanowicz[2], Edyta Szurowska[3], Ewa Szutowicz-Zielinska[4], Magdalena Koczkowska[5], Dawid Sigorski[6], Wojciech Kloc[7,8], Wojciech Rogowski[9], and Elzbieta Adamkiewicz-Drozynska[2]

Neuro-Oncology Advances

4(1), 1–14, 2022 | https://doi.org/10.1093/noajnl/vdac076 | Advance Access date 23 May 2022

Neuro-Oncol Adv, 2022

Detection of temozolomide-induced hypermutation and response to PD-1 checkpoint inhibitor in recurrent glioblastoma

Paul Daniel, Brian Meehan, Siham Sabri, Fatemeh Jamali, Jann N. Sarkaria*, Dongsic Choi, Delphine Garnier, Gaspar Kitange, Kate I. Glennon, Antoine Paccard, Jason Karamchandani, Yasser Riazalhosseini, Janusz Rak[1], and Bassam Abdulkarim[1]

Treatment phase 1:
Anticancer treatment

Surgery
Radiochemotherapy
Maintenance chemotherapy
ICD Immunotherapy

Imm

Imm

JITC, 2020

Consensus guidelines for the definition, detection and interpretation of immunogenic cell death

Lorenzo Galluzzi, Ilio Vitale, Sarah Warren, Sandy Adjemian, Patrizia Agostinis, Aitziber Buqué Martinez, Timothy A Chan, George Coukos, Sandra Demaria, Eric Deutsch, Dobrin Draganov, Richard L Edelson, Silvia C Formenti, Jitka Fucikova, Lucia Gabriele, Udo S Gaipl, Sofia R Gameiro, Abhishek D Garg, Encouse Golden, Jian Han, Kevin J Harrington, Akseli Hemminki, James W Hodge, Dewan Md Sakib Hossain, Tim Illidge, Michael Karin, Howard L Kaufman, Oliver Kepp, Guido Kroemer, Juan Jose Lasarte, Sherene Loi, Michael T Lotze, Gwenola Manic, Taha Merghoub, Alan A Melcher, Karen L Mossman, Felipe Prosper, Øystein Rekdal, Maria Rescigno, Chiara Riganti, Antonella Sistigu, Mark J Smyth, Radek Spisek, John Stagg, Bryan E Strauss, Daolin Tang, Kazuki Tatsuno, Stefaan W van Gool, Peter Vandenabeele, Takahiro Yamazaki, Dmitriy Zamarin, Laurence Zitvogel, Alessandra Cesano, Francesco M Marincola

J Neurooncol (2010) 98:395–405
DOI 10.1007/s11060-009-0093-0

CLINICAL STUDY - PATIENT STUDY

Transcranial electro-hyperthermia combined with alkylating chemotherapy in patients with relapsed high-grade gliomas: phase I clinical results

Caecilia Wismeth · Christine Dudel · Christina Pascher · Paul Ramm · Torsten Pietsch · Birgit Hirschmann · Christiane Reinert · Martin Proescholdt · Petra Rümmele · Gerhard Schuierer · Ulrich Bogdahn · Peter Hau

Received: 7 November 2017
Accepted: 9 July 2018
Published online: 31 July 2018

OPEN **Newcastle disease virus enhances the growth-inhibiting and proapoptotic effects of temozolomide on glioblastoma cells in vitro and in vivo**

Yang Bai, Yong Chen, Xinyu Hong, Xinrui Liu, Xing Su, Shanji Li, Xuechao Dong, Gang Zhao & Yunqian Li

---

Treatment phase 1:
Anticancer treatment

Surgery
Radiochemotherapy
Maintenance chemotherapy
ICD Immunotherapy

Treatment phase 2:
Immunization

Active specific Immunotherapy
Modulatory immunotherapy

Imm

Imm

Imm

OPEN ACCESS Freely available online   PLOS ONE

1 **Clinical Efficacy of Tumor Antigen-Pulsed DC Treatment for High-Grade Glioma Patients: Evidence from a Meta-Analysis** PLOS-One, 2014

Jun-Xia Cao, Xiao-Yan Zhang, Jin-Long Liu, Duo Li, Jun-Li Li, Yi-Shan Liu, Min Wang, Bei-Lei Xu, Hai-Bo Wang, Zheng-Xu Wang

3 Therapeutics and Clinical Risk Management   Dovepress
open access to scientific and medical research

REVIEW

Dendritic cell vaccines for high-grade gliomas

This article was published in the following Dove Press journal:
Therapeutics and Clinical Risk Management   Eagles ME, et al, 2018

5 CLINICAL CANCER RESEARCH | PERSPECTIVES

Once, Twice, Three Times a Finding: Reproducibility of Dendritic Cell Vaccine Trials Targeting Cytomegalovirus in Glioblastoma CCR, 2020

Kristen A. Batich, Duane A. Mitchell, Patrick Healy, James E. Herndon II, and John H. Sampson

2 **Dendritic Cell-Based Vaccine for the Treatment of Malignant Glioma: A Systematic Review**

Xuan Wang, Hong-Yang Zhao, Fang-Cheng Zhang, Yun Sun, Zhi-Yong Xiong & Xiao-Bing Jiang   Cancer Invest, 2014

4 **Assessment of efficacy of dendritic cell therapy and viral therapy in high grade glioma clinical trials. A meta-analytic review** JII, 2019

Bogdan Ionel Vatu, Stefan-Alexandru Artene, Adeline-Georgiana Staicu, Adina Turcu-Stiolica, Catalin Folcuti, Alexandra Dragoi, Catalina Cioc, Stefania-Carina Baloi, Ligia Gabriela Tataranu, Cristian Silosi & Anica Dricu

6 Contents lists available at ScienceDirect

International Immunopharmacology

journal homepage: www.elsevier.com/locate/intimp
ELSEVIER

Efficacy and safety of dendritic cell vaccines for patients with glioblastoma: A meta-analysis of randomized controlled trials IIP, 2020

Li Lv, Jiangchao Huang, Haipeng Xi, Xiangyang Zhou
Department of Neurosurgery, First Affiliated Hospital, University of South China, Hengyang 421001, Hunan Province, China

Treatment phase 1:
Anticancer treatment

Surgery
Radiochemotherapy
Maintenance chemotherapy
ICD Immunotherapy

Treatment phase 2:
Immunization

Active specific Immunotherapy
Modulatory immunotherapy

Treatment phase 3:
Maintenance and
Expansion of
Immune protection

ICD Immunotherapy

Modulatory immunotherapy
• Anti-inflammation: anti-HR1, Cox2 inhibitor, Curamun
• CPI if needed
• Risedronate
Neuron-Glioma axis
Metabolic cocktail
• Metformin
• Atorvastatin
• Mebendazol
Neuro-psycho-endocrino-immunology axis
Melatonin

Modulated Electrohyperthermia in
Integrative Cancer Treatment for Relapsed
Malignant Glioblastoma and Astrocytoma:
Retrospective Multicenter Controlled Study

Integrative Cancer Therapy, 2018

Integrative Cancer Therapies
1-11
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SAGE

Giammaria Fiorentini, MD[1], Donatella Sarti, PhD[1], Carlo Milandri, MD[1],
Patrizia Dentico, MD[1], Andrea Mambrini, MD[1], Caterina Fiorentini, MD[1],
Giammaria Mattioli, MD[1], Virginia Casadei, MD[1] and Stefano Guadagni, MD[1]

Dendritic cell–based immunotherapy targeting Wilms'
tumor 1 in patients with recurrent malignant glioma

J Neurosurg, 2015

Keiichi Sakai, MD, PhD,[1,11] Shigetaka Shimodaira, MD, PhD,[2] Shinya Maejima, MD, PhD,[3]
Nobuyuki Udagawa, DDS, PhD,[3] Kenji Sano, MD, PhD,[4] Yumiko Higuchi, PhD,[2]
Terutsugu Koya, MS,[2] Takanaga Ochiai, DDS, PhD,[5] Masanori Koide, DDS, PhD,[6]
Shunsuke Uehara, PhD,[7] Midori Nakamura, DDS, PhD,[7] Haruo Sugiyama, MD, PhD,[8]
Yoshikazu Yonemitsu, MD, PhD,[9] Masato Okamoto, DDS, PhD,[10] and Kazuhiro Hongo, MD, PhD[1]

Phase I/II Trial of Intravenous NDV-HUJ Oncolytic Virus in
Recurrent Glioblastoma Multiforme

Molecular Therapy, 2006

Arnold I. Freeman,[1] Zichria Zakay-Rones,[2] John M. Gomori,[3] Eduard Linetsky,[4,5]
Linda Rasooly,[1] Evgeniya Greenbaum,[2] Shira Rozenman-Yair,[6] Amos Panet,[2]
Eugene Libson,[7] Charles S. Irving,[6] Eithan Galun,[1,*] and Tali Siegal[5]

Phase IIa Study of SurVaxM Plus Adjuvant
Temozolomide for Newly
Diagnosed Glioblastoma

JCO, 2023

Manmeet S. Ahluwalia, MD[1]; David A. Reardon, MD[2]; Ajay P. Abad, MD[3]; William T. Curry, MD[4]; Eric T. Wong, MD[5];
Sheila A. Figel, PhD[6,7]; Laszlo L. Mechtler, MD[1]; David M. Peereboom, MD[1]; Alan D. Hutson, PhD[5]; Henry G. Withers, PhD[6];
Song Liu, PhD[5]; Ahmed N. Belal, MD[6]; Jingxin Qiu, MD, PhD[11]; Kathleen M. Mogensen, NP[1]; Sanam S. Dharma, PhD[6];
Andrew Dhawan, MD[11]; Meaghan T. Birkemeier, BS[6]; Danielle M. Casucci. BS[6,7]; Michael J. Ciesielski, PhD[6,7]; and
Robert A. Fenstermaker, MD[6,7]

cancers   MDPI

Article
Individualized Multimodal Immunotherapy for Adults
with IDH1 Wild-Type GBM: A Single Institute Experience

Stefaan W. Van Gool *, Jennifer Makalowski, Peter Van de Vliet, Stefanie Van Gool, Tobias Sprenger,
Volker Schirrmacher and Wilfried Stuecker

Table 3: Patient characteristics in the selected publications

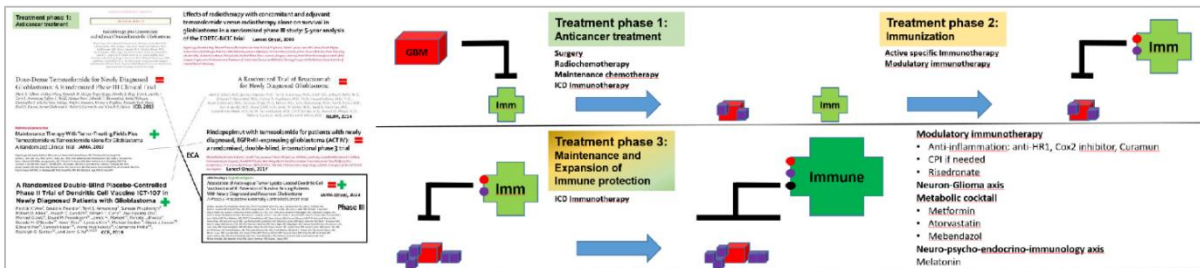|	n	Median age	F/M (%)	Median KPI	Meth (%)	Unmeth (%)	R0 (%)	<R0 (%)	ND (%)
Gilbert [283]	411	>50	42/58	>90	30	62	46	44	0
Gilbert [284]	309	>50	37/63	>90	28	69	59	41	0
Stupp [228]	229	57	31/69	90	42	51	54	46	0
Weller [285]	374	58	39/61	>70	35	58	56	44	0
Wen [81]	43	60	28/72	>90	42	56	74	26	0
Liau [10, 11]	232	56	41/59	>90	39	56	63	37	0
Van Gool [106]	50	48	46/54	80	44	56	28	52	20

F: female; M: male; KPI: Karnofsky performance index; Meth: MGMT promoter-methylated; Unmeth: MGMT promoter-unmethylated; ND: not documented; R0: complete resection; <R0: less than complete resection
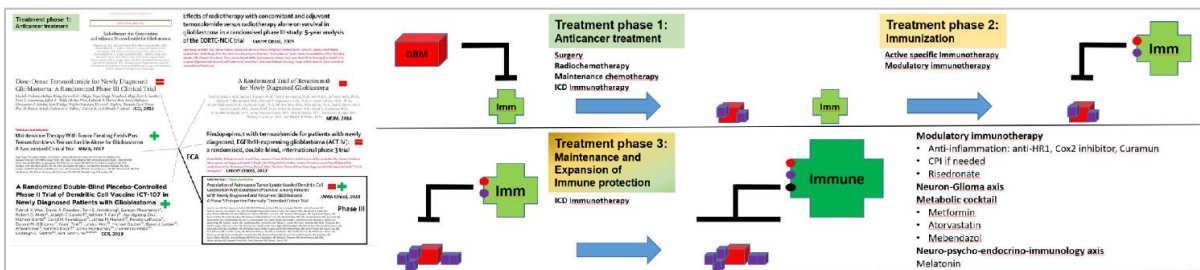
Table 4: Overall survival reported in selected publications

Reference		Unmethylated		Methylated	
		mOS (m)	2y OS (%)	mOS (m)	2y OS (%)
Stupp [195]	S + RT	11,8	1,8	15,3	23,9
RCT	S + RCT + CT	12,6	14,8	23,4	48,9
Stupp [228]	**S + RCT + CT**	**14,7**	**22,1**	**21,2**	**37,7**
RCT	S + RCT + CT + TTF	16,9	26,8	31,6	59,1
Liau [11]	**S + RCT + CT**	**14,6**	**21**	**21,3**	**42**
ECA	S + RCT + CT + DCVax®-L	14,9	19	30,2	58
Van Gool [106]					
RWD	S + RCT + CT + IMI	22,1	41,6	37,7	80,5

IMI: individualized multimodal immunotherapy; m: months; mOS: median overall survival RCT: randomized controlled trial; RDW: real-world data; S + RT: surgery + radiotherapy; S + RCT + CT: surgery + radiochemotherapy + chemotherapy; TTF: tumor-treating fields; 2y OS: two-year overall survival. Expected OS with standard of care treatment anno 2023 are marked in bold and gray background.
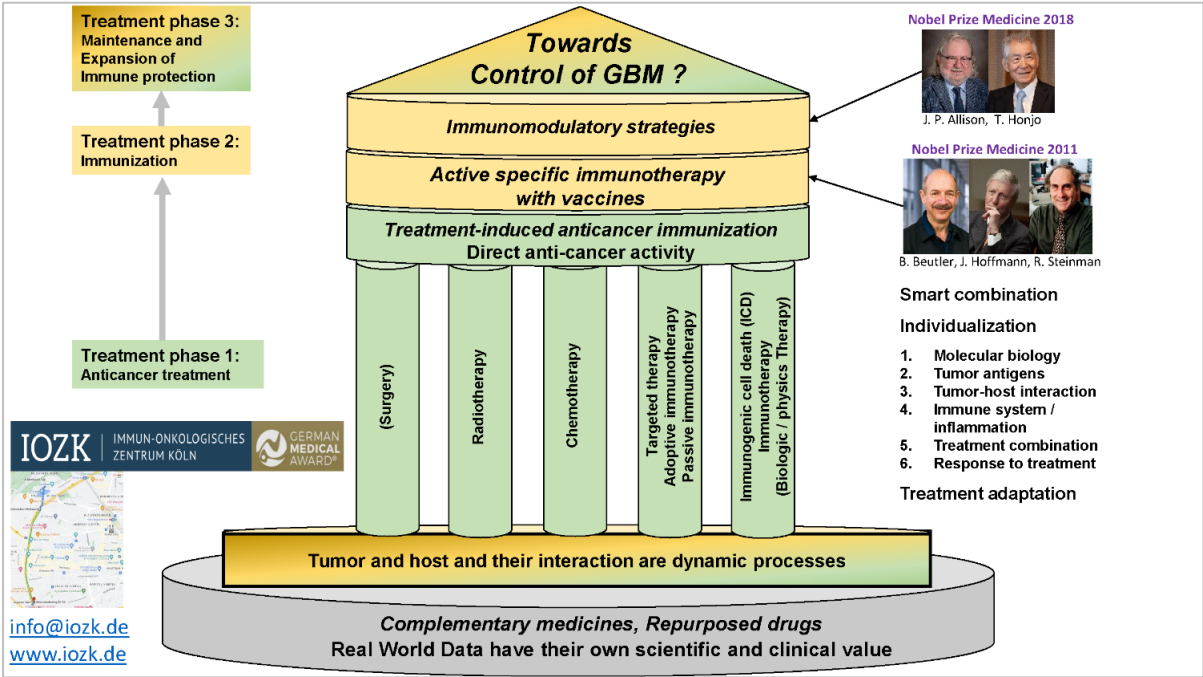
Palliative treatment containing modulated electro-hyperthermia alone or combined with chemotherapy versus second or third line of chemotherapy in patients with advanced pancreatic cancer: a multicenter retrospective observational comparative study on 217 patients - ESHO 2023 presentation

Fiorentini Giammaria

On behalf of International Clinical Hypertermia Society Italian Network

35° Annual Meeting European Society for Hyperthermic Oncology

26-29 September 2023- Cologne, Germany
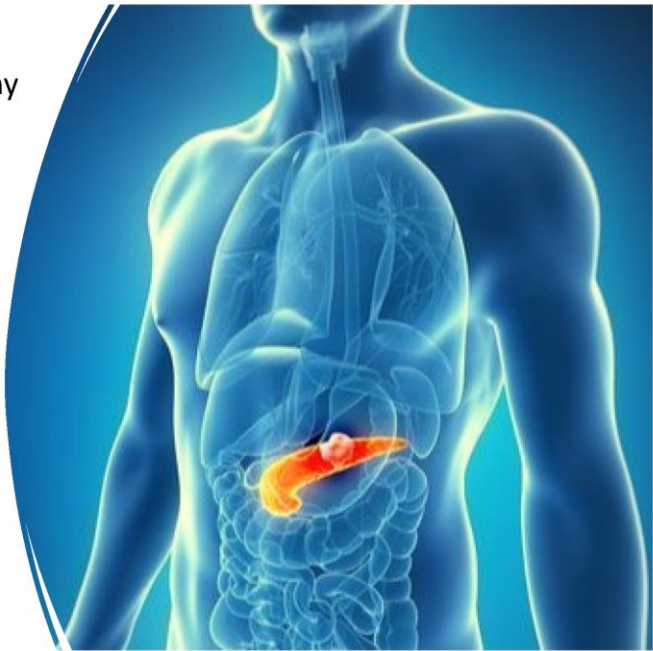
Palliative treatment containing modulated electro-hyperthermia alone or combined with chemotherapy versus second or third line of chemotherapy in patients with advanced pancreatic cancer: a multicenter retrospective observational comparative study on 217 patients

Fiorentini Giammaria M.D.
On behalf of
International Clinical Hypertermia Society
Italian Network
g.fiorentini2020@gmail.com

CANCER TYPES

Pancreatic ductal adenocarcinoma

Pancreatic Neuroendocrine Tumors (e.g. Insulinomas, etc.)
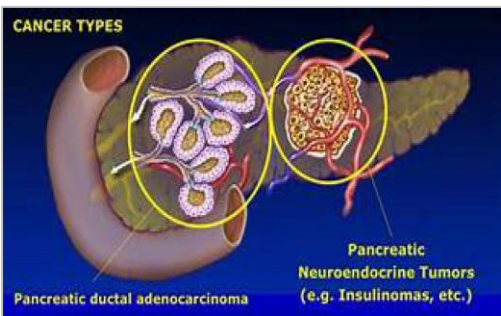
Istology : all patients treated are adenocarcinoma

Stage of cases treated in our study

stage	TNM classification	clinical classification (in terms of treatment)	median survival (months)
0	Tis, N0, M0	resectable	
IA	T1, N0, M0	resectable	24.1
IB	T2, N0, M0	resectable	20.6
IIA	T3, N0, M0	resectable	15.4
IIB	T1/2/3, N1, M0	locally advanced potentially resectable	12.7
III	T4, N0/1, M0	locally advanced unresectable	10.6
IV	T1/2/3/4, N0/1, M1	metastatic	4.5

Microenviroment in pancreatic cancer
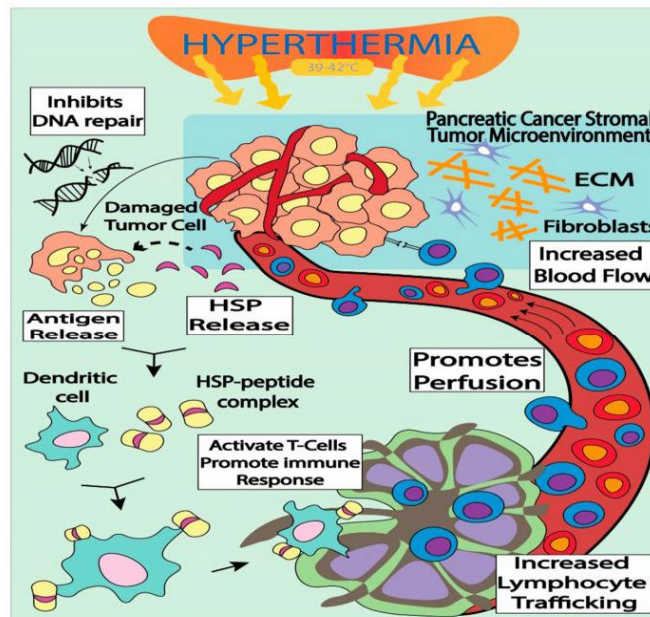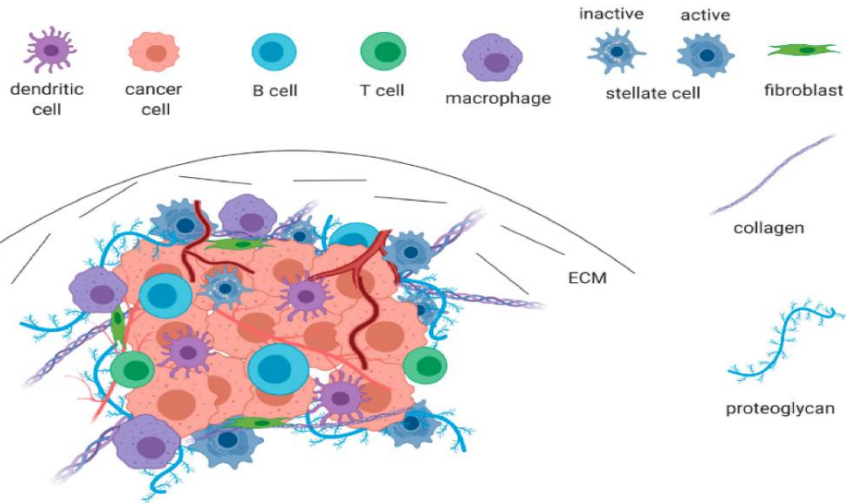
Rationale from praevious studies :

The clinical benefit of hyperthermia in pancreatic cancer: a systematic review

Astrid van der Horst, Eva Versteijne, Marc G. H. Besselink, Joost G. Daams, Esther B. Bulle, Maarten F. Bijlsma, Johanna W. Wilmink, Otto M. van Delden, Jeanin E. van Hooft, Nicolaas A. P. Franken, Hanneke W. M. van Laarhoven, Johannes Crezee & Geertjan van Tienhoven

Conclusions: Hyperthermia, when **added to chemotherapy and/or radiotherapy**, may positively affect treatment outcome for patients with pancreatic cancer. However, the quality of the reviewed studies was limited and future randomized controlled trials are needed to establish efficacy (2018).

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METHODS

This was a **multicenter retrospective observational comparative study**; data were collected for **patients with stage III-IV pancreatic cancer** that were **treated with mEHT alone or** in **combination with CHT from 2003 to 2021**

→ A total of 628 patients were treated in nine Italian Hospitals

→ 217 of them were included in this study

→ 89 (41%) of them received mEHT ± CHT (mEHTgroup)

→ 128 (59%) with CHT (no-mEHT group)

CHT was mainly gemcitabine-based regimens in both study groups

mEHT protocol and device

→ was performed using the EHY-2000plus device (CE0123, Oncotherm, Torisdorf, Germany)

→ applying a radiofrequency current of 13.56 MHz as carrier frequency that was modulated by time-fractal fluctuation

→ The energy was transferred by capacitive coupling, with precise impedance matching

The hyperthermia protocol included

→ three mEHT treatments/week for 2 mo

→ starting at a 60 W power for 40 min

→ Following treatments were performed by increasing the power up to 150 W and the time up to 90 min in 2 wk.

mEHT was administered **after CHT or within 48 h**, in order to **couple the high drug blood concentration** with the **modulated electro hyperthermia** and **optimize their synergy**

Patients: sites of metastases

SITE	Total	mEHT 89	no-mEHT 128		P
LIVER	132	70 53%	63 51%		n.s.
Peritoneum	55	35 27%	20 %19		n.s.
Lymphnodes	37	22 17%	15 15%		n.s.
OTHER	10	5 4%	5 5%		n.s.

Patients: praevious treatments

Patients	Total 217	mEHT 89		no-mEHT 128			P
Metastatic	142	70	79%	72	56%		0.004
RT	10	1	1.1%	9	7%		n.s
CHT	136	68	76%	68	53%		0,005
Surgery	51	22	24%	31	24%		n.s.

RESULTS:

→ Overall survival and progression free survival

- Overall survival (20 mo, range 1,6-24 *vs* 9 mo, range 0,4-56.25, *P* < 0.001)
- progression-free survival (7 mo, range2-24 *vs* 5 mo, range 0.4-41, *P* < 0.05)
- OS and PFS were **better for the mEHT+CHT group** compared to the CHT group.

RESULTS: Tumor response and Safety

Tumor response at three month follow up was available for:
- 87(98%) of mEHT
- 111 (88%) patients for non-mEHT group

→ mEHT patients showed a higher number of PR (45% vs 24%, P= 0.0018) and a lower number of progressions (PD) (4% vs 31%, P <0.01) than no-mEHT group

→ SD had similar value in both groups: 51% for mEHT and 45% for no-mEHT

→ Median mEHT sessions was 16.8 (range 6-25), resulting 1495 mEHT delivered sessions.

Tumor response at 3 months

	mEHT	N=87	no-mEHT	N=111	
	n	%	n	%	p
PR	39	45	27	24	0,0018
SD	44	51	50	45	0,8430
PD	4	4	34	31	<0,001

Side effects and toxicity

→Adverse events were reported in 2.6% of cases and included:
- G1 skin pain in 22 (1.5%) sessions
- G1-2 burns in 16 (1.1%) cases that resolved in few days

mEHT **did not increase** haematological, hepatic, pulmonary and metabolic toxicity due to CHT

Particularly no increased blood pressure or any other cardiac changes after adequate cardiological monitoring

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OS of mEHT and no-mEHT groups. Dots represent censors, cloud area represent CI 95%.
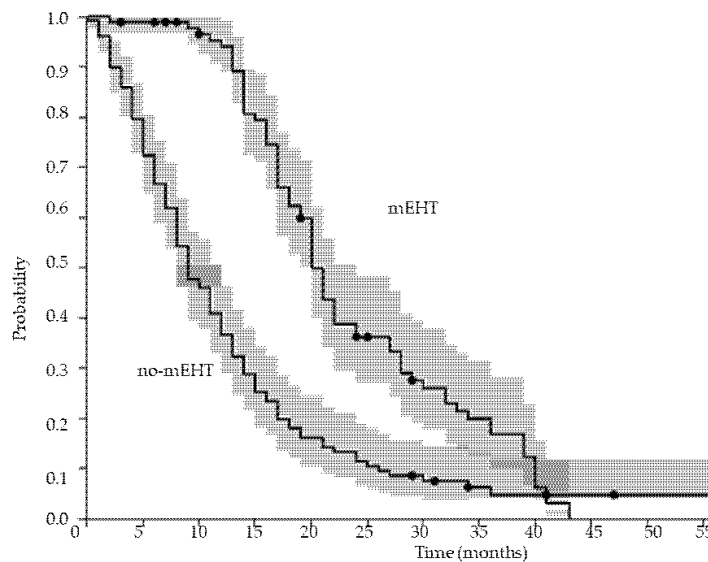
PFS of mEHT and no-mEHT groups. Dots represent censors, cloud area represent CI 95%.
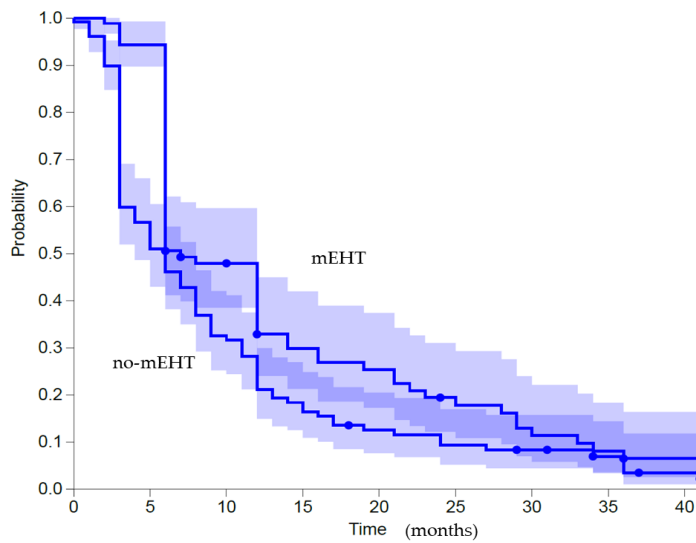
OS of mEHT and no-mEHT groups divided by age. Dots represent censors, cloud area represent CI 95%

The analysis of OS **by age less 70 years or more 70 years** showed that:

→ there was no difference in OS between mEHT less than 70 years (20 mo, range 2-43 m) and more 70years (20mo , range 3-27) P=0.235

→ whereas no-mEHT **patients with less than** 70 years had a higher OS than no-mEHT more than 70 years group (12 mo, range 1-56 vs 8 range 1-47, P= 0.01)

→ mEHT had a longer OS than no-mEHT group both among less than 70 years (20 mo range 3-27 vs 8 mo range 1-47, p <0.01) and more than 70 years (20 mo range 2-43 vs 12 mo range 1-56, P<0.01).
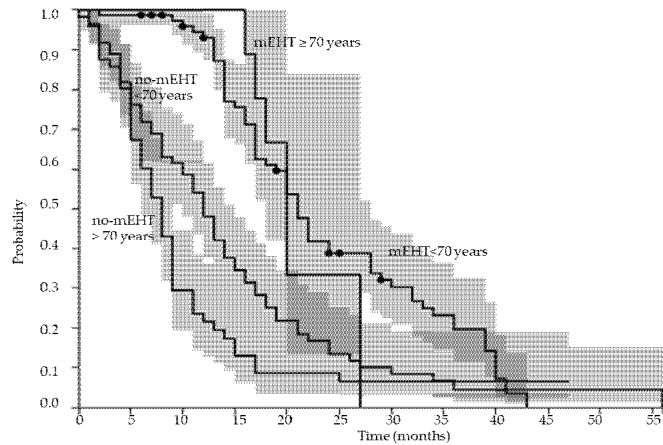
PT 33-PANCREATIC CANCER (HEAD) AFTER DRAINAGE RECEIVED MEHT (28 SESSIONS) PLUS GEM 9 C. SEE EVIDENCE OF RESPONSE

PT 26 - PANCREATIC CANCER (BODY) PROGRESSED AFTER 6 C. OF GEMOX, RESPONSE AFTER MEHT+ GEM (32 MEHT SESSIONS AND 8 C. OF GEM)
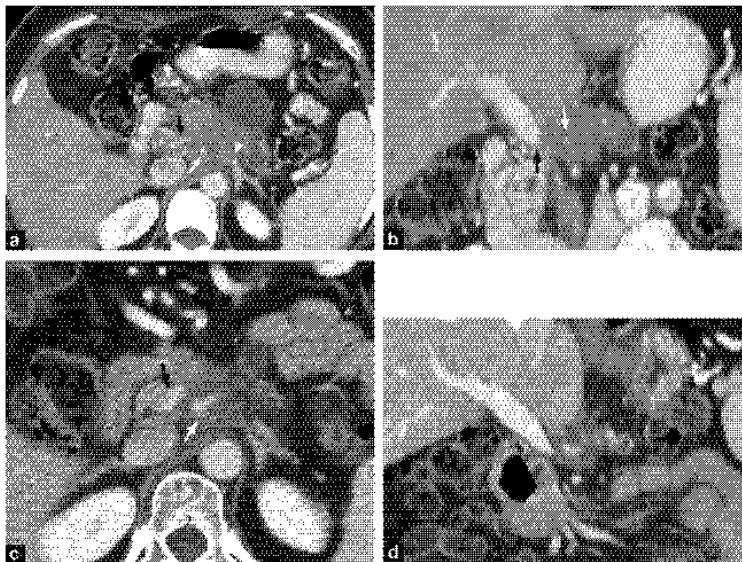
LOCOREGIONAL HYPERTHERMIA: SOME of ONGOING STUDIES IN PANCREATIC CANCER

1. NCT01077427: Hyperthermia European <mark>Adjuvant Trial (HEAT) in pancreatic cancer</mark> University Munich (Germany)
2. NCT02862015: Multicenter RCT of the Clinical Effectiveness of Oncothermia With Chemotherapy in <mark>Metastatic Pancreatic Cancer Patients</mark>. University Seul (S. Korea)
3. NCT02150135: Effect of Oncothermia on Improvement of Quality of Life in Unresectable <mark>Pancreatic Cancer Patients</mark>. University Seul (S. Korea)
4. NCT00178763 **Hyperthermia With Chemotherapy for Locally Advanced or <mark>Metastatic Pancreas Cancer</mark>** (Texas)
5. NCT02439593 <mark>**Concurrent Hyperthermia and Chemoradiotherapy in LAPC**</mark>**: Phase II Study (HEATPAC; Zurig, Swiss)**
6. NCT04889742 <mark>**Hyperthermia Enhanced Re-irradiation of Loco-regional Recurrent Tumors**</mark> **(HETERERO) Berlin, Germany**

Take Home Massage

→ The addition of mEHT to systemic CHT **improved overall and progression-free survival** and **local tumor control** with comparable toxicity

→ On the basis of this study and the other numerous studies in the literature, and the the ongoing trials it now seems time to organize an international randomized trial to evaluate the utility of electro-hyperthermia in this serious disease

COMPARISON OF THE EFFECTIVENESS OF INTEGRATIVE IMMUNOMODULATORY TREATMENTS AND CONVENTIONAL THERAPIES ON THE SURVIVAL OF SELECTED GASTROINTESTINAL CANCER PATIENTS

Ralf Kleef [1], Magdolna Dank [2], Magdolna Herold [2,3], Emese Irma Agoston [4], Julia Lohinszky [3], Emoke Martinek [2], Zoltan Herold [2], Attila Marcell Szasz [2]

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[4] Department of Surgery, Transplantation and Gastroenterology, Semmelweis University, Budapest 1082, Hungary.

In the last decade, the use of immunomodulating treatments (IMT) at integrative oncology providers (IOP) increased. IMTs are used to modulate the tumor microenvironment, which might lead to increased response-to-treatment, and the indication of immune checkpoint inhibitors might also be widened. The efficacy and safety of IMTs in advanced/metastatic gastrointestinal cancers were compared with conventional chemo(radio)therapy (CT). 21 colorectal- (CRC), 14 pancreatic- (PC), 5 cholangiocellular- (CCC), 5 gastric- (GC) and 4 esophageal cancer (EC) patients received IMT. IMT and CT were compared in CRC and PC. CT was administered at an academic oncology center. After the initiation of IMT, a median survival of ~ 20 (CRC, PC and EC) and ~ 10 months (CCC and GC) was observed. Of the IMTs, locoregional modulated electro-hyperthermia had the most positive effect on overall survival (HR: 0.3055; P = 0.0260), while fever-inducing interleukin-2, and low-dose ipilimumab showed a positive tendency. IMT was superior to CT in PC (HR: 0.1974; P = 0.0013), while modest effect was detected in CRC (HR: 0.7797; P = 0.4710). When the whole study population was analyzed, IMTs showed minimal effect on patient survival, still CT had the greatest effect if introduced as early as possible (HR: 0.0624; P < 0.0001). The integrative IMTs in the presented form have mild impact on gastrointestinal cancer patients' survival, however, we observed its benefit in PC, which warrants further investigations.

Gastrointestinal cancers account for 25.8% and 35.4% of all new cancer cases and cancer-related deaths, respectively[1]. To date, surgical resection (if possible) and chemotherapy/chemoradiotherapy and/or biological/ targeted therapies are the gold standards for their treatment, however, despite all the efforts in the latest drug development and state-of-the-art surgical methods, the 5-year survival rate of advanced stage gastrointestinal cancers is still under 15%[2]. In the last decades, a new trend in the treatment of cancers has emerged that takes advantage of the immune system and fights cancer by reactivating the body's natural immunity. It is suggested, that based on the amount of T-cell-infiltration, immunologically "cold" and "hot" tumors can be distinguished[3]. "Hot" tumors are known to have a high level of intratumoral infiltrating T-cells, a high level of tumor mutational burden, and a less immunosuppressive tumor microenvironment. In contrast, "cold" tumors have a low level or no T-cell infiltration at all, low tumor mutational burden, and a highly immunosuppressive tumor microenvironment[3-7]. The immunosuppression largely occurs through immune checkpoint molecules, which are expressed by the tumor itself helping it escape the immune surveillance mechanisms[2]. The majority of gastrointestinal cancers are known to be "cold" tumors[2-4], and only a very limited number with specific phenotypes are "hot" and respond well to immune checkpoint inhibitor (ICI) therapy[3]. Immunotherapy using ICIs in the "hot" subtypes is part of the standard of care. Moreover, numerous attempts have been made lately to make cancer of different origins immunologically "hotter", which may result in an overall better response to treatment, and in the wider applicability of ICIs[6-9]. In vitro cellular and in vivo animal research have found that artesunate[10], curcumin[11], dichloroacetate[12], high-dose vitamin C[13], interferon-γ[14], interleukin-2[15], ozone therapy16, and various forms of oncological hyperthermia1[7,18], including whole-body hyperthermia (WBH)[17] and modulated electrohyperthermia (mEHT)[18], can modulate the immune system and induce antitumoral mechanisms. Although, data on their clinical utility is very limited, it is important

to understand the basis of the off-label use of these products. Therefore, the available clinical data on them is reviewed in the Supplementary Materials of this article. It has to be emphasized, that, to our knowledge, several integrative oncology providers (IOP19) offer such therapies in various combinations, however, no literature data is available about these applications.

Although, in conventional oncology treatment centers none of these off-label treatment modalities are available, an emerging number of IOPs[19] offer patients these treatment modalities, despite the lack of safety and/or efficacy data from randomized or observational studies. Uniquely, we have published very promising results about an integrated immunomodulatory treatment (IMT) method previously[20]. However, to our knowledge, no further result(s) emerged in the literature since. Therefore, a retrospective pilot study was conducted in collaboration between an IOP (Dr. Kleef Medical Center, Vienna, Austria) and an academic oncology center (Semmelweis University, Budapest, Hungary) to collect data about the safety and efficacy of IMT, compared to conventional treatments. Further research questions included the direct comparison of the two approaches and attempts were made to identify their benefits and caveats.

RESULTS

Of the forty-nine gastrointestinal cancer patients who received IMT, 21 (42.86%) had colorectal cancer (CRC), 14 (28.57%) had pancreatic cancer (PC), 5 (10.20%) had cholangiocellular cancer (CCC), 5 (10.20%) had gastric cancer (GC), and 4 (8.16%) had esophageal cancer (EC). Those patients developing CRC had the highest probability of having the primary tumor removed, while, as expected, at least half of the patients had inoperable CCC, EC, GC, and PC. 18 (85.7%) of the 21 CRC, 9 (64.3%) of the 14 PC, 3 (60%) of the 5 CCC, 2 (40%) of the 5 GC, and 3 (75%) of the 4 EC patients received conventional chemo(radio)therapy (CT) prior IMT. The time between the diagnosis of the tumor and the initiation of IMT was the longest in CRC (23.08 ± 20.15 months), followed by CCC, PC, EC, and the shortest was in GC (4.02 ± 3.56 months). At the time of IMT initiation, most of the patients developed distant metastases. A 49-, 29-, 30-, 13-, and 31-month-long median survival for CRC, PC, CCC, GC, and EC was observed, respectively, if survival was calculated from the diagnosis of the tumor (Fig. 1). After the initiation of IMT, median survival was similar in CRC (20.01 months), EC (21.82 months), and PC (17.91 months), while shorter survivals could be observed in CCC (11.53 months) and GC (9.00 months). Detailed anamnestic and clinicopathological data of the five tumor cohorts can be read in Table 1.

Of the IMTs, locoregional mEHT (median: 16; range: 2–51) and high-dose vitamin C treatment (median: 15; range: 2–38) were used the most often, followed by low-dose ICIs (median: 3; range: 1–12) and WBH (median: 6; range: 1–25) treatments. IMT sessions are summarized in Table 2. The effect of the IMT modalities on patient survival was assessed, using an extended Cox model, where the IMTs were included as time-dependent coefficients, and different baseline hazards were assumed for the five tumor cohorts. This modeling approach was necessary due to the different combinations and initiation times of the IMTs. In addition, a few patients also received CT alongside (CRC: 5, PC: 3, CCC: 2, GC: 2, EC: 0) or after (CRC: 4, PC: 7,

CCC: 4, GC: 2, EC: 0) the IMT. Therefore, it was also added as a possible effector of patient survival.
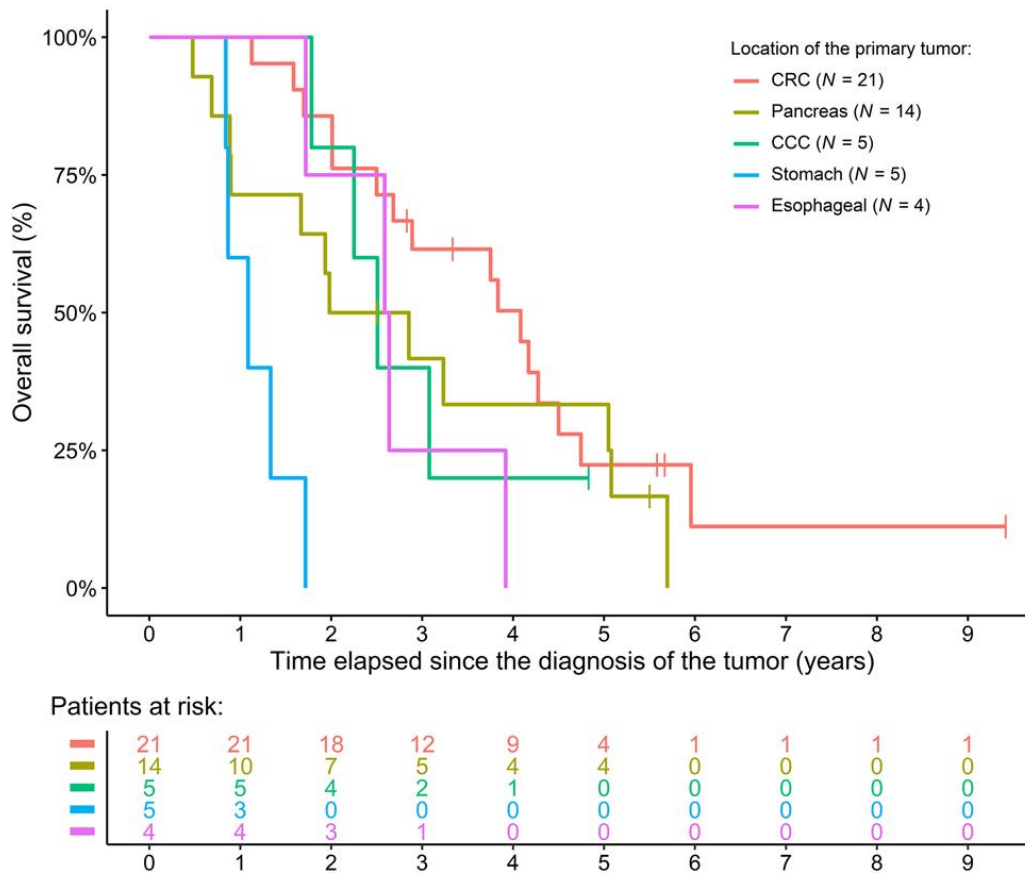

Figure 1. Overall survival of the five tumor cohorts treated with immunomodulatory treatments. CCC:
cholangiocellular cancer; CRC: colorectal cancer.

Parameter	CRC (N = 21)	Pancreatic (N = 14)	CCC (N = 5)	Gastric (N = 5)	Esophageal (N = 4)
Age (year)	49.00 ± 15.68	55.83 ± 9.61	53.96 ± 14.44	54.74 ± 13.72	52.02 ± 11.93
Sex (male : female)	7 : 14	5 : 9	2:3	1:4	0:4
Body-mass index (kg/m^2)	22.21 ± 3.62	22.60 ± 4.61	23.80 ± 1.82	22.36 ± 6.64	22.76 ± 2.76
Primary tumor					
Completely / partially resected	19/0	7/0	1/0	1/1	1/0
Inoperable or not operated	2	7	4	3	3
TNM[40]					
T: 1/2/3/4/irresectable	2/2/8/7/2	0/3/2/2/7	0/1/0/0/4	1/0/0/0/4	0/0/0/1/3
N: 0/1/2/irresectable	3/7/9/2	4/3/0/7	1/0/0/4	0/0/1/4	0/0/1/3
Metastasis: synchronous / metachronous	11/9	8/5	3/1	4/1	2/2
Location of metastases					
Liver	12	7	3	1	2
Lung	8	4	1	1	2
Distant lymph nodes	9	1	3	2	2
Peritoneal	7	3	0	3	0
Ovarium	3	1	0	1	0
Locoregional	4	3	0	0	0
Other (e.g., bone, brain)	0	2	1	3	0
Medical history prior cancer diagnosis					
Hypertension	4	3	2	0	0
Diabetes mellitus	4	0	0	0	0
Stroke / MI / other CV diseases [1]	0/3/3	0/0/2	0/0/3	0/0/0	0/0/0
Thyroid disease	4	2	1	0	0
Autoimmune disease	4	0	1	0	0
Time to first IMT (month)	23.08 ± 20.15	12.53 ± 14.07	19.69 ± 10.69	4.02 ± 3.56	13.03 ± 10.31
Median survival time since the					
Tumor diagnosis (month)	48.99	28.98	30.09	13.01	31.34
1st day of IMT (month)	20.01	17.91	11.53	9.00	21.82
Last day of IMT (month)	15.41	9.31	10.51	1.15	5.73
Survival rate since 1st IMT					
1-year (%)	57.1	64.3	40.0	20.0	75.0
2-year (%)	41.9	21.4	20.0	0	25.0
3-year (%)	27.9	0	0	0	0

Table 1. Anamnestic and survival data of patients receiving immunomodulating therapy (IMT). Unless otherwise indicated, continuous and count data are presented as the mean ± standard deviation and the number of observations, respectively. 1 Other CV diseases included, e.g., congestive heart failure, atrial fibrillation, peripheral artery disease, etc. CCC: cholangiocellular cancer; CRC: colorectal cancer; CV: cardiovascular disease; MI: myocardial infarction, TNM: Tumor − Node − Metastasis staging system.

Model results showed that locoregional mEHT (HR: 0.3055; 95% CI 0.1075−0.8680; P = 0.0260) and CT (HR: 0.2688; 95% CI 0.0873−0.8280; P = 0.0221) had a significantly positive effect on patient survival, while fever-inducing interleukin-2 had tendentially positive effect (HR: 0.2212; 95% CI 0.0402−1.2150; P = 0.0826). Furthermore, a second model was also created, where the low-dose ICIs were not investigated together, but as separate model predictors. In addition to the previously detailed effects of mEHT, CT and interleukin-2, the use of ipilimumab was also associated with tendentiously longer survival times (HR: 0.0720; 95% CI 0.0038−1.3620; P = 0.0794).

It was also investigated whether the addition of further clinicopathological characteristics into the survival models as explanatory parameters influences on the IMTs. The survival models described in the previous paragraphs were extended with the following: age, sex, when the metastasis occurred (none / metachronous / synchronous), and the location of the metastases (liver, distant lymph node, peritoneal and/or locoregional). In both cases, the effect of peritoneal metastases on patient survival was too large, therefore it was excluded from the final models. In the model, where the two ICIs were investigated together, mEHT (HR: 0.4122; 95% CI 0.1279–1.3282; P = 0.1377) and CT (HR: 0.2368; 95% CI 0.0641–0.8755; P = 0.0308) was tendentiously and significantly associated with longer survival times. Neither the other IMTs, nor the newly introduced clinicopathological parameters had any effect on patient survival. Similarly, where ipilimumab and nivolumab were investigated separately, mEHT (HR: 0.3388; 95% CI 0.1005–1.1424; P = 0.0809), ipilimumab (HR: 0.0697; 95% CI 0.0030–1.5956; P = 0.0954) and IL-2 (HR: 0.1840; 95% CI 0.0264–1.2814; P = 0.0873) had tendentiously, and CT (HR: 0.2640; 95% CI 0.0737–0.9464; P = 0.0409) had significantly positive effect.

The most common adverse events (AEs) related to the IMTs were fever with chills, rashes, vomitus, weakness, malaise, neutropenia, and hyperthermia-related local skin reactions: skin redness and grade I burns.

Therapy	CRC (N=21)	Pancreatic (N=14)	CCC (N=5)	Gastric (N=5)	Esophageal (N=4)
Locoregional mEHT	16.57 (6–42)	19.71 (2–47)	20.20 (13–35)	25.40 (13–51)	16.25 (5–44)
wIRA hyperthermia	2.10 (0–19)	1.93 (0–16)	2.60 (0–13)	5.80 (0–17)	0.25 (0–1)
Mild WBH	3.52 (0–8)	4.43 (1–8)	4.80 (3–7)	5.60 (0–15)	5.25 (2–10)
Moderate WBH	1.71 (0–15)	1.79 (0–13)	2.60 (0–9)	0.00 (0–0)	0.25 (0–1)
Long duration moderate WBH	1.00 (0–3)	2.14 (1–12)	1.20 (1–2)	2.60 (1–4)	1.00 (0–2)
Low-dose ipilimumab [1]	3.57 (0–12)	3.36 (1–6)	4.20 (3–6)	5.00 (3–12)	6.00 (3–9)
Low-dose nivolumab [1]	4.09 (1–12)	3.43 (1–7)	3.60 (2–6)	6.60 (3–12)	6.00 (3–9)
Interleukin-2	3.86 (1–22)	3.93 (1–14)	7.40 (1–18)	2.80 (1–5)	3.25 (1–6)
High-dose vitamin C	14.81 (2–35)	16.86 (4–38)	19.40 (13–36)	22.20 (13–34)	15.00 (5–37)
Curcumin	2.00 (0–21)	1.14 (0–12)	4.20 (0–15)	1.00 (0–5)	2.50 (0–10)
Dichloroacetate	1.71 (0–8)	3.14 (0–22)	5.40 (0–12)	1.60 (0–4)	4.00 (0–13)
Artesunate	2.05 (0–8)	0.86 (0–8)	5.20 (0–11)	1.00 (0–5)	0.00 (0–5)
Ozone therapy	0.81 (0–4)	1.07 (0–7)	2.40 (0–5)	0.00 (0–0)	0.50 (0–2)
Interferon-γ	1.57 (0–13)	2.64 (0–13)	3.80 (0–9)	1.00 (0–5)	0.00 (0–0)
Metronomic chemotherapy	0.57 (0–11)	3.93 (0–15)	1.20 (0–3)	5.40 (0–18)	2.75 (1–5)

Table 2. Number of immunomodulating therapy sessions. Data are presented as mean (range). 1 A single patient in the CRC cohort received nivolumab as monotherapy. CCC: cholangiocellular cancer; CRC: colorectal cancer; mEHT: modulated electro-hyperthermia; WBH: whole-body hyperthermia; wIRA: water-filtered infrared-A.

Less common AEs included sleeping problems, limb edema, elevated liver enzymes, and diarrhea. In a single case, bloody stool without further occurrence was observed after the first administration of fever-inducing interleukin- 2. A total of three serious AEs occurred: (i) Reversible kidney failure due to ICI-associated nephritis21, (ii) bleeding of a rectal tumor requiring hospitalization after the first administration of fever-inducing interleukin-2, and (iii) psoriasis exacerbation.

Comparison of CRC patients treated with conventional and IMT therapy

The 21 IMT-treated CRC patients were compared to age, sex, TNM staging, tumor location, and metastasis occurrence time (none, synchronous, metachronous) matched cohorts, who received only CT. As the CT cohort was significantly larger, namely 835 patients, in addition to 1:1 matching, it was also feasible to perform 2:1, 5:1 and 10:1 matching. The inclusion of higher matching ratios into the analysis was done to reduce selection bias. Clinicopathological data and comparison results of the IMT and matched CRC cohorts are summarized in Tables S1 and S2. Except for the following, all cohorts were basically identical. In the 1:10 pairing, the non-IMT patients were older than their IMT pairs (IMT: 49.00 ± 15.68; non-IMT: 59.48 ± 10.94; P = 0.0067).

Survival analysis revealed no difference between the IMT and 1:1 matched cohorts (P = 0.4710; Fig. 2A), and similar results were found for the 2:1 (P = 0.9830; Fig. 2B), 5:1 (P = 0.9050; Fig. 2C), and 10:1 (P = 0.8980; Fig. 2D) cohorts. If analyzed in a time-dependent manner, neither the effect of all (P = 0.4650) nor of individual IMTs could be verified, while the early introduction of CT within the course of the disease was the most effective predictor of longer patient survival (HR: 0.0779; 95% CI 0.0290–0.2090; P < .0001). All model results stayed the same, even if age, sex, and metastasis occurrence were added to the models.

Comparison of PC patients treated with conventional and IMT therapy

Three cohorts were compared: the 14 IMT-treated PC patients, and 14–14 age, sex, tumor location, and metastasis occurrence time (none, synchronous, metachronous) matched PC patients, who were treated with CT with or without concomitant mEHT. Clinicopathological results and their comparison of the three PC cohorts are summarized in Tables S3 and S4. It has to be noted, that in the IMT-treated cohort the number of patients with an irresectable PC was less common, compared to that of the control cohorts. Therefore, in all subsequent analyses, all models were corrected for this parameter.

The IMT cohort had longer survival than the CT (reduced risk of the IMT cohort vs. CT: HR: 0.1974; 95% CI 0.0736–0.5295; P = 0.0013), but had the same survival as the CT + mEHT cohort (IMT vs. CT + mEHT: HR: 0.4892; 95% CI 0.1858–1.2880; P = 0.1478). The shortest survival was found in the CT-only cohort (survival advantage of the CT + mEHT cohort vs. CT only: HR: 0.4035; 95% CI 0.1695–0.9606; P = 0.0403). The naïve Kaplan–Meier curves of the three cohorts are drawn on Fig. 3. When analyzing the various IMT options and their effect on patient survival separately, mEHT had tendentious effect on the survival of PC patients (HR: 0.1082; 95% CI 0.0098–1.1954; P = 0.0696). Extending the models with age, sex, and metastasis occurrence time yielded consistent results. None of the additional clinicopathological parameters had any effect on survival, and the same tendencies regarding the patient cohorts [IMT (ref.) vs. CT: P = 0.0007; IMT (ref.) vs. CT + mEHT: P = 0.0794; CT (ref.) vs. CT + mEHT: P = 0.0372] and mEHT (P = 0.0546) could be justified in every extended model.

A

B
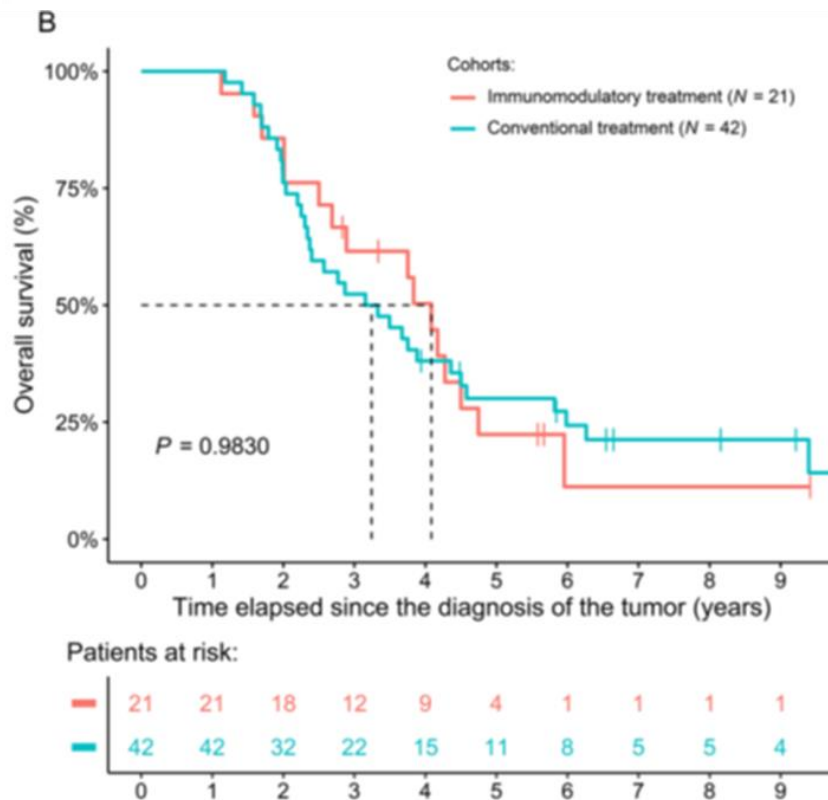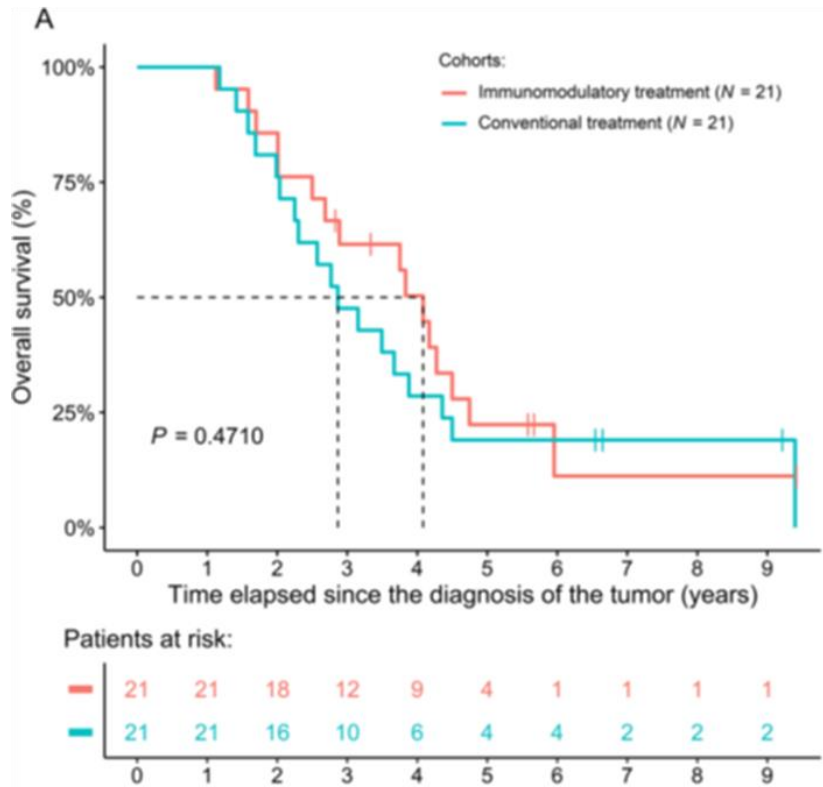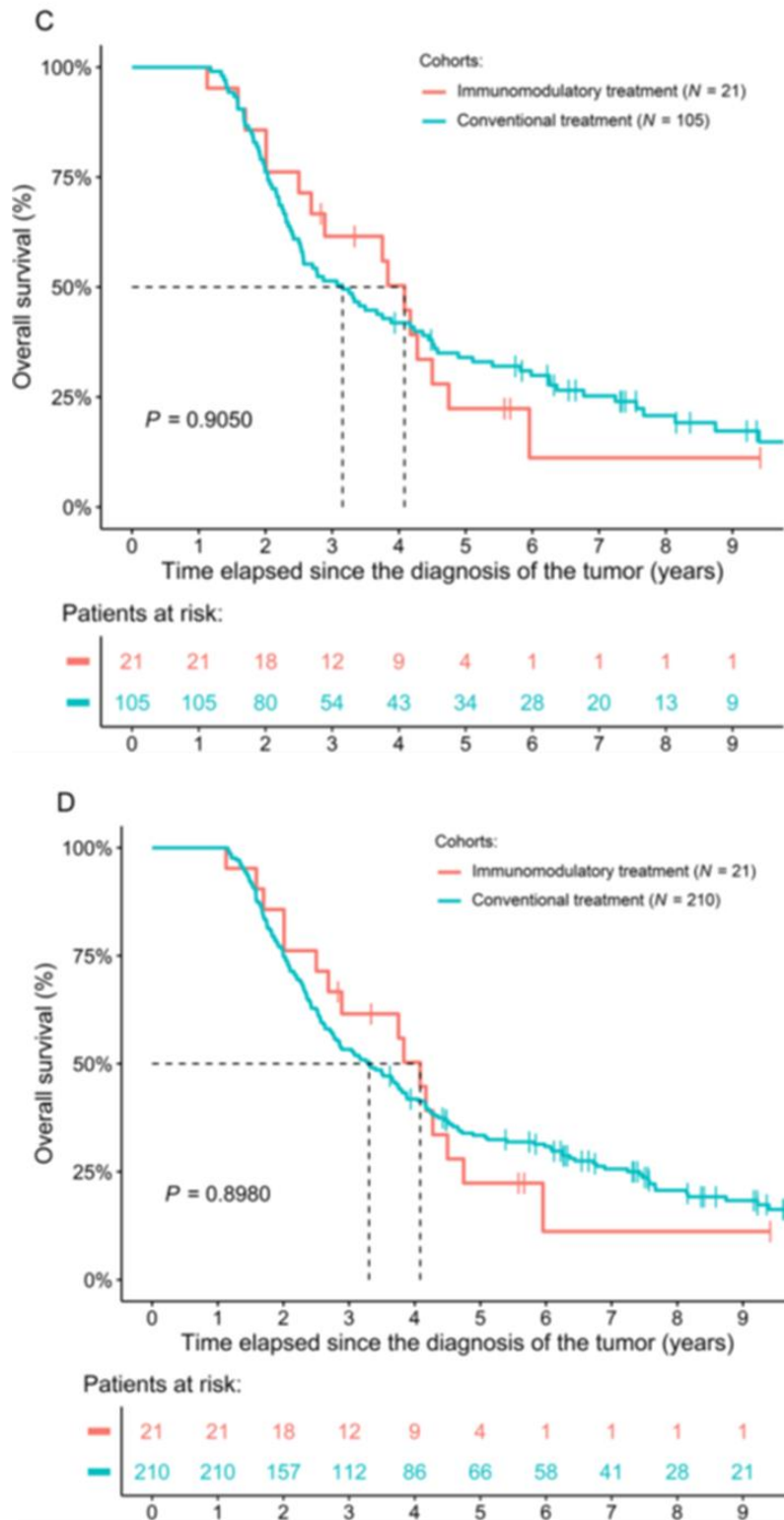
Figure 2. Comparison of survival data of colorectal cancer patients treated with and without immunomodulatory treatment (IMT) modalities. The IMT cohort was matched with a significantly larger (N = 835) cohort of patients having only conventional chemo(radio)therapy, therefore, it was feasible to investigate the differences in (A) 1:1, (B) 2:1, (C) 5:1, and (D) in 10:1 matched cohorts.

What effect does IMT have on patient survival?

As detailed above, most IMT-treated patients received CT during the course of their disease: either prior, alongside or after IMT. Therefore, it was possible to test the effect of IMT and CT on patients' survival as follows. We chose two approaches, first, a simplified time-dependent extended Cox survival model with adjusted baseline hazards for the different tumor cohorts was analyzed, in which we specified the intervals when the patients received either CT, IMT, simultaneously both, or no therapy at all. Results showed that IMT in general had no effect on patient survival neither if introduced close to cancer diagnosis (P = 0.9980), nor if used in a later stage of the disease (P = 0.8350). In contrast, CT had the best positive effect on patient survival if it was administered shortly after tumor diagnosis (HR: 0.0624; 95% CI 0.0193–0.2022; P < 0.0001), while its late introduction/use had a less obvious effect (P = 0.5710).

Second, the individual therapeutic options were analyzed after tumor diagnosis. We could justify the positive significant effect of mEHT (HR: 0.1984; 95% CI 0.0558–0.7051; P = 0.0124) and CT (HR: 0.0895; 95% CI 0.0262–0.3063; P = 0.0001). Moreover, metronomic chemotherapy had a marginal association with longer survival times (HR: 0.2244; 95% CI 0.4985–1.0100; P = 0.0516). None of the remaining IMTs showed any effect.

If the two models described in the above paragraphs were extended with further, clinicopathological parameters, the following was found. In the extended model, where we investigated the effect of combined IMT, CT, age, sex, and metastasis data, the same was observed as above. IMTs had slight effect over patient survival: neither if introduced closer to the cancer diagnosis (P = 0.9988), nor if introduced at a later time (P = 0.9552). CT had the most positive effect if introduced early (HR: 0.0395; 95% CI 0.0080–0.1947; P < 0.0001), and older age of the patients (HR: 1.0440; 95% CI 1.0030–1.0880; P = 0.0351) was also a significant predictor. Similarly, the second model also predicted the same as before: mEHT (HR: 0.2496; 95% CI 0.0569–1.0960; P = 0.0659), CT (HR: 0.0817; 95% CI 0.0206–0.3240; P = 0.0004) and metronomic chemotherapy (HR: 0.2162; 95% CI 0.0408–1.1460; P = 0.0718) being tendentious/significant effectors.
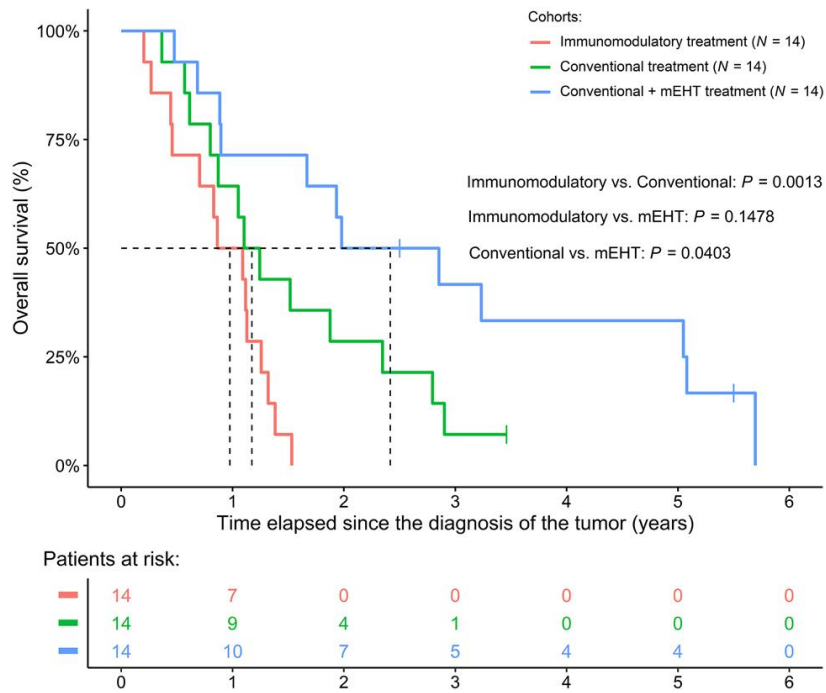
Figure 3. Survival difference of pancreas cancer patients between the immunomodulatory treatment and matched control cohorts with and without modulated electro–hyperthermia (mEHT) treatment. It has to be noted that naïve Kaplan–Meier curves were drawn on the figure, while the P-values were obtained using baseline hazard adjusted Cox regression models. Baseline hazard adjustment was performed due to the fact, that in the "Conventional" and "Conventional + mEHT" cohort the number of patients with inoperable pancreatic tumors were higher.

ADDITIONAL OBSERVATIONS

We could identify the following additional information and trends from the documentation of the IMT-treated patients: The socioeconomic status of these patients was high. Long-lasting stable disease was observed in several cases. It should be noted, however, that we could not identify a standardized protocol for the selection of treatment options and the later introduction of additional treatments seemed also random. Moreover, compared to the strict schedule of CT, the various IMT treatments could often be postponed based on the preference of patients, and the diagnostic tests used for treatment selection often had no normal values or acceptable standards.

DISCUSSION

Treating advanced-stage cancer is a challenging task for the medical staff, patients, their families and the society at large. Compared to conventional oncology centers, which operate on strict guidelines and evidence-based treatment options only, IOPs offer a more patient-accepted approach[22]. In addition to dietary supplements, herbal medicines, and traditional treatments, the use of off-label drugs is also very common [19,22]. Although more and more IOPs

offer some kind of immunotherapy[19,23,24,] and in previous research we could have demonstrated improved patient survival and curative effects in stage IV cancer patients[20], to our knowledge, this is the first report in gastrointestinal tumors that analyzed and compared integrative IMTs to conventional oncological treatments.

A large set of literature data supports that these—in most cases off-label—therapeutic solutions have some effect in the treatment of gastrointestinal tumors on their own [10- 18], however, the lack of randomized clinical trials and/or evidence-based data prevents their use in CT. In contrast, IOPs around the world often offer these drugs either standalone or in various combinations. In the current study we found that of the administered IMTs, mEHT evidently had a significant effect on patients' survival, which was comparable to that of CT, followed by the promising, tendentiously positive effect of interleukin-2 induced artificial fever therapy and low-dose checkpoint inhibitors (ipilimumab + nivolumab). However, we were not able to demonstrate sufficient certainty about the other IMT modalities that their usage significantly promotes longer survival of patients, while CT had a strong positive effect on patient survival in all of the analyses we performed. The same result was obtained in those models where the analysis was "simplified" to compare CT and IMTs in general: mild effect of IMTs could be observed, while CT is the most effective if it is introduced after the diagnosis of the tumor as early as possible. Similarly, no effect of IMT on survival was found when the CRC cohort was investigated separately, even with varying matching ratios, while the superiority of the IMT approach over CT without mEHT was found in PC, but the same survival was found when CT was supported by concomitant mEHT. No statistical difference was observed between the survival of the IMT and CT + mEHT cohorts. The number of PC patients included in the present study was relatively low, but besides the differences in the treatment, we could not prove any other effector that could cause this difference. We hypothesize a possible strong effect of the combined use of mEHT, ipilimumab and nivolumab, however, the data in the literature about ipilimumab and nivolumab in PC are controversial and scarce [25- 28]. Therefore, this observation needs further investigation as soon as possible.

It was also investigated whether the extension of the survival models with further clinicopathological parameters changed the above-detailed results. The same results could have been justified, even if known strong predictors, such as age, presence / development time of metastases, etc. were included. All these consistencies ultimately strengthen that CT is the most important factor in a cancer patient's survival, and of the IMTs mEHT, fever inducing interleukin-2 and low-dose ICIs seem to be those, that need further investigations. In the literature, most data is available about mEHT: e.g., the studies investigating mEHT in PC have found the same positive tendencies over patient survival[29- 33] as detailed in the results of the current study. Therefore, it is strongly suggested to further investigate the positive effects of concomitant mEHT in (gastrointestinal) cancer.

As detailed in the previous paragraphs, except for mEHT, IMTs had no statistically justifiable effect on patients' survival. Based on the data collected, we can only speculate the reason behind this observation. During the course of the disease, the various types of IMTs were introduced randomly, sometimes closer to the diagnosis, while in other cases significantly, even years later. Moreover, the various drugs were often used with low doses, and their application was often based on whether the patient could afford it or not. These are some of those biases, that we hypothesize might have significantly affected the efficacy of these

therapeutic options. Therefore, systematic, multicenter research conducted in the future would be ideal to further investigate the effect of these therapeutic options. Furthermore, we believe, the strong advantage of CT, which was found in the current research, might also arise from these biases of the IOPs. During the administration of CTs the medical staff needs to follow strict rules, and all therapeutic decisions are made based on evidence-based tools, tests, etc. While, in the philosophy of IOPs, the patient's decision/preference comes first. All of these points to that the introduction of standardized protocols is essential and necessary in the case of IOPs as well.

The following side effects and adverse events were registered throughout the study. Local skin reactions (redness and grad I burns) were mostly associated with mEHT, while weakness, malaise, and sometimes fever were associated with WBH, in line with previous descriptions[34]. Similarly, the occurrence of fever with chills, rashes, vomiting, neutropenia, sleeping problems, limb edema, elevated liver enzymes, and diarrhea are known frequent side effects of the drugs used during IMT[35-38]. In addition, three previously described serious AEs occurred, namely, an ICI associated nephritis[21], a psoriasis exacerbation manageable using standard psoriasis treatments[39], and a bleeding of a rectal tumor requiring hospitalization after the first administration of feverinducing interleukin-2[40]. It can be determined that there is a strong relationship between the described serious AEs and the applied treatments. As all serious AEs were known from the literature and particular attention was paid to known signs and symptoms. In the article of Belliere et al.[21], it was suggested that renal monitoring of all patients receiving ICI treatments is necessary. In the current study, regular blood samples were taken before, during and after the treatments, including creatinine and eGFR testing. Similarly, psoriasis flares manageable with standard treatments[39] and grade 3 or 4 hemorrhages[40] are known side effects of ICIs and interleukin-2, respectively. Although in all cases, the serious AEs resolved without sequelae, it is important to draw attention to the fact that the treatment of serious AEs always requires hospitalization, often at a different, external institution. Most IOP centers themselves are not sufficiently prepared to handle serious AEs, as these events require specialized tools, wards, and personnel, such as an intensive-care unit. Furthermore, comorbidities, such as autoimmune diseases make oncology treatments difficult even in traditional centers, therefore, treating such patients at IOPs requires extra attention and/or the involvement of specialist(s). On the basis of the AE cases processed during our research, for the protection of patients, it is extremely important for every IOP to have an emergency cooperation partner. We recommend that during these collaborations, the partner healthcare institution should be adequately informed about the treatments and drugs used at the IOP, that way they can adequately prepare for the care of these patients and (serious) AE events.

Based on the observations of the current study, the main shortcoming of IOPs is the lack of guidelines, followed by the excessive influence of patients on therapeutic decision processes, socioeconomic considerations, and the lack of public and/or private health insurance support for the applied therapies, which has also been criticized by others before[19,41]. The problem of the lack of guideline(s) was evident at the very beginning of our research. As described in "Results", even though we had access to the complete treatment data, we were unable to define a uniform protocol. This ultimately resulted in the increased heterogeneity of the investigated patient population and the obtained data could only be analyzed using much more advanced, computationally demanding, and skill-intensive statistical (modeling) methods. Based on the experiences available from CT centers, if treatment protocols based

on strict rules are available, they make the decision-making processes easier for both the practicing oncologists and the patients. The strict rules must also include what conditions (imaging studies, laboratory tests, etc.) the given therapy is based on. The second most influential factor affecting the treatment of patients was the various socioeconomic status of the patients. All patients receiving integrative IMT at the Dr. Kleef Medical Center had higher socioeconomic status than average, including a better financial situation and family support. Most off-label treatments offered by IOPs are expensive and not covered by public and/or private health insurance. Patients often must travel abroad and stay for a longer period to achieve optimal/effective treatment, and regular help/support from family and/or friends during those trips/treatments is essential.

It must be mentioned though, that, naturally, not everything is black and white in the case of CT either. Since the beginning of their use, it is a known fact that they have many side effects, including but not limited to alopecia, mucositis, myelosuppression, gastrointestinal side-effects such as nausea, vomiting, and diarrhea, fatigue, sterility, infertility, infusion reactions, and increased risk to infections[42]. Due to the strict regulations and guidelines patients have significantly less input and/or decision-making opportunities about their therapy, and for the same reason, the therapeutic agents the practicing oncologist can use are more limited compared to that of the IOPs.

Limitations

Limitations of our study include the retrospective design, the high heterogeneity of patients, and the small sample sizes. The biasing effect of heterogeneity was somewhat reduced by using various robust statistical techniques, such as adjusting the baseline hazards in the survival models or performing comparisons with different matching ratios. E.g., by using these two techniques alone, we were able to significantly reduce the resulting variance that originated from the non-uniform tumor cohorts, the differences between the two main populations, and selection biases. Due to the heterogenous and low number of cases in the IMT cohort statistical significance might be biased in some comparisons, however, as previously said, the use of robust methods and consequent results from all models strengthens all tendencies that were found. It must be noted, that due to the extremely high price of nivolumab/ipilimumab, the number of patients who could afford to buy them at the IOP was even lower, which was the reason behind the small sample size of patients receiving IMT. The socioeconomic status of the two centers was significantly different, which might further affect the heterogeneity of patients. To counterbalance these effects, during the comparison of IMT and CT, propensity score matching was used to reduce confounding effects as much as possible, and baseline hazard correction was always used in all survival models when comparing the two main cohorts.

Conclusion

Summarizing the results of the current study, a retrospective cohort analysis was performed to compare complex integrative IMT and CT with the participation of an IOP and a conventional oncology center. It was found that, except for mEHT, IMTs have mild effect on patient survival

in most gastrointestinal cancer cases, compared to that of CT. However, the superiority of IMT, mainly mEHT, seemed to appear in PC. This latter observation needs further confirmation as this patient population has a poor prognosis in all aspects. To our knowledge, this is the first occasion to report a positive effect of immunomodulation in pancreatic cancer. In addition, the following general observations were made: The lack of detailed/strict protocols at the IOPs makes the analysis of scientific data significantly more challenging. The development of standardized guidelines for IOPs is strongly recommended, which should be influenced less by patient preference. Moreover, these newly developed protocols should rely on evidence-based results, imaging studies, and laboratory results that have proper control and/or normal ranges. Of course, it is not only the IOPs that need to change, but we also encourage conventional oncology centers to use off-label drugs more often if they have the opportunity, and creating a more patient-oriented environment is also extremely important. We also recommend the development of complex oncology treatment programs involving psychologists, dieticians, physiotherapists, and other specialists in addition to the oncologist, ultimately improving the quality of life of patients.

Materials and methods
Ethics approval

The research was approved by the Regional and Institutional Committee of Science and Research Ethics, Semmelweis University. The prospective and retrospective data collection on patients treated with concomitant mEHT was granted on February 16, 2017 (SE TUKEB 8/2017) and renewed on January 9, 2023 (SE TUKEB 8–1/2017). The retrospective data collection on colorectal cancer patients was granted on June 9, 2015 (SE TUKEB 133/2015) and on February 23, 2021 (SE TUKEB 21–14/1994). Patient consent at Semmelweis University was waived due to the retrospective, anonymized design of the study, while all patients treated at the Dr. Kleef Medical Center signed an informed consent form for future analysis of their anonymized data for research purposes. The consent patients signed also included that the Dr. Kleef Medical Center may anonymously transmit treatment data to cooperating third parties for research purposes. The research was conducted in accordance with the regulations of the WMA Declaration of Helsinki and the General Data Protection Regulation issued by the European Union.

Patients and study design

A retrospective pilot study was conducted with the inclusion of the following three cohorts: 1.) 49 gastrointestinal tumor patients, who received IMT including nivolumab and/or ipilimumab at the Dr. Kleef Medical Center, Vienna, Austria, between 2015 and 2021. 2.) 78 pancreatic cancer (PC) patients treated at the Division of Oncology, Department of Internal Medicine and Oncology, Semmelweis University, Budapest, Hungary, between 2015 and 2019, and 3.) 835 colorectal cancer (CRC) patients, who attended the Department of Internal Medicine and Hematology, Semmelweis University, Budapest, Hungary, and at the Division of Oncology, Department of Internal Medicine and Oncology, Semmelweis University, Budapest, Hungary between 2006 and 2018. Detailed inclusion and exclusion criteria of the two non-IMT treated

cohorts were further detailed in[31] and in[43]. All patients included in the study had an Eastern Cooperative Oncology Group (ECOG) performance status score ≤ 2.

Patients of the second and third cohorts were used for propensity score–based matching. Matching was based on age, sex, staging data (only for CRC), location of the tumor, and when metastases developed (synchronous vs. metachronous). For the comparison with the 14 IMT PC patients, 14–14 PC patients treated with conventional therapeutic options with and without concomitant mEHT treatment were selected from the second cohort. 21, 42, 105, and 210 CRC patients of the third cohort were compared with the 21 IMT CRC patients after 1:1, 2:1, 5:1 and 10:1 matching, respectively. The latter was possible due to the larger sample size (N = 835) of the non–IMT CRC cohort. This technique was used to reduce the selection biasing effects of propensity score matching. Bias might arise after the selection of the matched pairs, as the non–observed samples might still be systematically different between the two groups. Therefore, performing the pairing with different one-to-many ratios can reduce the effect of selection bias without losing the optimal matching balance[44]. Balance statistics were determined for all matching ratios, and a slight imbalance was only observed in the case of the 1:10 pairs, where the non–IMT patients were older.

Description of the immunomodulatory treatments

The IMT modalities were used in different combinations, and their selection was determined as described previously[20]. In brief, next-generation sequencing analysis on tumor biopsies, circulating tumor cell assays, and tumor chemosensitivity assays were used. The following therapeutic options were available:

- Low-dose ICI therapy: 0.3 mg/kg ipilimumab (anti-cytotoxic T-lymphocyte-associated protein 4) plus 0.5 mg/ kg nivolumab (anti-programmed cell death protein 1) with 3 × 250 mL 2% taurolidine solution (equivalent of 15 g taurolidine). In the case of contraindication, such as ECOG > 1 or abnormal laboratory findings, ipilimumab or nivolumab monotherapy was used.

- Locoregional mEHT using either the Oncotherm EHY2000 (Oncotherm Kft., Budaörs, Hungary) or the Synchrotherm RF 600 T (Synchrotherm di Rolando Susanna, Vigevano, Italy) devices for 60 min.

- Water-filtered infrared-A hyperthermia (wIRA) using the Iratherm 1000 (Von Ardenne Institute of Applied Medical Research GmbH, Dresden, Germany) device.

- WBH uses the Heckel-HT2000 and the Heckel-HT3000 (Heckel Medizintechnik GmbH, Esslingen am Neckar, Germany) WBH devices. Based on the temperature reached during the treatment and the time spent in the device, three treatment variants can be distinguished: mild WBH (< 38.5 °C for up to 2 h), moderated WBH (38.5 °C–40.5 °C between 2 and 4 h), and long duration moderate WBH (38.5 °C–40.5 °C for up to 8 h). Furthermore, long-duration moderate WBH was performed under light sedation, and 300 mg/ m2 cyclophosphamide infusion was also administered.

- Artificial fever therapy: using interleukin-2 (Proleukin®) with 2 × 250 mL 2% taurolidine solution (equivalent of 10 g taurolidine, to mitigate the possible cytokine storm induced

by interleukin-2[45]). A total dosage of 5–14 million IU/m2 interleukin-2 was applied via a motor-syringe pump to reach a maximum fever temperature of 38.5 °C. Continuous body core temperature, blood pressure, heart rate and oxygen saturation monitoring was performed.

- Ozone therapy was administered using the HAB HERRMANN Hyper Medozon comfort (HERRMANN Apparatebau GmbH, Elsenfeld, Germany) device.

- Metronomic chemotherapy: a weekly dose of, e.g., 500 mg/m2 gemcitabine, or GemTaxol (500 mg/m2 gemcitabine + 60 mg/m2 paclitaxel). The chemotherapy agents were selected according to international guidelines.

- Other intravenous drugs:

  o High-dose vitamin C therapy: 0.5 g/kg vitamin C (capped at a total dose of 37.5 g per treatment) with 400 mg magnesium and 600 mg α-lipoic acid.

  o Curcumin: 150 mg in 500 mL 0.9% saline solution.

  o Dichloroacetate: 25 mg/kg in 500 mL 0.9% saline solution over 60 min.

  o Artesunate: 250 mg in 500 mL 0.9% saline solution over 90 min.

- Recombinant interferon-γ 1b (Imukin®): 0.5 × 106 IU, administered subcutaneously.

DETAILS OF THE CONVENTIONAL TREATMENTS

Conventional chemo(radio)therapy (CT) was based on national and ESMO guidelines. For CRC, local radiotherapy (only for rectal cancer, if feasible and needed); a cytotoxic doublet with or without a biological agent (bevacizumab or anti-EGFR recombinant chimeric monoclonal antibody) as the first-line and second-line treatment; and irinotecan + cetuximab and regorafenib or trifluridine/tipiracil as third-line or above was administered[46–48]. For PC, radiotherapy (if feasible and needed), gemcitabine, gemcitabine + nab-paclitaxel, or the 5-fluorouracil (5-FU) + irinotecan + oxaliplatin (FOLFORINOX) regimens were used[49].

CLINICOPATHOLOGICAL DATA

Medical history data including co-morbidities and recent medications were collected. Staging of the tumors was given by histopathological examination of surgical specimens and imaging studies; the 8th edition of the American Joint Committee on Cancer Staging was used[50]. Detailed location of the tumors was recorded for PC and CRC only, moreover, in CRC the sidedness of the tumor was defined as previously described[51]. Overall survival of patients was calculated from the diagnosis of the tumor until the death of the patient or until the termination of data collection (October 31, 2022). Patients alive at the time were right-censored.

STATISTICAL ANALYSIS

Statistical analyses were performed within the R for Windows version 4.2.2 environment (R Foundation for Statistical Computing, 2022, Vienna, Austria). Matching of the cohorts was performed via propensity score matching (R-package "Matching" version 4.10-8). Cohort comparisons were performed using Welch two sample t-tests, Fisher exact tests and Cochran-Mantel–Haenszel tests. Survival data of the cohorts were compared using "simple" and extended Cox survival models with time-dependent coefficients (R package "survival" version 3.4-0). To reduce the heterogeneity of the data, which could bias the model results, stratification was used to harmonize baseline hazards. If proportionality was violated, the survival models were extended with step functions, as described by Therneau et al.[52]. Naïve Kaplan–Meier curves were drawn with the R-package "survminer" (version 0.4.9). $P < 0.05$ was considered statistically significant. Survival, continuous, and count data were expressed as the hazard ratio (HR) with a 95% confidence interval (95% CI), the mean ± standard deviation, and the number of observations (percentage), respectively.

ETHICS DECLARATIONS

The study was approved by the Regional and Institutional Committee of Science and Research Ethics, Semmelweis University (SE TUKEB 133/2015, approval date: June 9, 2015; SE TUKEB 8/2017, approval date: February 16, 2017; SE TUKEB 21–14/1994, approval date of latest modification: February 23, 2021; and SE TUKEB 8–1/2017, approval date of latest modification: January 9, 2023).

CONSENT TO PARTICIPATE

Patient data were retrieved anonymously in a retrospective manner. At the Dr. Kleef Medical Center, all patients signed informed consent to the off-label treatment they received including consent to evaluate their data retrospectively for scientific publication. At Semmelweis University, due to the retrospective design of the study, signed informed consent was waived given the anonymized, de-identified data, after the approval of the Regional and Institutional Committee of Science and Research Ethics, Semmelweis University.

DATA AVAILABILITY

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Hyperthermia combined with chemotherapy vs chemotherapy in patients with advanced pancreatic cancer: A multicenter retrospective observational comparative study

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Citation

ABSTRACT

BACKGROUND

Several studies report the useful therapeutic results of regional hyperthermia in association with chemotherapy (CHT) and radiotherapy for the treatment of pancreatic cancer. Modulated electrohyperthermia (mEHT) is a new hyperthermia technique that induces immunogenic death or apoptosis of pancreatic cancer cells in laboratory experiments and increases tumor response rate and survival in pancreatic cancer patients, offering beneficial therapeutic effects against this severe type of cancer.

AIM

To assess survival, tumor response and toxicity of mEHT alone or combined with CHT compared with CHT for the treatment of locally advanced or metastatic pancreatic cancer.

METHODS

This was a retrospective data collection on patients affected by locally advanced or metastatic pancreatic cancer (stage III and IV) performed in 9 Italian centers, members of International Clinical Hyperthermia Society-Italian Network. This study included 217 patients, 128 (59%) of them were treated with CHT (no-mEHT) and 89 (41%) patients received mEHT alone or in association with CHT. mEHT treatments were performed applying a power of 60-150 watts for 40- 90 min, simultaneously or within 72 h of administration of CHT.

RESULTS

Median patients' age was 67 years (range 31-92 years). mEHT group had a median overall survival greater than non-mEHT group (20 mo, range 1.6-24, vs 9 mo, range 0.4-56.25, $P <$ 0.001). mEHT group showed a higher number of partial responses (45% vs 24%, $P = 0.0018$) and a lower number of progressions (4% vs 31%, $P < 0.001$) than the no-mEHT group, at the three months follow-up. Adverse events were observed as mild skin burns in 2.6% of mEHT sessions.

CONCLUSION

mEHT seems safe and has beneficial effects on survival and tumor response of stage III-IV pancreatic tumor treatment. Further randomized studies are warranted to confirm or not these results.

KEY WORDS:

Modulated electro hyperthermia; Locally advanced pancreatic tumor; Overall survival; Tumor response; Gemcitabine; Apoptosis; Immunogenic cell death

CORE TIP:

Pancreatic cancer has very poor prognosis with a 5-year overall survival of 5% and a median overall survival (OS) time of 8-12 mo. The concomitant use of modulated electro-hyperthermia (mEHT) in addition to chemotherapy has been introduced. mEHT has specific antitumor effects, increasing survival and tumor response. This was a retrospective data collection on 217 patients affected by locally advanced pancreatic cancer performed in 9 Italian centres, aiming to assess survival, tumour response and toxicity. The mEHT group had a greater OS (20 mo vs 9 mo, P < 0.001), higher number of partial responses (45% vs 24%, P = 0.0018) and a lower number of progressions (4% vs 31%, P < 0.001) than no-mEHT group. Adverse events were observed in 2.6% of mEHT sessions. mEHT have beneficial effects on survival and tumor response of stage III-IV pancreatic tumor patients, without adding toxicity.

INTRODUCTION

Pancreatic cancer (PC) is a particularly serious disease with poor prognosis[1,2]. Main risk factors of pancreatic cancer are non-hereditary and environmental factors, such as increased age, diabetes, obesity, smoking, alcohol intake and diet high in fat and low in vegetables. Only 10% of PC are familial or hereditary. Numerous molecular biology studies are underway the genes and pathological conditions involved in PC onset, in particular, the most studied are: Lynch syndrome, hereditary pancreatitis, Peutz-Jeghers syndrome, cystic fibrosis and breast cancer gene (BRCA)[2].

The Lynch syndrome is an inherited condition that is associated with 5% of colon cancer cases. Patients with this syndrome have about 10-fold increased risk of developing PC[3].

Hereditary pancreatitis is another rare inherited condition that is usually diagnosed in young individuals (< 20 years); its main symptoms are frequent episodes of severe inflammation of the pancreatic gland, leading to chronic pancreatitis. This pathology increases of about a 50% the risk of developing PC; this risk is increased when associated to smoke[4].

Peutz-Jeghers Syndrome is characterized by polyps in the small intestine and pigmented spots on nose and lips. Patients with this syndrome have a 10%-35% risk of developing PC[5]. Cystic fibrosis induces pancreatic insufficiency and chronic pancreatitis and increases of 5 to 6 times the risk of developing pancreatic cancer[6].

BRCA 1 and 2 mutations are often related to inherited ovarian and breast cancer. It is well known that the BRCA1 mutation can also induce an increased risk (4-9 times) of developing PC[7].

Over the past twenty years, PC prognosis has been improved by early diagnosis, the use of neoadjuvant, adjuvant and palliative therapies and the increased number of centres expert in hepaticpancreatic surgery.

Today PC is the seventh cause of cancer-related death worldwide with a median overall survival < 1 year[1,2]. Incidence and mortality of this cancer have increased significantly in the

last two decades[2]. The most common PC histology is adenocarcinoma that accounts for about 90% of cases. Radical surgery followed by adjuvant radiotherapy (RT) and/or chemotherapy (CHT) is considered the gold standard treatment for this tumor; however, surgery is indicated only in 10% of patients at the first diagnosis, whereas, 90% are non-resectable because they are locally advanced or metastatic. Neoadjuvant CHT or a combination of RT and chemo-therapy can be performed in locally advanced PC without evidence of distant metastases, in order to allow surgical approach[8,9].

FOLFIRINOX (leucovorin, fluorouracil, irinotecan, oxaliplatin) and the combination of nab-paclitaxel and gemcitabine are the more suitable upfront CHT for PC patients with good performance status (PS). These protocols have partial activity, resulting in similar overall survival (32-54 mo), but they have frequently severe side effects[8-10].

Recent studies on cisplatin therapeutic use in PC patients with BRCA 1 and 2 mutations report clinical benefit, although superiority over other chemotherapeutic treatments and optimal dosing and combination therapies are still ongoing. PARP inhibitors such as niraparib and olaparib have also promising results in this setting. They prevent single-strand DNA break repair, resulting in doublestranded DNA breaks that cannot to be repaired by homologous recombination deficiency tumors, causing cell cycle arrest and apoptosis. Olaparib increases progression-free survival, when used as a maintenance therapy for PC patients responding to first-line platinum-based therapy. Although these drugs show interesting results, however their efficacy is undermined by drug resistance and high cost [7].

PC is generally refractory to CHT and RT because of its low perfused and hypoxic microenvironment that is mainly due to large accumulation of non-tumor cells (stroma), obstructing vascularization and the delivery systemic chemotherapeutics. This also reduces oxygen delivery and hence the sensitivity to CHT and RT, impacting negatively on prognosis. In vitro experiments have shown that hyperthermia can increase the effectiveness of conventional treatments[15-19].

Regional hyperthermia (RHT) optimize the distribution of drugs in cancer cells, improves blood circulation, increases district oxygen level, reduces DNA repair and promotes cancer cell death[16]. RHT increases temperature inside the tumor up to 39.5/43 °C, using an external radiofrequency equipment. RHT is often associated to CHT and RT, in order to increase responses and overall survival (OS) in PC [20-24]. A systematic review shows the benefits of hyperthermia added to CHT and/or RT for PC patients from 14 clinical trials, resulting in median OS of 6-18.6 mo that is longer than that observed in the control cohorts and improved tumor response with 31.3% overall response rate that is greater than the control cohort. These data suggest that this combination positively affect tumor response and OS for PC patients[24-28].

Modulated Electro- Hyperthermia (mEHT) is obtained with a 13.56 MHz capacitive equipment and is a relatively new RHT technique (introduced in clinical practice in the last decade)[25,27,28-30]. This type of RHT is more selective in killing tumor cells and overcoming the limited tissue permeation of radiofrequency. There is no direct way to measure the temperature inside the tissues in vivo, however it can be predicted from input power applied,

because of the high efficacy of the electric field[29–34]. mEHT increases the temperature of the targeted malignant cells over 3 °C higher than surrounding environment[29].

The high heterogeneity of PC cells is counteracted by mEHT, which selectively damages the external membrane of cancer cells that have different energy uptake on the membrane rafts from healthy tissue and different electron impedance compared to normal cells[17–19].

Data from the literature show that mEHT is safe and feasible and has not only palliative but also therapeutic effects in several advanced cancer and also in pancreatic adenocarcinoma in monotherapy or in combination with CHT and/or RT[25,27,28,32–35]. This method seems to re-sensitize patients who are refractory to CHT and RT and improve palliation, increasing survival time, tumor response and quality of life (QoL), prolonging OS and improving QoL[25,27,28,32–34,36–39].

The purpose of this multicenter observational comparative study was to assess the advantages of mEHT in association to CHT compared to CHT alone in locally advanced or metastatic pancreatic tumors. This study was carried out by members of International Clinical Hyperthermia Society–Italian Network and created the platform for a large randomized phase III trial to compare outcomes with or without mEHT application.

Materials and Methods
Sample selection

This study was multicenter, retrospective, observational, comparative and case-control, aiming to assess survival and tumor response of CHT or mEHT plus CHT for the treatment of locally advanced or metastatic pancreatic tumors. Inclusion criteria were: Older than 18 years, informed consent signed, histological diagnosis of locally advanced or metastatic pancreatic tumor, PS of 0–2, treatment with CHT, mEHT or mEHT plus CHT, data on tumor response and survival. From 2003 to 2021, 628 patients with locally advanced or metastatic pancreatic tumor were treated in nine Italian centers, 217 of them were included in this study, 89 (41%) of them were treated with mEHT plus CHT (mETH group) and 128 (59%) with only CHT (no-mEHT group). CHT regimen was mostly gemcitabine-based in both study groups (Table1). The majority (95%) of gemcitabine-based treatments were administered on the same day of mEHT treatment. In a minority of patients (5%), it was administered the following day or within the following 72 h because of precarious clinical conditions and geographic accessibility. Even if gemcitabine had a half-life of 42–94 min and was eliminated within 5–11 h after infusion, the pharmacokinetic elimination half-life for dFdU varies between 2 and 24 h, and it is still present systemically in concentrations greater than 1 μmol/L up to 1 wk after infusion.

This was a retrospective data collection, CHT type was chosen by singular physician, we collected the data when the treatments (CHT and mEHT) were already done.

mEHT treatment protocols

mEHT was performed with EHY-2000 plus device (CEO123 Oncotherm, Torisdorf, Germany) and a radiofrequency current of 13.56 MHz as previously described[28–31]. mEHT treatments

were performed 1-3 times/week for a total of 4-6 wk, starting at 60 W/40 min and increasing up to 150 W/ 90 min in 2 wk[28]. Power increase was performed in the initial mEHT sessions, in order to assess patient treatment tolerance. Starting with the increased potency could have caused a feeling of heat and pain on the scars, as indicated in previous protocols that we have set up[32,36]. Power increase is performed in the initial mEHT sessions and was not used for efficacy purpose, the efficacy was measured after reaching the final power of 150 W/90 min. The computer connected to the hyperthermia machine has a program that calculates and converts the Kilojoules dispensed by the machine into degrees of temperature at the treatment site. This was represented on the screen and printed on graphs. Temperature on the target was 41–42.5°C during mEHT sessions as they were assessed in previous publications from the manufacturer of the device[38]. As concerning metastases, most patients had disease located in the upper abdomen and for this reason the treatment was suitable because we used an applicator-antenna of 30 cm diameter that allowed to treat the entire upper abdomen and multiple liver or nodal metastases at the same time.

OUTCOME MEASURES

The primary objective was OS that was measured from date of diagnosis to date of death or last available follow up date. The analysis of OS was made for the whole mEHT and no-mEHT group or by age ≥ 70 years or < 70 years. Secondary objectives were tumor response and treatment tolerability. Progression free survival (PFS) was computed from date of treatment start to progression.

Tumor response was measured with the Response Evaluation Criteria in Solid Tumors version 1.4 at three months follow up imaging. In case of multiple tumors, the target lesion was considered that with the largest diameter in outcome measures. Complete response was obtained if every target lesion disappeared; partial response (PR) if tumor diameter decreased by > 30%; progressive disease (PD) was observed if tumor size increased by > 20%, or one or more new lesions appeared. Stable disease (SD) was considered in all the other cases.

Since no data were available on cardiac toxicity due to radiofrequency disturbance from mEHT applied on upper abdomen; twenty-one patients out 89 treated with mEHT were monitored with electrocardiogram and echocardiogram before and after treatment in order to evaluate any alterations in cardiac rhythm and morphology.

As concerning the other adverse events were all monitored according to clinical practice and classified using the Common Terminology Criteria for Adverse Events version 5.0.

STATISTICAL ANALYSIS

Survival and patients' age were indicated as median and range values, whereas frequencies were indicated as percentages. Kaplan-Meier method and log-rank test were used or OS analysis, with survival probability on the Y axis and time (months) on the X axis. Mann–Whitney test and Student's test for proportions were used to assess statistical significance ($P \leq 0.05$) among differences of patient characteristics. $\chi2$ test was used to assess statistical significance ($P \leq 0.05$) among differences of tumor response.

Results

Sample description

The sample included 217 pancreatic patients: 122 (56%) were males and 95 (44%) were females, 89 (41%) of these were treated with the combination of CHT and mEHT (mETH group) and 128 (59%) with only CHT (no-mEHT group). Their median age was 67 years (37-89 years, range). Most patients were metastatic (65%). In the 217 patients, 235 metastatic sites were observed, with liver being the most frequent metastatic site (132/235, 57%). Previous CHT was administered to 136 (63%) patients, previous RT to 10 (5%) and surgery to 51 (24%). Most frequent CHT regimen was gemcitabine-based regimens: Gemcitabine-oxaliplatin (35%), gemcitabine (29%) and gemcitabine-abraxane (9%). Inclusion criteria indicated both radio and CHT in concomitant used with mEHT, however, none of the patients included in the study received RT in association to mEHT. The two groups had similar characteristics (Table 1).

Overall survival and progression free survival

mEHT group had a median OS greater than non-mEHT group (20 mo, range 1.6-24, vs 9 mo, range 0.4- 56.25, $P < 0.001$) (Figure 1). mEHT group had a median PFS greater than non-mEHT group (7 mo, range 2-24, vs 5 mo, range 0.4-41, $P < 0.05$) (Figure 2). The analysis of OS by age ≥ 70 years or < 70 years showed that there was no difference in OS between mEHT ≥ 70 years (20 mo, 2-43 mo range) and < 70 years (20 mo 3-27 mo range) $P = 0.235$, whereas no-mEHT < 70 years had a higher OS than no-mEHT ≥ 70 years group (12 mo range 1-56 vs 8 mo range 1-47, $P = 0.01$) (Figure 3). mEHT had a longer OS than no-mEHT group both among ≥ 70 years (20 mo range 3-27 vs 8 mo range 1-47, $P < 0.01$) and < 70 years (20 mo range 2-43 vs 12 mo range 1-56, $P < 0.01$).

Tumor response

Tumor response at three months follow-up was available for 87 (98%) of mEHT and 111 (88%) patients for non-mEHT group. mEHT patients showed a higher number of PR (45% vs 24%, $P = 0.0018$) and a lower number of progressions (PD) (4% vs 31%, $P < 0.01$) than no-mEHT group. SD had similar value in both groups: 51% for mEHT and 45% for no-mEHT (Table 2).

mEHT safety

Median mEHT sessions was 16.8 (range 6-25), resulting 1495 mEHT delivered sessions. Adverse events were observed in 2.6% of cases and included: Low grade (G1) skin pain in 22 (1.5%) sessions and lowmild grade (G1-2) burns in 16 (1.1%) cases that resolved in a few days. Hyperthermia did not increase haematological, hepatic, pulmonary and metabolic toxicity due to CHT.

In particular no increased blood pressure or any other cardiac changes were observed for mEHT sessions in patients who received adequate cardiological monitoring including clinical examination, electrocardiogram and echocardiogram.

DISCUSSION

Hyperthermia involves the application of heat-generating energy to tumors, increasing their temperature to 39–43.5 ∘C and improving the response to systemic therapies. Hyperthermia has been studied for cancer treatment since the 80s, especially for the benefits observed in laboratory experiments, clinical case series and phase II studies. The results of these studies show that hyperthermia enhances CHT, RT and immunotherapy efficacy (tumor response and survival) for several cancers and also in pancreatic adenocarcinoma[16–20,24–28,32–36].

Hyperthermia can be performed with different electromagnetic devices/techniques that can be classified as loco-regional or whole-body methods, according to the size of the body treated[26,31,40– 44]. First studies on hyperthermia effects in PC were made mainly using whole-body hyperthermia, whereas, more recent clinical studies apply mainly loco–RHT, such as mEHT that is non–invasive technique that balances low–power thermal effects and non–thermal electric processes, operating with capacitive coupled impedance on a radiofrequency of 13.56 MHz[25–31,37–38]. Current mEHT protocols show that optimal treatment is obtained when the mEHT is performed two or three–times a week, resulting in improvements of OS, disease control and QoL and PFS[25-28,33,39,40]. mEHT has also benefits in tumor control when used as neoadjuvant therapy in combination with CHT and RT[42].

Table 1 The sample, n (%)

	Whole sample, $n = 217$		mEHT, $n = 89$		No-mEHT, $n = 128$		P value[1]
	Median	Range	Median	Range	Median	Range	
Age (yr)	67	34-89	64	38-82	69	34-89	NS
Student's test for proportions							
M	122	56	58	65	64	50	NS
F	95	44	31	35	64	50	NS
Metastatic	142	65	70	79	72	56	0.004
Previous chemotherapy	136	63	68	76	68	53	0.005
Previous Radiotherapy	10	5	1	1	9	7	NS
Current chemotherapy type							
Gemcitabine/oxaliplatin	76	35	34	38	42	33	NS
Gemcitabine	62	29	26	29	36	28	NS
Gemcitabine/abraxane	39	18	9	10	30	23	NS
Gemcitabine/5-fluorouracil	5	2	4	4	1	1	NS
Gemcitabine/cisplatin	10	5	2	2	8	6	NS
Gemcitabine/Nab-paclitaxel	2	1	0	0	2	2	NS
FOLFIRINOX	5	2	1	1	4	3	NS
Other	10	5	5	6	5	4	NS
None	8	4	8	9	0	0	NS
Site of metastases	($N = 235$)		($N = 132$)		($N = 103$)		
Liver	132	57	70	53	63	61	NS
Peritoneum	55	23	35	27	20	19	NS
Lymph nodes	37	16	22	17	15	15	NS
Other	10	4	5	4	5	5	NS

[1]Mann–whitney test.

FOLFIRINOX: Folinic acid/fluorouracil/irinotecan/oxaliplatin; NS: Not significative.

Table 2 Tumor response, n (%)

	mEHT	$n = 87$	no-mEHT	$n = 111$	P value
PR	39	45	27	24	0.0018
SD	44	51	50	45	0.8430
PD	4	4	34	31	< 0.001

PR: Partial response; PD: Progression disease; SD: Stable disease; mEHT: Modulated electro–hyperthermia

mEHT can counteract heterogenicity of PC and its resistance to systemic therapy, because it targets selectively tumor cells (heating homogeneously the target tissues), exploiting their several biophysical differences from normal cells, such as energy absorption and damage–associated molecular patterns[16- 19,37,38]. mEHT induces programmed or immunogenic apoptosis of tumor cells, increasing DNA fragmentation, MAPK/ERK signaling pathways and pro–apoptotic Bcl–2 activation, low mitochondrial membrane potential, the concentration of intracellular Ca2+, Fas and c–Jun N–terminal kinases and the expression of pro–apoptotic genes (EGR1, JUN, and CDKN1A), while silencing other genes that are associated with cytoprotective functions[16–19,3O].
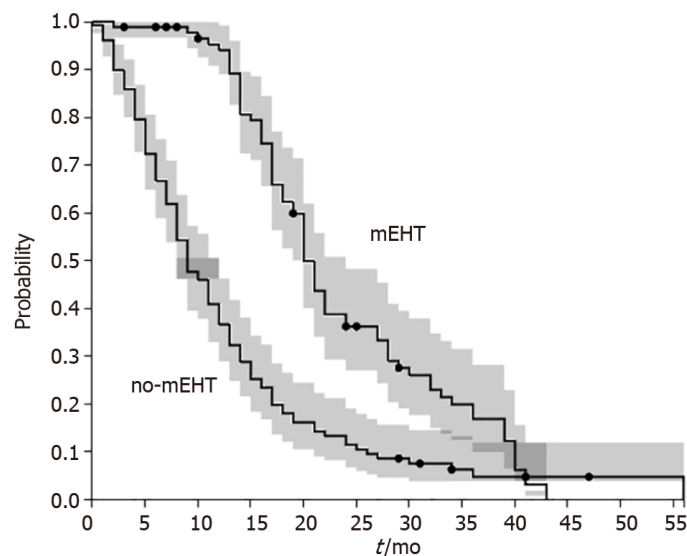
Figure 1 Overall survival of modulated electro–hyperthermia (mEHT) and no–mEHT groups. Dots represent censors, cloud area represent 95%CI. mEHT: Modulated electro-hyperthermia.
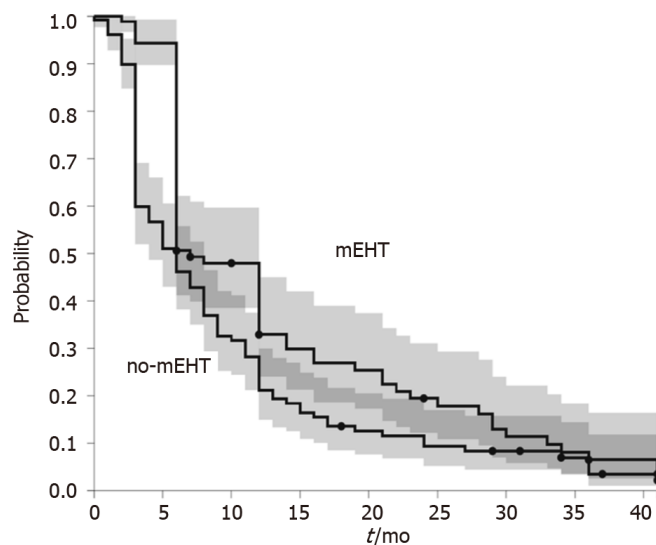
Figure 2 Progression free survival of Modulated electro–hyperthermia (mEHT) and no–mEHT groups. Dots represent censors, cloud area represent 95%CI. mEHT: Modulated electro–hyperthermia

In our study, mEHT group had a longer OS than the no-mEHT group (20 mo vs 9 mo, P < 0.001). This effect was present also among ≥ 70 years patients (20 mo, range 3-27 vs 8 mo, range 1-47, P < 0.01). mEHT group had also a median PFS greater than non-mEHT group (7 mo, range 2-24, vs 5 mo, range 0.4-41, P < 0.05). OS improvement was observed in other studies when mEHT was associated with CHT, resulting in an OS of 12.9 mo (95%CI: 9.9-15.9) and disease control rate of 50% in pancreatic cancer treatment [35-38]. Other studies on mEHT reported an OS of 8.9-19 mo and a PFS of 3.9-12.9 mo in advanced pancreatic adenocarcinoma [25-28,32,33]. The results of our study are in agreement with the above data, even if they used different types of deep hyperthermia devices but similar CHT regimens.

OS improvement was of particular interest for elderly patients who suffer most from side effects of CHT. The analysis of OS by age ≥ 70 years or < 70 years showed that there was no difference in OS between mEHT ≥ 70 years and < 70 years, whereas no-mEHT < 70 years had a higher OS than no-mEHT ≥ 70 years group (12 mo range 1-56 vs 8 mo range 1-47, P = 0.01). These data would recommend to use mEHT in elderly patients instead of a second or third line of CHT with heavy side effects.

The combination mEHT with CHT also improved tumor response, disease control rate (DCR = 96%), as it was reported in other studies (DCR = 71%-96%)[21,23,24,29]. mEHT group had a higher number of PR (45% vs 24%, P = 0.0018) and a lower number of progressions (PD) (4% vs 31%, P < 0.001) than the no-mEHT group. This was also reported in other studies, showing higher DCR of mEHT group than that of no-mEHT group (96% vs 77%, P < 0.05)[16-19].
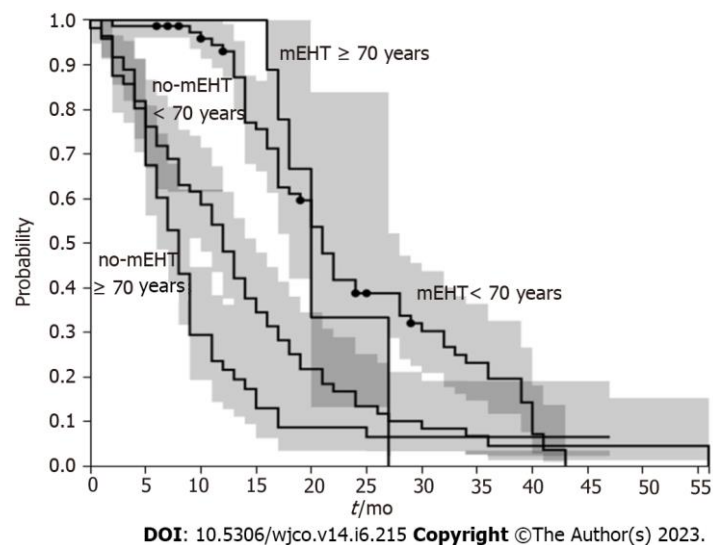
Figure 3 Overall survival of modulated electro-hyperthermia (mEHT) and no-mEHT groups divided by age. Dots represent censors, cloud area represent 95%CI. mEHT: Modulated electro-hyperthermia.

Adverse events that were related to hyperthermia were observed in 2.6% of the sample, showing G1- 2 pain and a skin burns. These data agree with toxicity reported in other studies (5%) and suggest that mEHT-related toxicity is low[25,28,33]. Little has been published on the possible cardiotoxicity of radiofrequencies produced by mEHT when applied to the upper

abdomen, in locations close to the heart. Twenty-one patients out 89 treated with mEHT were evaluated by electrocardiogram, echocardiogram before and after treatment with mEHT. We observed that upper abdominal mEHT does not generate rhythm disturbance, changes in electrocardiogram, cardiac morphology and blood pressure elevation.

The novelty of this study is the accurate reporting of a significant number of patients treated with mEHT compared with an equally large number of patients who received second and third-line CHT. The data on such a large total number of patients (217) and on the therapeutic effect of mEHT against PC has not been published before in the literature, of particular interest is also the improvement the of older patients' prognosis of older patients that reaches values comparable to that observed in younger patients.

The observational and retrospective nature of the study is the main limitation together with the long period of observation and inclusion from 2003 to 2021 during which many diagnostic and therapeutic approaches were modified and the still low number of patients treated with mEHT due the limited number of centers using mEHT in Italy. Future immunological research for PC treatment should be directed on circulating tumor cells (CTCs). It should be of interest characterize the transcriptomes of human pancreatic ductal adenocarcinoma CTCs, primary, and metastatic lesions at single-cell scale. Cell-interaction analysis and functional studies in vitro and in vivo reveal that CTCs and natural killer (NK) cells interact via the immune checkpoint molecule pair HLA-E:CD94-NKG2A. The breakdown of this interaction by blockade of NKG2A or knockdown of HLA-E expression could enhance NK-mediated tumor cell killing in vitro and prevents tumor metastasis in vivo[43].

Other methods of hyperthermia when the tumor is metastatic are currently being studied such as whole body hyperthermia but still seem to be at an early stage of application in clinical practice or burdened with excessive complexity[44].

Our study would like to open a new avenue in the treatment of locally advanced pancreatic cancer including mEHT in therapeutic options, since the results of CHT alone are not satisfactory. It is also necessary to know that the centers that practice hyperthermia at a good level are few both in Europe and in the World. Therefore, it is not easy to reach case studies with numerous patients to implement randomized trials. Future studies with a greater number of patients and randomized protocols are needed to confirm these results.

Conclusion

Skilled doctors who use RHT on a daily basis argue that the reasons for the progress hampered in this field are not the lack of effectiveness of technique but the paucity of qualified hyperthermia centres, lack of quality assurance processes, poor temperature monitoring and heterogeneous practices, lack of funding, poorer tolerance of older technology, limited access for the patients.

To overcome these limitations, we performed this study on a disease with high lethality where current therapeutic options in advanced stages are still unsatisfactory.

Therefore, our observations do not have the value of a prospective randomized study with defined and adequate times and methods of unfolding but, this study, despite many limitations, showed that mEHT group had improved OS and disease control rate in stage III–IV pancreatic cancer. These data are also reported for elderly patients. These data suggest that the association of mEHT to CHT is effective for the treatment of pancreatic tumors. mEHT was safe and had only mild toxicity. Notably not adding any toxicity compared to CHT. We hope that the results of our study will orient the scientific community to perform prospective, randomized, clinical study to further define the mEHT safety and efficacy in pancreatic cancer patients.

ARTICLE HIGHLIGHTS
RESEARCH BACKGROUND

Modulated Electro- Hyperthermia (mEHT) optimize the distribution of drugs in cancer cells, improves lood circulation, increases district oxygen level, reduces DNA repair and promotes cancer cell death. mEHT is effective for different types of tumors and also in pancreatic adenocarcinoma resulting in better tumor response and longer survival, as reported in clinical case series and phase II studies.

RESEARCH MOTIVATION

Pancreatic cancer is generally refractory to chemotherapy (CHT) and radiotherapy because of its low perfused and hypoxic microenvironment that reduces the efficacy and sensitivity to these treatments. The high heterogeneity of pancreatic cancer cells is counteracted by mEHT, which selectively damages the external membrane of cancer cells that have different energy uptake on the membrane rafts from healthy tissue, inducing immunogenic death of cancer cells or apoptosis.

RESEARCH OBJECTIVES

The aim of this study was to assess the advantages of mEHT in association to CHT compared to CHT alone in locally advanced or metastatic pancreatic tumors.

RESEARCH METHODS

This was a retrospective data collection on patients affected by metastatic or locally advanced pancreatic cancer performed in 9 Italian centres. This study included 217 patients, 128 (59%) of them were treated with CHT (no-mEHT) and 89 (41%) patients received mEHT alone or in association with CHT. mEHT treatments were performed applying a power of 60–150 watts for 40–90 min two or three times a week for 4–6 wk.

RESEARCH RESULTS

mEHT group had a median overall survival (OS) greater than non-mEHT group (20 mo, range 1.6-24, vs 9 mo, range 0.4-56.25, P < 0.001). mEHT group showed a higher number of partial responses (45% vs 24%, P = 0.0018) and a lower number of progressions (PD) (4% vs 31%, P < 0.001) than the no-mEHT group, at the three months follow-up. Adverse events were observed in 2.6% of mEHT sessions.

RESEARCH CONCLUSIONS

The results obtained in this study provided new evidence that mEHT is safe and has beneficial effects on survival and tumor response of stage III-IV pancreatic tumor treatment. Further studies are warranted.

RESEARCH PERSPECTIVES

This multicentre retrospective observational comparative study on 217 patients provides further evidence that mEHT improved OS and disease control rate in stage III-IV pancreatic cancer. This study would like to open a new avenue in the treatment of locally advanced pancreatic cancer including mEHT in therapeutic options, since the results of CHT alone are not satisfactory.

FOOTNOTES

Author contributions: Fiorentini G, Sarti D and Guadagni S wrote the paper; Fiorentini G and Sarti D performed the formal analysis; Fiorentini G, Sarti D, Bonucci M, Hammarberg Ferri I, Mambini A, Sciacca P, Ballerini M, Bonanno S, Milandri C, Nani R, Guadagni S, Dentico P and Fiorentini C collected the data; Fiorentini G, Sarti D and Bonucci M administered and supervised the project administration; Fiorentini G, Sarti D, Bonucci M, Ferri I, Mambini A, Sciacca P, Ballerini M, Bonanno S, Milandri C, Nani R, Guadagni S, Dentico P and Fiorentini C reviewed and edited the final manuscript.

Institutional review board statement: The study was approved by our Institutional Review Board.

Informed consent statement: Patients were not required to give informed consent to the study because the analysis used anonymous data that were collected retrospectively after each patient agreed and performed the treatment after signing the written consent for the treatment.

Conflict-of-interest statement: The authors declare that they have no conflict of interest.
Data sharing statement: The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

STROBE statement: The authors have read the STROBE Statement—checklist of items, and the manuscript was prepared and revised according to the STROBE Statement—checklist of items

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The Immunogenic Connection of Thermal and Nonthermal Molecular Effects in Modulated Electro-Hyperthermia

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ABSTRACT

Hyperthermia in oncology is an emerging complementary therapy. The clinical results depend on multiple conditional factors, like the type of cancer, the stage, the applied treatment device, and the complementary conventional therapy. The molecular effect could also be different depending on the temperature, heating dose, kind of energy transfer, and timing sequences compared to the concomitant treatment. This article examines the molecular impacts of a specific technique used in oncological hyperthermia called modulated electro–hyperthermia (mEHT). What sets mEHT apart is its emphasis on harnessing the combined effects of thermal and nonthermal factors. Nonthermal energy absorption occurs through the excitation of molecules, while the thermal component ensures the ideal conditions for this process. The applied radiofrequency current selects the malignant cells, and the modulation drives the nonthermal effects to immunogenic cell death, helping to develop tumor–specific antitumoral immune reactions. The synergy of the thermal and nonthermal components excites the lipid–assembled clusters of transmembrane proteins (membrane rafts) as the channels of transient receptor potentials (TRPs), the heat-shock proteins (HSPs), the voltage–gated channels, and the voltage–sensitive phosphatases (VSPs). All these transmembrane compartments channeling various ionic species (like calcium and proton) interact with the cytoskeleton and are involved in the apoptotic signal pathways.

KEYWORDS

Thermal, Nonthermal, Membrane Rafts, TRP, VSP, HSP, Cytoskeleton, Polarization, mEHT, Immune Effects, Abscopal Effect

I. INTRODUCTION

Electromagnetism appears as a continuous challenge in biology. The search for the use of electromagnetic effects for therapies ignited considerable research and hypotheses [1]. The ionizing radiation beam (high energy electromagnetic spectrum) shows immediate effects. The absorbed energy spectacularly destroys the biomaterial in the path of the beam. The non–ionizing radiation has less energy and more complexity. It modifies the chemical compounds and reactions and could impact enzymatic processes [2]. The complexity of living structures with physiological self–regulations challenges the therapeutic applications of non–ionizing effects. The challenges highlight the physiological importance of electric currents, deriving much intensive research, including neuroscience [3] and controlling the cellular effects [4]. A general hypothesis of "biologically closed electric circuits" (BCEC) introduced bioelectromagnetic homeostasis based on the existence of intrinsic electric currents in the body [5] [6], modified by malignant diseases [7] [8]. The pathological disorders [9] and wounds induce intrinsic injury currents [10], driven by the automatic biological charge transfers induced by the tissue– repair process [11] [12]. Observations have been made regarding the biological effects of low–level, non–stationary magnetic fields [13] [14]. The bioelectromagnetic effects may have resonance characters [15] [16] [17].

All electromagnetic interactions deliver energy to the biomaterials. The energy could be realized by heat (which may increase the temperature) and electron excitation (which makes chemical changes). These effects are naturally combined. The bioelectromagnetic interactions partly modify the chemical bonds and structure of compounds with electromagnetic forces, while the part of the energy absorption heats the target. The preferences may change the treatments. For example, radiotherapy breaks the DNA strands, modifying the chemical bonds, where the heating is an adverse effect, while the focus of hyperthermia is to heat and neglects the direct chemical effects of the electric field. Initially, hyperthermia used both the field and heat effects combined in the middle of the 18th century, but later it split by dominant electric (by French doctor Arsene d'Arsonval) and heat (by Danish doctor Kristian Overgard) effects. To produce, control, and understand the heat effects were more accessible, promoting its worldwide spread and helped by some industrial devices manufactured by Siemens in the early 20th century.

Nowadays, a novel approach tries again to combine the thermal and nonthermal factors of non–ionizing radiation using modulated radiofrequency (RF) signals [18]. The method (modulated electro–hyperthermia, mEHT [19]) applies definite heating in the fever range [20] and bioelectromagnetic effects in the bioprocesses using the nonthermal electromagnetic activity [21] [22] in the energy range selectively exciting transmembrane proteins on the malignant cells [23]. The principal selectivity of mEHT concentrates on the physiologic specialties of malignant cells and how they differ thermally and electrically from healthy ones. The malignant cells have a higher metabolic rate that drives the RF current by high ionic density in the tumor–cell microenvironment (TME). Moreover, healthy cells maintain homeostatic electrolyte concentrations in various regions by wellcontrolled electrolyte balance having body electrolytes in the right concentrations regulated by heart, kidney, and neurological function, controlling the acid– base, fluid concentration balance, oxygen delivery, carbon dioxide transport, and other processes in the complex human body. The kidneys maintain a massive sodium regulation, which balances the important Na/K balance and calcium concentration for cellular functions. Calcium is involved in the function of enzymes and serves in signal transduction pathways, acting as a second messenger, in neurotransmitter release from neurons, in contraction of all muscle cell types, and in fertilization. Cancer cells alter the electrolyte balance concentrations. Some tumors have hypercalcemia, and the dysregulated pH causes electrolyte imbalance in cancer.

The measured impedance between healthy and cancerous tissues exhibits significant differences [24]. This impedance assists in selecting appropriate radiofrequency (RF) parameters [25] and enhances current density within the cancerous tissue [26] [27]. MRI images the selection showing high RF current density in the tumor [28] and prove the self–selection of the malignant region by the current flow [29] [30]. Electrical impedance tomography pro provides further feasibility of focusing on impedance differences [31]. The preclinical experiments in various investigations show the temperature differences between the tumor and its surroundings (Figure 1).

The growing temperature on the membrane makes a particular thermal impact compared to the conventional heating (water–bath, wHT) (Figure 2).

The mEHT focuses on the complex equilibrium of the human body [39] with an appropriate technical solution [40], synergizing thermal and nonthermal energy components [41] by strong interaction of heat production with field effects [42]. The central concept of mEHT uses the natural homeostatic control of the human body [43], using a low–frequency modulation to stimulate healthy homeostatic regulation [44] [45].
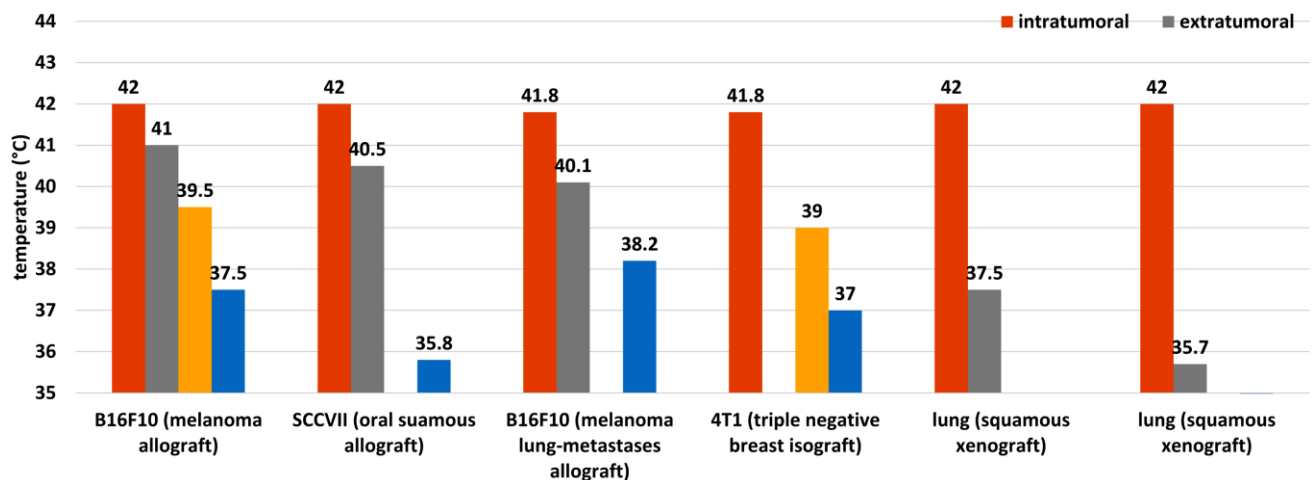
Figure 1. Temperature differences between the tumor and its surroundings, B16F10 (melanoma allograft) [32], SCCVII (oral squamous allograft) [33], B16F10 (melanoma lung–metastases allograft) [34], 4T1 (triple negative breast isograft) [35], lung (squamous xenograft) [36], lung (squamous xenograft) [37].
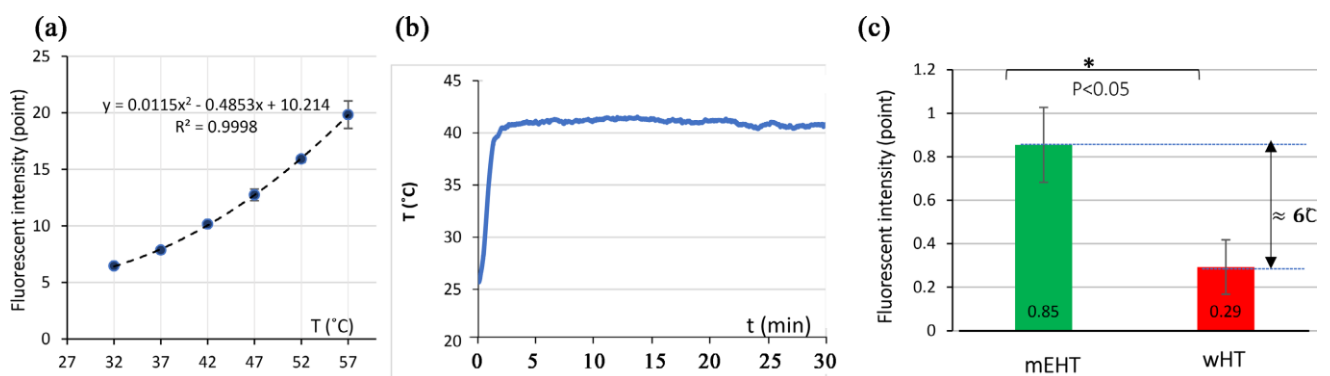
Figure 2. Measurement of the membrane temperature [38]. (a) Calibration of the membrane temperature by 10 µM DIL dye (RPMI + 10% FCS + 1% L–glutamine + 0.4% gentamycin). HT29 (human CRC cell line). Dilutions were kept at discrete temperatures for 30 minutes. (b) The temperature of the medium of the cell culture. (c) Significantly higher membrane temperature was achieved with mEHT than with wHT.

2. THERMAL IMPACT

The intensive metabolic activity of the malignant cells [46] increases electric conductivity by the ionic density in the TME. Furthermore, the tumor has a higher water content [47], which further increases the electric conductivity of the tumor. In this way, the entire tumor conducts better than its neighbor [48] [49] [50] [51]. An additional selection factor is that the malignant

processes destroy the networking orders [52] [53] [54]. The presence of the disorder leads to an increase in the dielectric permittivity (ε) of the microregion [55] [56] [57] [58]. Consequently, the electric current will naturally follow the most accessible route, which is typically the most conductive path, thereby flowing through the tumor. The water content within the tumor microenvironment (TME) interacts with the membrane [59], forming various bonds [60] and significantly impacting the membrane's functionality. This phenomenon results in a low specific absorption rate (SAR) but a high voltage drop [61], facilitating the excitation of raft proteins [62] by the signal. The electrostatic charge of the membrane attracts ions from the extracellular matrix (ECM), producing a diverse effect that is sufficient to establish a transmembrane potential [63].

Selective raft heating makes a higher cell-killing rate with apoptotic processes than conventional homogeneous water bath heating (wHT). A calibration curve by wHT describes the apoptotic rate by temperature. The mEHT heterogeneous heating has a higher impact on the membrane proteins than wHT (Figure 3).
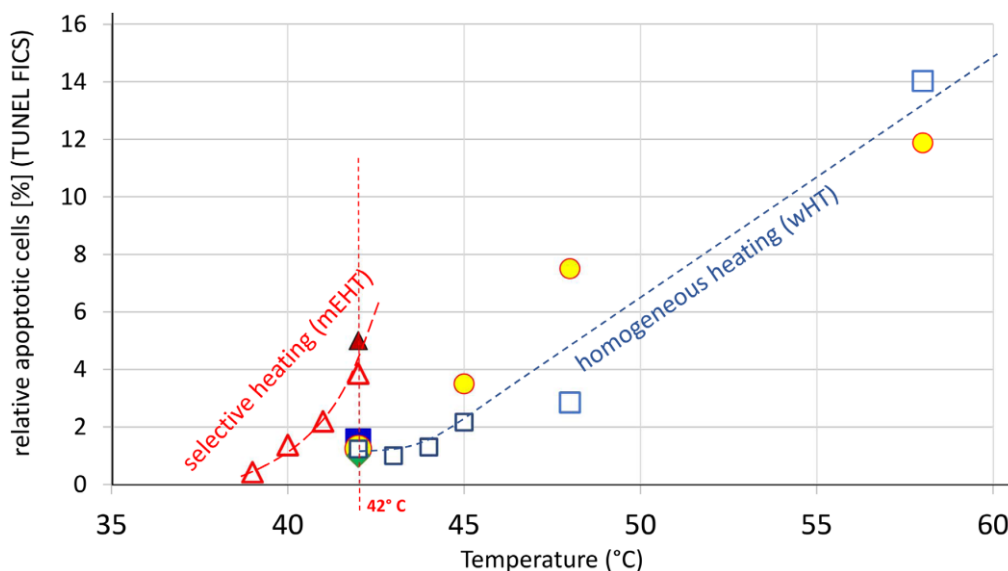
Figure 3. Thermal calibration with wHT measured with cell-lines U937 (□) [64], HepG2 (○) [65], CT26 (◆) [66], and the mEHT with U937 (△) [64], and HepG2 (▲) [65], cell-lines. RF homogeneous heating with a conventional capacitive device for HepG2 cells is also given (■) [65].

The transient receptor potential (TRP) channels are a set of transmembrane proteins and form a family of cation control channels [67]. These channels rectify the ionic transport, mainly calcium Ca2+, through the membrane. The rectification parameters primarily depend on thermal conditions sensing the relative to homeostasis oppositely in hot and cold temperatures. The hot sensing shifts the Ca2+ ion-flux to the opening direction [68], while the cold one shifts oppositely [69] to the closing side. TRPs regulate the membrane polarization, function as primary thermal sensors of cells [70], inducing action potential for physiological sensation, and cover chemo-, mechano-, and photosensation [71] of individual cells. The intracellular organelles and cellular compartments also have TRP channels in various vesicular processes [72] [73]. The Ca2+ intra and extracellular transmembrane ionic exchanges have a decisional role [74], allowing the individual cells to react to all intra and extracellular stimuli

signals. The intracellular TRPs actively participate in membrane fusion and fission, signal transduction, and general vesicular homeostasis [73]. The TRPV5 and TRPV6 are the only TRPV channels that are highly selective for Ca2+ [75]. Others have low or no selection on this ion. The TRPV1 is also a proton channel [76], which lowers the pH of the cytosol [76]. However, the Ca2+ –selective ORAI channel [77] has tight interactions with non and weak Ca2+ selective TRP channels and may activate the TRPCs while that may localize the ORAI [78].

The TRP channels have an exceptionally high temperature–coefficient Q10 [79]. It is notable that both the enthalpy and entropy components of its transition through the ion–permeability barrier are high [79].

The vanilloid receptors (TRPVs) cover the temperature sensing in mammals from low skin temperature (~25°C – 45°C TRPV4) to the necrotic high up to ~50°C – 60°C, TRPV2 [80] [81]. The hyperthermia fits TRPV3 (~24°C – 34°C) and TRPV1 (~41°C – 50°C) ranges [81].

The cell-membrane rafts became in focus [82] and well-studied [83]. The TRP receptors could also be a part of these clustered microdomains in the membrane and present effective thermosensors of the cell [84]. There are essential observations indicating a coherent cluster structure of a large number (~10^5) of voltage– gated ionic channels [85] [86], and it could have transient receptor potential (TRP) receptors in one temperature–sensing domain [84]. Small temperature changes may affect the TRP channels with membrane lipid assistance in the raft microdomain. The membranes are inhomogeneous, which is enhanced by the mild temperature change. Notably, the activated TRPV1 channel's ionic current may disappear at the multiple repetitions of the thermal ignition [87]. The opening of the TRPV channel for ionic current needs a relatively large enthalpy, while its closing depends less on the provided energy [88]. This asymmetry works oppositely in cold–activated channels like TRPM8 [89].

The primary energy absorbers in the mEHT method are the cytoplasmic membrane rafts, heating them selectively. The selection is based on these microdomains' high specific absorption rate (SAR). The thermal influence of mEHT has traditional hyperthermic functions, promoting the cell death in various ways [90]. Nevertheless, the difference is significant: the mEHT selectively heats the tumor. The selection focuses on membrane rafts, the cholesterol–stabilized microdomain cluster of transmembrane proteins [82], participating in the membrane dynamics [83]. The rafts have exceptionally high energy absorption from the RF current [91], allowing cellular selection of malignant cells with significantly high raft density [92] without substantially heating the healthy ones [93]. The concentrated heating of the molecular groups in the membrane rafts creates a heterogenic situation, where the rafts heat the entire cell and, in a second step, the tumor [94]. While the tumor, on average, remains in the <40°C fever range, the rafts reach < 3°C higher temperatures [95].

The temperature increase of the nano parts in the target is negligible with homogeneous heating [96]. However, when the heating is heterogenic (which is the case of all nanoparticle heating and in the mEHT too), the local SAR could be extremely high due to the small particle size. This is used in nanoparticle heating when the SAR on the nanoparticles could be as much as more than 1 MW/g (1,000,000,000 W/kg), depending on the absorber's concentration [97].

The inhomogeneous electric field in the case of mEHT, where the dielectrophoretic force drifts the rafts forwards, gives an additional factor to increase the micro–heterogeneity of cellular heating (Figure 4). This process exhibits a significant level of selectivity because the dielectric permittivity of the transmembrane proteins is at least two orders of magnitude higher than the permittivity of the surrounding membrane through which they traverse [98].

The TRP regulative processes are dynamic. The transmembrane protein displaces by the temperature action [99]. In malignant cells, the motility of transient receptor potential (TRP) channels is more pronounced compared to their healthy counterparts [100]. As a result, the drift movement of these channels indicates regions of higher energy density, where the specific energy absorption (SAR) is also elevated. The SAR values increase specifically at these points of the membrane known as micro–contacts (Figure 5(a)).
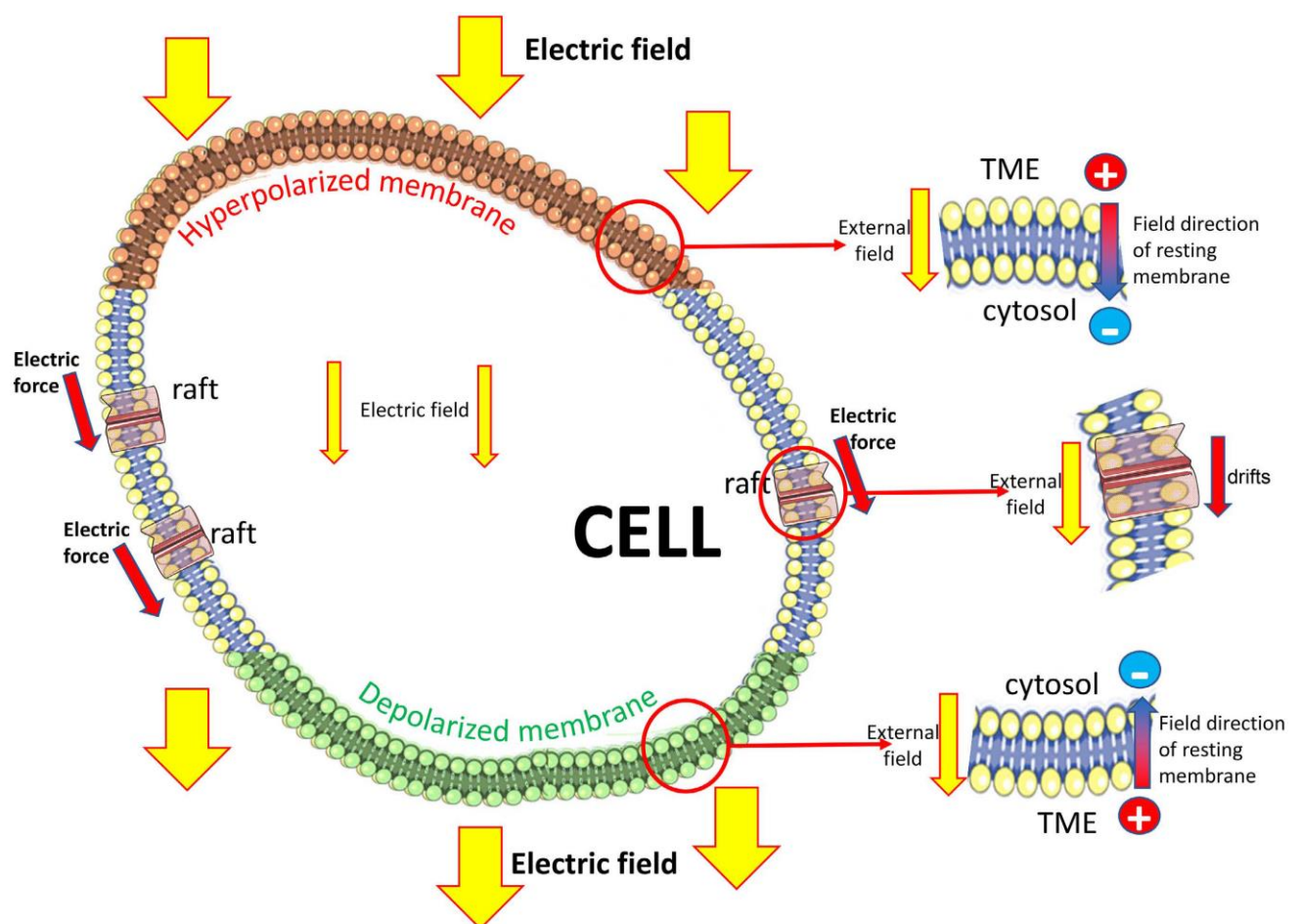

Figure 4. The electric field polarizes the membrane. Hyperpolarizing happens on one and depolarizing on the opposite side of the cell. The rafts have the electrophoretic force to drift by the electric force. The mEHT uses a 13.56 MHz carrier frequency. Consequently, the direction of all processes changes by ~0.07 μs, and so the movable proteins will be enriched in both sides of the cell.
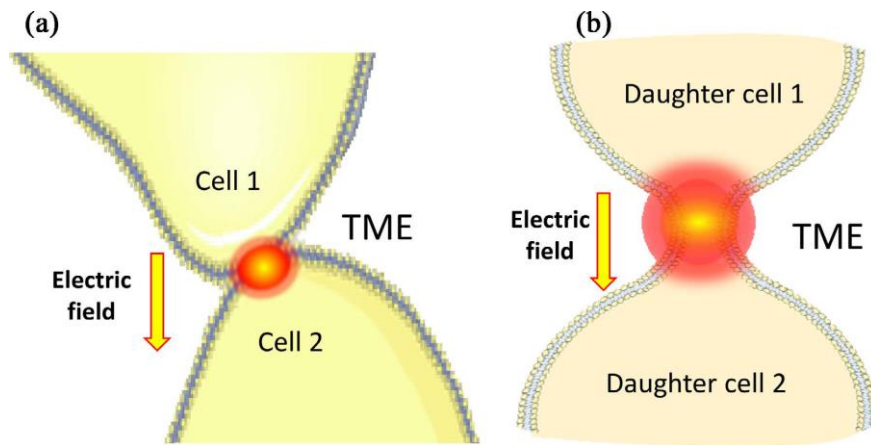
Figure 5. Developing extra hot spots on the cell membrane. (a) The cells touch each other, which enhances the SAR value and increases the spot's temperature. (b) The cytokinetic phase of mitosis has a "neck" between the forming daughter cells. This small area behaves like a touching point shown in Figure 5(a).

The telophase → cytokinesis phase forms another significant vital direct contact during the mitotic spindle, which has high importance in the proliferative malignant cells. The neck between the just-forming daughter cells induces cataphoretic forces [101]. The small cross-section of the neck may absorb an extremely high SAR when its directional position matches to electric-field vector. The absorbed energy by the cytokinetic "neck" depends on the cellular orientation, having a maximum when the field lines are directly parallel to the cytokinetic "neck" [102] [103]. The exceptionally concentrated energy may arrest the cytokinesis and block malignant proliferation [104] (Figure 5(b)).

In the outer membrane, dominantly the TRPV1, while in the membrane of intracellular compartments, the TRPV3 is thermally activated by mEHT's heterogeneous heating. The TRPs actively participate in membrane fusion and fission, signal transduction, and general vesicular homeostasis [75].

The thermal load has another well-known consequence: the development of protective chaperoning heat-shock proteins (HSPs) [105] [106]. The HSPs are also part of the complex regulation of the living organization, resulting in cellular defense or promoting cell death [107]. The complexity of HSPs questions their role as a "friend" or "foe" [108] [109] [110]. This dual behavior [111] [112] appears to decide their function as inflammatory or anti-inflammatory, pro-tumor or antitumor, immune-stimulatory or immune-suppressant, etc. The intensive thermal stress secrets membrane [113] and extracellular HSPs [114], which may reverse their cell-protecting activity [115]. The HSP expression can link to the plasma membrane processes by mild heat [116], which may cause non-specific clustering [117] by fever-like temperatures, where TRPs are particularly sensitive.

3. Nonthermal Impact

The electromagnetic effects differ between healthy and cancerous tissues. The essential differences appear in the conductivity and dielectric properties of the tissues. The breaking

healthy cellular network in cancer better conducts the radiofrequency current, and its dielectric permittivity (polarizability) is also significantly higher. Further differences appear in the electromagnetic excitability of the signal pathways in the cells due to the expressive contrasts of the healthy and malignant cellular membranes. The low membrane potential and high number of intercellularly unconnected transmembrane proteins appear in malignancy which interacts profoundly differently with the external electric field than the healthy cell. Using the apoptotic calibration in Figure 3, the impact of mEHT for cell–killing is rather significant (Figure 6). The basic structural disruption of healthy order makes the tumor also distinguishable by its electromagnetic interactions.

Any other than thermal stress that influences the homeostatic equilibrium also activates the HSP synthesis [118], which induces the cellular chaperoning function with HSPs. Living objects have not only thermal interactions. The dominant number of living regulation effects is not feasible with thermal effects. Enzymatic reactions and other molecular changes are mostly nonthermal, and their functions are mandatory for life. Thermal conditions are responsible for optimizing the nonthermal chemical reactions, and many biological processes synergize thermal and nonthermal components [119]. RF radiation induces nonthermal effects [120] together with the well–known thermal one for conventional hyperthermia treatments [121]. There is a large family devoted to voltage- gating ionic control [122] [123], which has an also large subfamily of voltage- gated calcium channels [124].
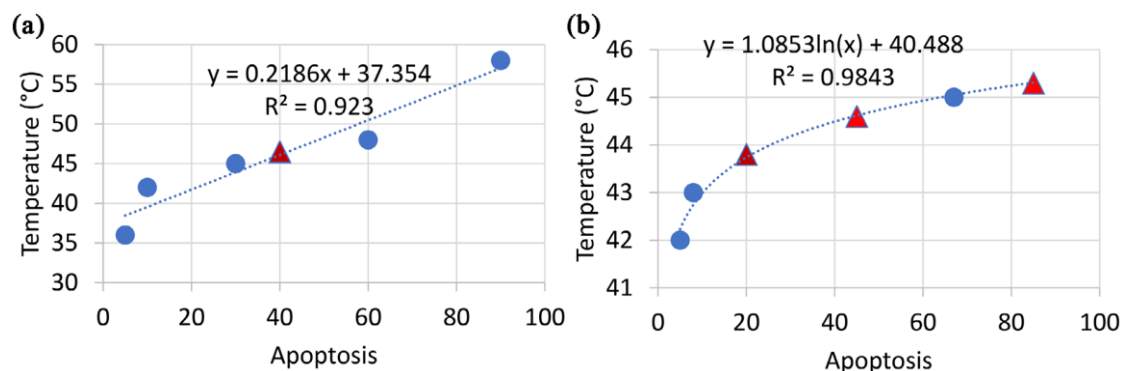
Figure 6. The heterogenic impact of mEHT ( ) compared to homogeneous heating ( ). The percentage of apoptosis shows much higher temperature of the process than the measured 42°C in the cell culture medium. (a) HepG2 [65], (b) U937 [64].

Even the thermally sensitive TRP channels also have nonthermal voltage dependence [125], which is effective in different temperature ranges. The high value of the transition entropy of TRPVs [126] shows that the transition follows Eyring's theorem [127]. This transition explains how the enzymatic reactions decrease the energy barrier with quantum–mechanical effects (e.g., tunneling [128]) and what mechanism is behind the reaction rate changes of catalyzes. The interdisciplinary applications [129] could use the quantum–mechanical considerations [130] [131] of the transition process, making possible a first–order phase transition when only the entropy changes the overall reaction rate, and the temperature remains constant when a new phase appears. The process is nonthermal, at least in its part. In this way, the large entropy in the TRPV transition allows the nonthermal transition when the temperature is unchanged but optimizes the complete process. The high entropy points the structural changes, which have a critical role in the nonthermal activity [132]. The probability of changes has a similar

expression for temperature and electric field [133], which further supports the nonthermal activity. Notable, while the repetition of thermal stimuli decreases the signal level [90], the repetition of the nonthermal chemical stimuli increases it [68].

The electric field produces such excitations, which is impossible with thermal conditions [21] [22]. A notable example showing the exceptional excitation ability of electromagnetic field is that using a broad-band (0.2 – 20 MHz) signal increases the HSP70 expression [134] in such volume that to produce the same rise of HSP70 by temperature, the perturbation should have been 14 orders of magnitude greater [135]. The electric field significantly modifies the cells [136], manipulates protein expressions, and induces extrinsic molecular pathways [137]. The modulated electric field's vibrational effect, along with the associated electro- osmotic process, operates within the intracellular environment [138], potentially triggering various processes within intracellular compartments. The polarization and polymerization may affect the cytoskeleton structure and the pathways that use it. The network of cytoskeleton forms and changes by the various signal transmissions in the cells. These polarizable parts of the cytoskeleton (their main components are the microfilaments, intermediate filaments, and microtubules, and their building blocks the tubulin dimers the actin subunits, and the fibrous subunits) are all interacting with external fields and are all capable of rapid growth or disassembly. The field-sensitive polymerization processes are the most basic of the mitotic rebuilding of the cell-structure of the newly born daughter cells. The membrane rafts incorporating thermosensitive TRPVs could considerably increase the Ca^{2+} influx to the cytoplasm by heating. The Ca^{2+} ionic balance has an important controlling function in homeostasis. It controls several processes in tumorigenesis [139]. It participates in gene transcription [140], cellular motility [141], invasion [142], cell-cycle regulation [143], and angiogenesis [144]. The balance between cell proliferation and apoptosis is tightly regulated by the influx of calcium ions Ca^{2+}. The intracellular concentration of Ca^{2+} plays a crucial role in determining whether a cell undergoes division or apoptosis [145]. Several steps of the killing of cancer cells are Ca^{2+} dependent [146]. The cytosolic and mitochondrial Ca^{2+} overload strongly stimulates the apoptotic processes [147] [148], but, as is usual in complex processes, the low nM Ca^{2+} concentration may help the survival of malignant cells by promoting proliferation by lowering the membrane potential [149] [150] and increasing the malignant differentiation [151]. On the contrary, the rise of intracellular Ca2+ concentrations to µM may support apoptosis [152].

The regulation of various pathways by Ca^{2+} together with its concentration dependence, a temporal component has an important role in tumorigenesis [153]. The amplitude and duration of Ca^{2+} signals involved are different proinflammatory activation of B lymphocytes [154] by decoding its information of amplitude and duration. Amplitude modulation of the Ca^{2+} signal may produce positive or negative antigen response in gene activation of B cells [155] [156].

Bioelectromagnetic interactions make numerous molecular excitations and chemical changes, which thermal interactions cannot achieve [157]. For example, the various intensities of electromagnetic signals make an entirely different activity of the hormone 5-hydroxytryptamine, which could even cause transepithelial potential oscillation [157].

These nonthermal effects characterize the mEHT method [21] [22] and appear in all molecular changes made by thermal effects, even in the TRPV thermal sensors [158] and HSPs [159] [160].

The mEHT uses the gaining possibility of the cell membrane. According to in-silico models, the electric field-strength gain, which refers to the ratio of the induced field within a material compared to the externally applied field, is highest in the cell membrane [161]. Specifically, for frequencies up to a few tens of MHz, the gain remains approximately $\approx 5 \times 10^3$ and follows a power-law decrease of 1/f as the frequency increases [161]. Inmore realistic tissue models, the membrane gain varies depending on the cell's position within the tissue, but it never falls below 10^2 in tissue arrangements [162]. In the case of cancer cells, while the intracellular gain is comparable to that of non-cancerous cells, the membrane gain in malignant cells is twice as high as that of their healthy counterparts [162].

The TRPV investigation of activating and deactivating potentials proves their voltage dependence [163] and that thermal conditions optimize voltage action [164]. In addition to heat and electric effects (like potential change or proton influence), the chemical effects (like capsaicin) and proinflammatory cytokines may activate the TRPV1 [165], which is upregulated in many cancer types [166]. The multiple influences make synergy [130] [167]. The multi-sensing behavior makes these channels critical for communication with the changes in TME. The voltage alone cannot wholly activate the TRPV1 channel [168]; the thermal component works for it in synergy, in the complexity of the membrane potential, ligand binding, mechanical force, and temperature [169].

An essential aspect of TRPs activity is its interplay with the cytoskeleton [103], which is based on electromagnetic interactions [170]. The role of the cytoskeleton in signal transduction and its connective role between the intra and extracellular information exchange makes it especially important. The connecting structure of TRP and cytoskeleton allows Ca2+ independent signaling [171]. TRPV1 essentially regulates the dynamics of the cytoskeleton by colocalization and stable binding with microtubules when there it is resting. However, in an excited state, TRPV1 rapidly disassembles the microtubule polymers [169].

The microtubules of the cytoskeletal network have a polymer structure [172]. The loss of the polymerization order of the cytoskeleton probably causes the high motility of cancer cells [173] because it makes the cells especially soft and detachable [174]. The increasing motility induces high metastatic potential and high deformability [175]. The extracellular matrix (ECM) plays a role in the cellular motility of cancer cells connected to its rigidity [176]. The heightened motility observed in cells is likely attributed to the loss of polymerization order within the cytoskeleton [172], resulting in increased cell softness and mobility [173].

The polymerization process follows a chain polymerization model known as Einstein's polymer [177] [178]. However, this model is unable to account for multi-bonding processes where chemical bonds can form branches in tubulins, leading to the creation of various space-filling structures. The reorganization of the cytoskeleton also promotes the formation of multi-strand cases. Multi-strand structures have longer chains compared to single-strand structures due to their multiple free ends and energy centers, making them energetically less favorable. As a result, according to Boltzmann statistics, the concentration of multi-strand chains is lower than that of single-strand chains. There is a relationship between the polymer concentration $[M_n]$ and the polymer length, expressed in terms of the number of monomers, denoted as "n" as

$$[M_n] \propto e^{-\frac{n}{n_0}}$$

where n_0 is constant. Consequently, the high concentration has shorter polymers.

The modulation employed in modulated electro–hyperthermia (mEHT) amplifies the effective electric field, thereby supporting the polymerization and reorganization of the cytoskeletal network. When using 1/f fractal noise modulation [15] [44] [45], this effect becomes more potent due to the continuous spectrum of frequencies present in the non–discrete noise signal. The application of noise modulation bears a resemblance to the harmonizing method [179], which is gaining recognition in the field of physiology [180]. In cancer cells, cytoskeletal polymerization holds particular significance, as the destabilized and incomplete polymerization of the cytoskeleton contributes to increased cell motility and facilitates metastatic spread.

The influence of the modulated electromagnetic field on the cytoskeleton can also involve voltage–sensitive phosphatase (VSP) [181]. Field–controlled phosphorous hydrolysis mediated by VSP could play crucial roles in cytoskeletal restructuring and exhibit resonant–type behavior. VSP, a macromolecule with a voltage sensor and cytoplasmic phosphatase domains [182], regulates the influx of calcium ions Ca^{2+} into cells [183]. VSP is sensitive to external fields and operates within the cytoplasm, allowing the transmission of external field effects to the cell interior. This process generates biochemical signals that may contribute to intracellular organization. Through these signals, it is possible to generate biochemical cues within the cytosol that can control internal processes, most likely including cytoskeletal polymerization. The fundamental mechanism involves membrane depolarization leading to phosphoinositol hydrolysis [184]. This is a reversible decomposition reaction that the external electric field may modify.

Phosphorylation plays a crucial role in regulating the activity of microtubule- associated proteins (MAPs) within the cytoskeletal network. The activation or deactivation of phosphorous groups controls the functioning of MAPs. Specifically, the phosphorylation of MAPs destabilizes microtubules by weakening the internal bonds that contribute to their structural stability [185]. The membrane potential of proliferative cells has a lower absolute value than that of quiescent neighbors [186]. Due to this, the malignant cells present low membrane potential [187]. Consequently, the VSPs influence the cytoskeleton in the permanently depolarized cancerous cells. The low level of cytoskeletal polymerization supports the proliferation and mobility of malignant cells.

The phosphorylation of MAPs not only affects microtubule stability but also plays a crucial role in the proper functioning of various ion channels, transporters, and vesicle movement within the cell. This mechanism enables the active modulation of intercellular electrolyte levels and protein connections in response to external electric fields. The dynamic stability of the system is governed by the Le Chatelier principle: a sudden change in membrane potential triggers phosphorylation, leading to an increase in potassium transport and the simultaneous suppression of sodium transport. This intricate process aims to restore the original membrane potential and effectively interacts with the cell proliferation process. The phosphorylation of VSP is energized by ATP. This energy consumption decreases ATP concentration, which

increases the depolarization of the cell membrane by suppressing the other, ATP-dependent active membrane transport of ions. The VSP has a role in anaerobic glycolysis and cancerous transformation when permanent stress conditions massively demand ATP. These conditions may activate the oncogenes, inhibiting apoptosis and producing high concentrations of stress proteins. This situation combats normal homeostatic regulation, so it is ideal for developing cancer.

The electric field polarizes the cytoskeleton's fibers [188], so it reorganizes the cytoskeleton in static (direct current, DC-field) [189] and dynamic (alternating current, AC-field) conditions [188]. The AC has the greatest influence at around 1 Hz [190], while in amplitude modulation of high frequency, it is optimal around 16 Hz (Adey window) [191]. This phenomenon exhibits resonant effects that can be described by stochastic resonance [192]. It assumes a bistable two-position state of voltage-sensitive phosphatase (VSP), like voltage-gated ion channels [193]. In the presence of a DC electric field, the membrane polarizes in opposite directions at different sides of the cell relative to the field vector. One side becomes hyperpolarized while the opposite side becomes depolarized. Depolarization triggers phosphorous hydrolysis, initiating cytoskeletal formation on the hyperpolarized side. However, in the presence of an AC electric field, both sides exhibit stochastic resonance and become hyperpolarized, leading to cytoskeletal reorganization. In the belt region perpendicular to the DC field, where there is no resonance, phosphorylation proceeds normally, creating a general phosphorous gradient in this region. The reorganization of the cytoskeleton is driven by specific forces, resulting in a pattern perpendicular to the pattern induced by the DC field.

The AC electric field induces resonance-like reorganization of the cytoskeleton, with a distinct peak at a specific frequency dependent on thermal noise [193]. It has been rigorously demonstrated that amplitude-modulated carrier frequencies can generate stochastic resonance, leading to various biological effects [193]. This phenomenon selectively stimulates enzymatic reactions, activates and deactivates voltage-gated ion channels, and reorganizes cytoskeletal polymerization processes [43] [45]. Moreover, the amplitude-modulated carrier can modify complex processes [44]. The modulating signal alone may also produce resonances. However, the requested low frequency alone, due to the impedance barrier of the skin, does not penetrate deep enough into the body. The 0.1 – 15 MHz high frequency has enough penetration to the human body and, as a carrier, delivers the signal for stochastic resonances. The optimal carrier frequency, as used in mEHT, may select the cancer cells with β, δ frequency dispersions [119] [194] and cause definitive apoptotic cell destruction of malignant cells [23].

Another approach is when the cytoskeleton polarization is optimized without modulation in high frequency (~0.2 MHz). These tumor-treating fields (TTF) target the cytokinetic "neck" with nonthermal polarization effects [195]. The electric field generated by TTF (Time-varying Tumor Treating Fields) exerts an influence on the polarizable microtubules and actin fibers within the cell. It has the potential to reorient these structures and, importantly, can impede the polymerization process of the cytoskeleton and hinder the assembly of the mitotic spindle [105]. The process does not use considerable SAR as mEHT does to arrest the proliferation by targeting the cytokinetic neck. This difference affects the treatment protocol. The TTF must be applied 18 hours/day for months, while mEHT with thermal optimizing has only 10 – 12 treatments for 60 min each.

4. Immune Effects

Immunogenic cell death is one of the main advantages of mEHT [196], which produces damage-associated molecular pattern (DAMP) [197] by extrinsic excitation of apoptotic pathways through various channels, including the TRAIL-R2 (DR5) death receptors [198] with the complex interaction of FAD + FASS molecules [41] [199]. The concomitant immune-stimulative treatments with dendritic cells [33] [66] and another stimulator [200], with the applied bioelectromagnetic forces by modulated RF signal [19] [44] [45] [201], improve the immunogenic processes. The immunogenic effects represent the oncology trend, especially oncological hyperthermia oncologic hyperthermia [18].

Due to the missing apoptosis, intensive proliferation is the assertive behavior of cancer. The mEHT, with its bioelectromagnetic excitations, promotes a massive preference for apoptosis against proliferative survival [23] [38] [202] [203]. Multiple excitable transmembrane proteins exist in the malignant cells (Figure 7(a)). The excitation could be thermal or nonthermal, but dominantly the synergy of the two, when the thermal process ensures the optimal conditions for the nonthermal excitation. The apoptosis may go through various signal pathways (Figure 7(b)).

The apoptosis uses an extrinsic pathway through Caspase-8 (Cas-8), an intrinsic pathway (Cas-9) finally, with Cas-3, to the programmed cell death (Figure 8). The caspase-independent way through apoptosis-inducing factor (AIF) is also activated, making it possible to execute the apoptosis when the caspase paths are blocked.

When one pathway is blocked by the malignant evasion of apoptosis, the other pathways may completely substitute the missing line.

The elevated levels of BAX observed in the affected cells [205] [206] further support the apoptotic effect induced by the treatment. The increased presence of BAX suggests the activation of apoptotic pathways and reinforces the notion that the treatment is triggering programmed cell death in the affected cells. The selective energy absorption of mEHT produces heterogenic membrane temperature, intensively heating the transmembrane proteins, including the TRPV channels. The TRPV promoted Ca^{2+} influx massively overloads the intracellular conditions with Ca^{2+} concentration. The thermal and nonthermal synergy of mEHT ensures the requested apoptotic level of Ca^{2+} overload.
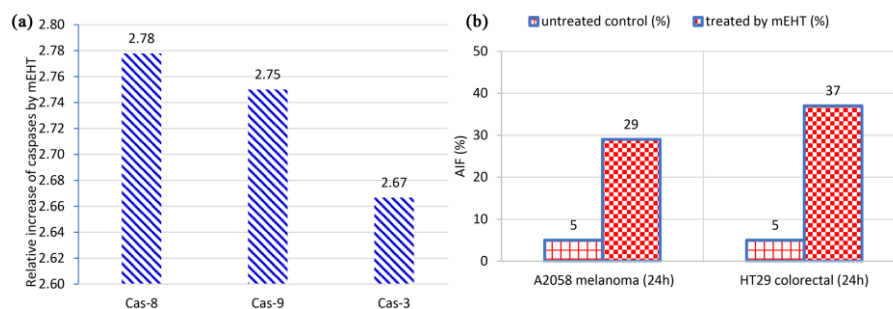

Figure 7. The critical protein involvements in apoptosis by mEHT. (a) The caspase involvements relative to the untreated samples (HepG2 cell-line in vitro [65]). (b) The AIF

percentages in the samples from in vivo xenograft experiments for A2058 melanoma [204] and HT29 colorectal carcinoma [205] tumors.
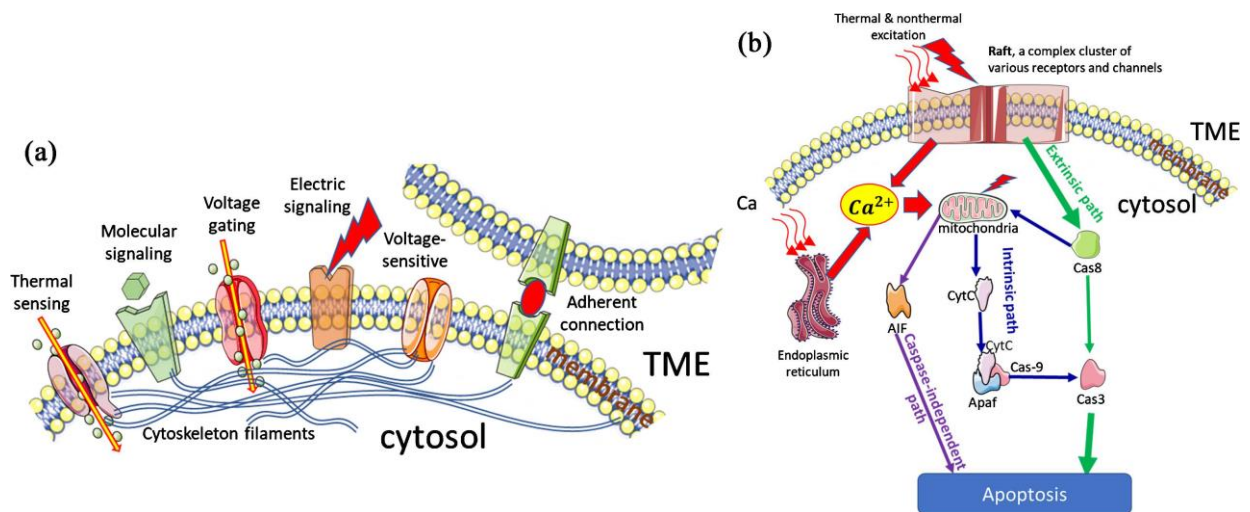

Figure 8. Forming apoptotic signals. (a) Multiple excitable transmembrane proteins accept the thermal and nonthermal effects from mEHT. (b) Multiple signal pathways are available, ensuring that a block in one can not terminate the apoptotic process.

The thermal and nonthermal stresses imposed on homeostatic control typically stimulate the production of chaperone stress proteins. The chaperoning HSPs are exhausted by mEHT and cannot meet the chaperone function to protect the cells against apoptosis [207]. A part of HSP70s is secreted on the membrane [119], and this localization [208] promotes apoptosis [209] and has a vital role in the membrane "fluid' to keep it functional [210].

While cancer has strong proliferation, it is weak in its loneliness. The cellular autonomy is a weak side, which offers a correction possibility. Immune surveillance is critical in attacking the weakness of malignancy and guaranteeing homeostatic balance. The counterbalance of the evasion of immune effects by malignant cells needs local and systemic activity, which rebuilds the standard healthy conditions. The autonomy of malignant cells shows the breaking of adherent and junctional connections with the neighboring cells, substantially modifying the homeostatic electrolyte composition and concentration in the TME [26], which offers an electric selection factor [211] [212]. The reorganization of the cytoskeleton by nonthermal electric polarization of mEHT promotes the form (β-catenin + E-cadherin) complexes [65] [213], giving a possibility to reestablish the lost adherent connections and fix the cancer cells in their position, block the dissemination.

Immune control creates an additional possibility to block the cellular autonomy of malignant cells by destroying the freely moving cancer cells. The great and unique advantage of tumor–specific immune activity is its ability to find and eliminate distant micro and macro metastases locally and systemically. The frequently applied CAR–T method has the same purpose [214], preparing tumor- specific T-cells from the patient's blood sample. Immune effects integrate the TRP channels for multiple purposes [215]. All immune cells, including NK cells [216], T-cells [217], and dendritic cells [218]), have TRPV1 channels with significant functions. The CD34+ hematopoietic stem cells express TRPV2, which is also expressed in

granulocytes, monocytes, and CD56+ natural killer cells and orchestrates the Ca^{2+} signals in CD4+ and CD8+ T-cells and CD19+ B lymphocytes [219]. Moreover, TRPV1 modulates macrophage-mediated responses [220].

The adaptive immune reaction was measured, detecting significant development of DC cells (S100) for maturation (antigen-presenting) CD3+ CD4+, CD8+ T-cells and suppressing Treg cell-population (Foxp3) (Figure 9) [33].

The TRPV1 and TRPV4 channels have been implicated in T-cell activation and the production of effector cytokines. These channels play a role in suppressing the release of tumor necrosis factor (TNF) and interleukin-2 (IL-2), which are important immune signaling molecules involved in inflammation and immune responses [217]. Furthermore, the TRP channels have a critical role in controlling phagocytosis, the production of chemokines and cytokines, and cell survival [221].

The HSPs are not less important in cell fate and immune activity than TRPs. The thermal and nonthermal stress combination overloads the malignant cells with chaperoning HSPs, which have much more function than only chaperoning, depending on their position in the cell [210].
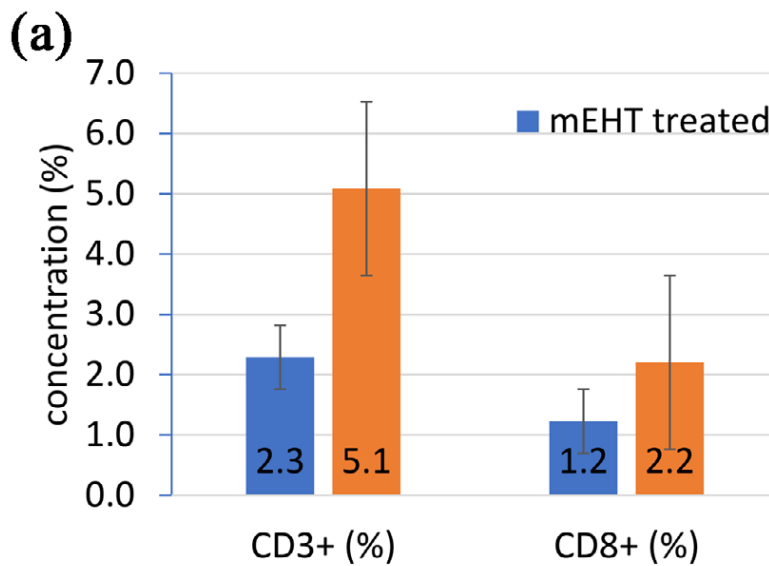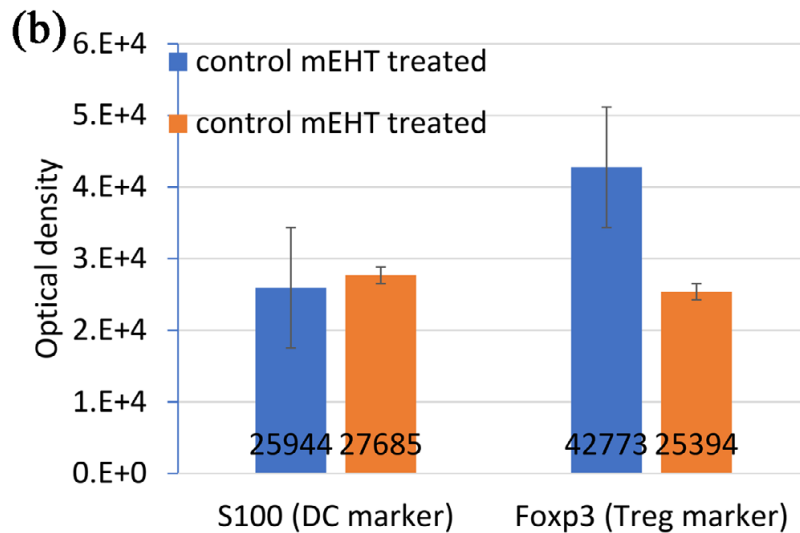
Figure 9. The relative development of the critical immune-surveillance cells in SCCVII allograft after mEHT [33]. Measured with (a) flow cytometry and (b) optical density.

To initiate the apoptotic process, the mitochondrial heat shock proteins (mHSPs) must first bind to a complex formed by tumor peptides [222]. The membrane-localized HSP70s [223] are mainly localized in the rafts [224] and activate the NK cells in the immune response [204] [225].

A part of the HSPs may leave the cells and become extracellular [226]. Their importance in building up an appropriate immune response is crucial. These released molecules deliver tumor-specific information for antigen presentation to develop antitumoral immune reaction [115], which process attracts much attention in studies of the overall immune reactions of the bio-systems [227] [228]. The antigen-presenting cells (APCs) develop the tumor-specific CD8+ killer and CD4+ helper T-cells, which are delivered by the bloodstream and combating with the cancer cells in the entire body (abscopal effect) [229]. The local treatment developed a whole-body effect by mEHT [230] (Figure 10). The in-situ and real-time production of tumor-specific immune activity is the advantage of mEHT [231].

Significant development of the DAMP molecules characterizes the results of mEHT treatment. The abscopal effect of distant, untreated tumors was observed when immune stimulation was added to the protocol (Figure 11) [200].

The thermal and nonthermal effects work in synergy to produce the in-situ immune effects with mEHT. In the thermal aspect, the relative mild tissue temperature is crucial for immune development. The enhanced temperature eases the enzymatic processes and increases the molecular reaction rate, but a higher temperature than 40°C blocks the activity of the immune cells [232] and so does not allow the real-time processes for APC prepared by immunogenic cell death [231]. Furthermore, the > 40°C temperature paralyzes the NK cells to attack the cells with the marks of the HSP70s on their membrane [233]. The immune cells may restore their activity with elapsing time [234] or by bloodstream replace them from the non-treated parts of the body, but the real-time processes with the simultaneous reactions vanish. The heterogenic heating of mEHT with the high temperature of rafts and simultaneously mild of

the tumor microenvironment (TME) solves the contradictory demands of the hyperthermic temperature range and the immune requirements.
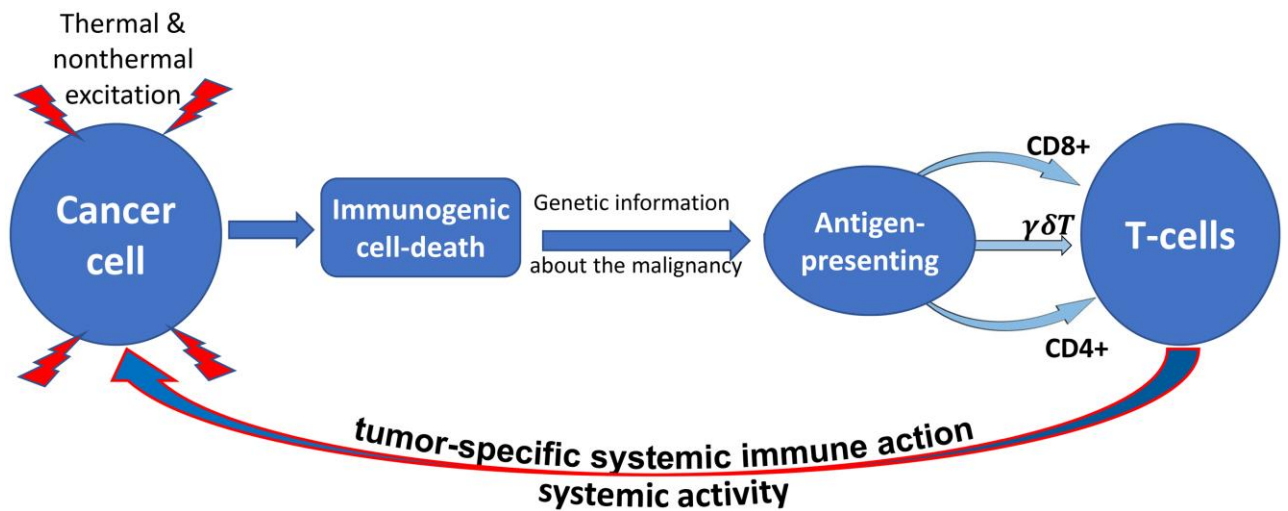
Figure 10. The thermal and nonthermal processes develop immunogenic cell death. The genetic information used by antigen–presenting produces tumor–specific T–cells. In this way, the adaptive immune machinery starts a systemic attack of malignant cells all over the system.

(a)

Calreticulin (12 h): 1.1, 0.9
HMGB1 (24 h): 1.0, 1.0
HSP70 (24 h): 1.0, 1.9
S100+ (DCs) cells (x10) [%]: 1.3, 0.9

Sham in treated tumor   mEHT untreated tumor

(b)

Calreticulin (12 h): 54, 34
HMGB1 (negative) (24h) [%]: 20, 60
Apoptrosis (24 h) /x100: 39, 1
S100+ (DCs) (48h) x10 [%]: 20, 7
T-cells (CD3+) (72h): 27, 8

mEHT treated location   untreated location
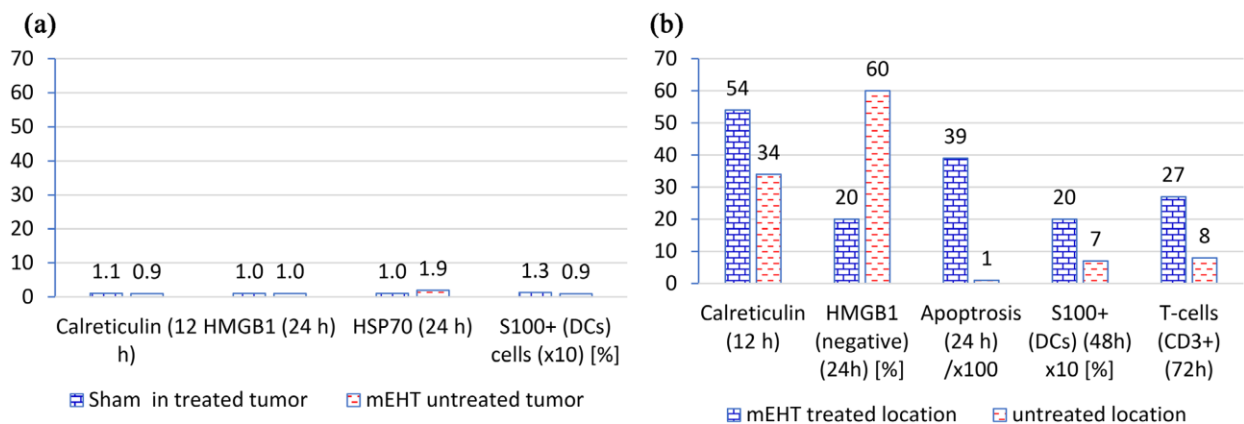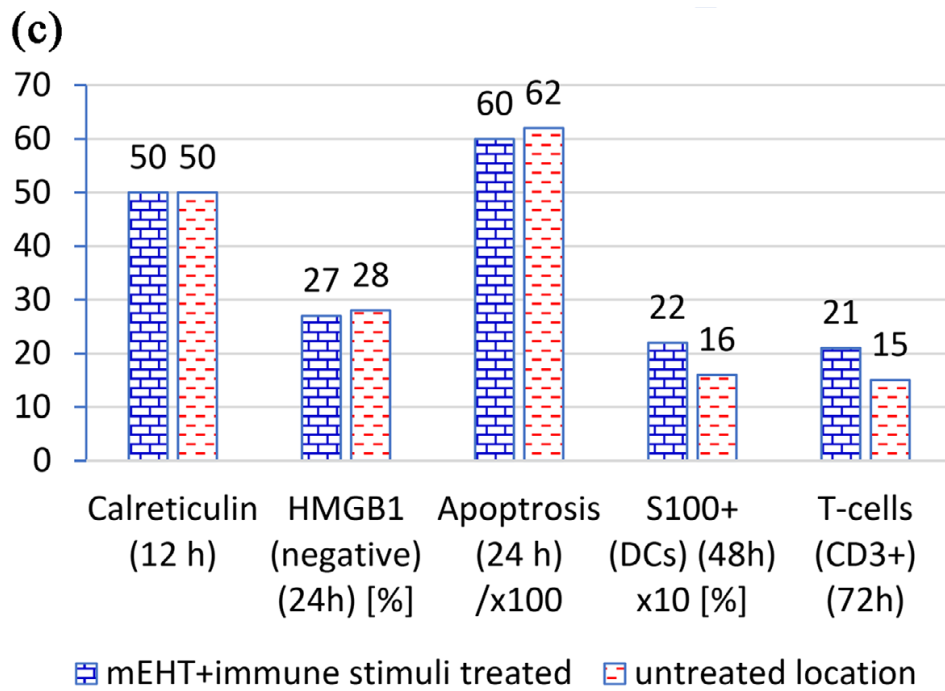
Figure 11. Development of DAMP molecules in colorectal allograft preclinical experiment of C26 tumor [22, 200]. (a) The sham treatment and untreated tumor on the same animal had treatment at a distant location. (b) Development of the DAMPs in the treated and untreated tumors on mice. (c) Development of DAMPs in treated and untreated locations when immune stimulants were added to the protocol.

The nonthermal processes in mEHT are also essential for the abscopal effect. The excitation of the TRP channels sensitizes the immune lymphocytes [215] and promotes apoptosis [101], helping with the ICD processes. The substantial nonthermal stress exhausts the evasion of apoptosis in cancer cells and produces transmembrane HSP70, which also supports apoptosis [209]. It activates ligands for NK cells [208], forming an innate immune response [235], especially in tumor cells [223]. The well–forming temporal order by mEHT of membrane secretion calreticulin, the extracellular release of HMGB1 and HSP70 developing the APCs for an active adaptive immune response [231].

Numerous preclinical studies prove the specialties of mEHT (Figure 12). A recent review summarizes the results [236].

Based on the regulatory conditions the mEHT method received the necessary certifications and numerous clinical trials were performed in different hospitals in various countries (Figure 13). Some protocols allow geriatric and pediatric considerations, too. A recent review summarizes the results up to 2019 [242].
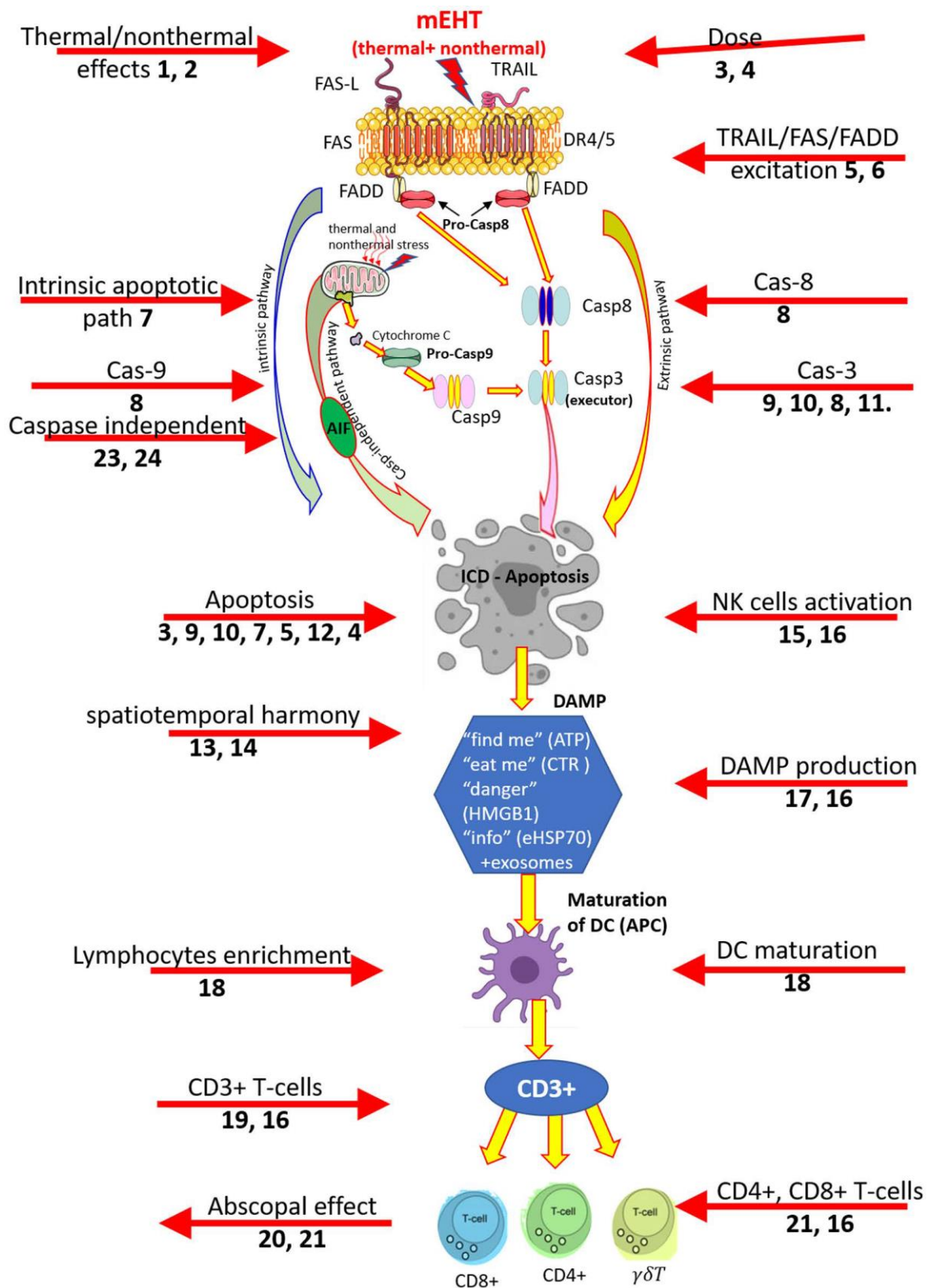
Figure 12. Some preclinical experiments with mEHT method. The numbers refer on the references: 1. = [42], 2. = [237], 3. = [238], 4. = [94], 5. = [202], 6. = [198], 7. = [239], 8. = [65], 9. = [207], 10. = [203], 11. = [240], 12. = [64], 13. = [207], 14. = [206], 15. = [204], 16. = [32], 17. = [205], 18. = [66], 19. = [241], 20. = [200], 21. = [33], 23. = [205].

5. Conclusion

The modulated electro-hyperthermia focuses on the nonthermal and thermal effects synergy when the thermal component provides optimal conditions for the nonthermal electric molecular excitation. In this review, we concentrate on the role of the ionic channels as TRPs, VSPs, and voltage-gated channels in the selective antitumoral processes. These transmembrane compartments primarily promote the Ca^{2+} and the H + influxes, interact with the cytoskeleton and are involved in the apoptotic signal pathways. The DAMP forming TRAIL–FAS-FADD excited extrinsic apoptotic signal combined with the Ca^{2+} induced apoptosis ensures a "gentle" distortion of the malignant cells, which, together with the fully functioning other DAMP molecules, uses the membrane secreted and extracellularly released HSPs with the exhaustion of their intracellular chaperoning to form APCs. The innate and adaptive tumor-specific immune activity appears by the membrane HSP70 and the APC-produced killer and helper T-cells. The
bloodstream-delivered T-cells attack the cancer cells all over the body (abscopal effect), so the immunogen processes transform the local mEHT to systemic.
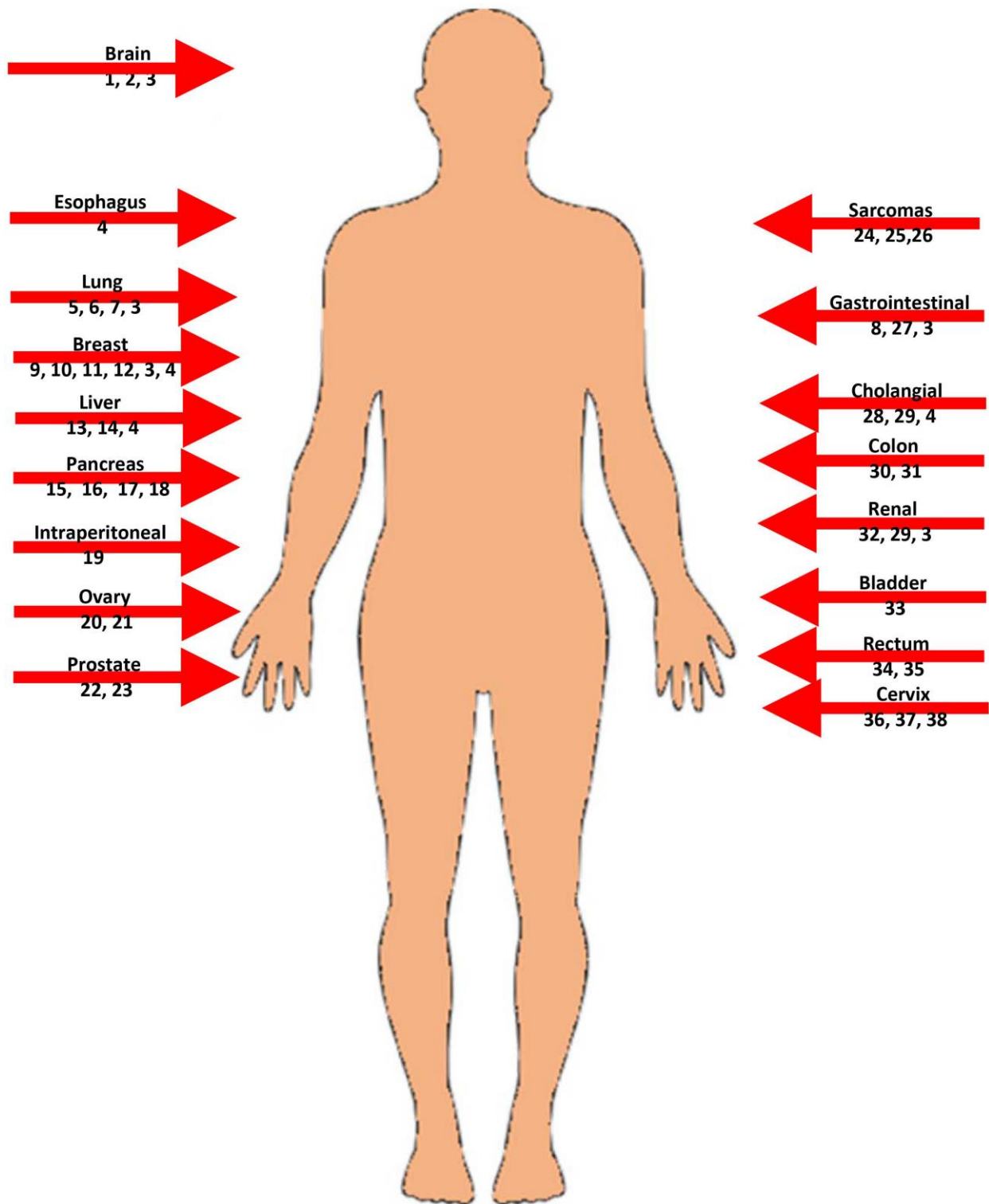
Figure 13. The clinical studies with mEHT. It contains various levels of evidence including case reports and phase II/III trials. The numbers refer to the references: 1. = [243], 2. = [244], 3. = [245], 4. = [246], 5. = [247], 6. = [248], 7. = [249], 8. = [250], 9. = [251], 10. = [252], 11. = [253], 12. = [254], 13. = [255], 14. = [256], 15. = [257], 16. = [258], 17. = [259], 18. = [260], 19. = [261], 20. = [262], 21. = [263], 22. = [264], 23. = [265], 24. = [266], 25. = [267], 26. = [268], 27. = [269], 28. = [270], 29. = [254], 30. = [271], 31. = [272], 32. = [273], 33. = [274], 34. = [275], 35. = [276], 36. = [277], 37. = [278], 38. = [20].

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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Elektromágneses eljárások a hasnyálmirigyrák kezelésében: eminens vagy renitens?

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Citation

Az előrehaladott stádiumú hasnyálmirigytumorok kezelésére korlátozott lehetőségek állnak rendelkezésre. Korábbi vizsgálatok alapján a modulált elektro-hipertermia (mEHT) alkalmazása előnyös e betegpopulációban. Az optimális kezelésszámról és a kezelés megkezdésének optimális időpontjáról azonban nincs adat. Retrospektív megfigyeléses vizsgálatunkban 96 fő mEHT-kezelt, illetve 86 fő kontroll, korban és nemben illesztett, előrehaladott stádiumú pankreásztumoros beteg kezelési adatait elemeztük. A minimális mEHT-kezelésszám alapján korban, nemben és tumorlokációban illesztett mEHT-kezelt és kontroll betegpárokból álló kohorszokat alakítottunk ki. Legalább 10, 20, 30 és 40 kezelésben részesült 76, 57, 38 és 33 beteg. Eredményeink alapján a legalább 30 (HR: 0,5011; p = 0,0041) és 40 (HR: 0,5048; p = 0,0085) mEHT-kezelésen átesett betegek túlélése szignifikánsan hosszabb, a várható medián túlélésük közel kétszerese a kontrollcsoporténak (10 vs. 18 hónap). Az mEHT bevezetése a diagnózist követő első (HR: 0,5382; p = 0,0056) és második (HR: 0,7861; p = 0,0031) 6 hónapban jár a legnagyobb előnnyel. Magy Onkol 67:194–201, 2023

The treatment of advanced-stage pancreatic cancers is limited. Previous studies have found that the use of modulated electro-hyperthermia (mEHT) is beneficial in this patient population. However, there is no data on the optimal treatment number and initiation period. Therefore, a retrospective study was conducted with the inclusion of 96 mEHT-treated and 86 age- and sex-matched control pancreatic cancer patients. 76, 57, 38 and 33 patient pairs were enrolled into propensity score matched cohorts, whether they received at least 10, 20, 30 and 40 mEHT treatments, respectively. The survival of patients with at least 30 (HR: 0.5011; p = 0.0041) and 40 (HR: 0.5048; p = 0.0085) mEHT treatments was significantly longer, median survival was almost twice as long (10 vs. 18 months). The introduction of mEHT had the greatest benefit in the first (HR: 0.5382; p = 0.0056) and second (HR: 0.7861; p = 0.0031) 6 months after diagnosis.

BEVEZETÉS

A hepatopankreatobiliáris daganatok olyan halálos kimenetelű betegségek, melyeket nagyon alacsony 5 éves túlélési arány jellemez (1). A pankreász duktális adenokarcinómája (PDAC) fordul elő a leggyakrabban (~90%), melynek ötéves túlélési aránya kevesebb mint 10%, és az előrehaladott stádiumban szenvedő betegek várható medián túlélése mindösszesen 4–6 hónap (1–3). A betegség sok esetben rezisztens a legtöbb hagyományos kemo- és sugárterápiás kezeléssel szemben. Jellemző továbbá az immunszuppresszív mikrokörnyezet (4), és csak igen kevés számú,

korai stádiumú beteg operálható meg teljes mértékben (2, 5). A betegségben alkalmazott leggyakoribb kemoterápiás kezelések a FOLFIRINOX protokoll, illetve a gemcitabin kombinációja platinaszerekkel vagy nab–paklitaxellel (6, 7). Sajnálatos módon PDAC-ban nem létezik igazán hatékony gyógyszeres kezelés, mint más szolid malignitásokban, és az alkalmazott kezelés(ek) alatt a várható életminőség is jellemzően rosszabb. Mindezek fényében szükség van új, hatékony terápiák bevezetésére. Az elmúlt évtizedekben számos új technika és lehetséges multimodális terápia jelent meg, amelyek kiegészítik a hagyományos sebészi reszekciót és a kemoradioterápiát. Ilyenek például a különböző termikus ablációs módszerek, mint például a rádiófrekvenciás és mikrohullámú abláció, a hipertermikus intraperitoneális kemoterápia, a perkután etanolinjekció, a transzkatéteres artériás kemoembolizáció és a különböző típusú hipertermiás eljárások (8). A hipertermia az onkológiában a daganat hőmérsékletének mesterséges, körülbelül 39–42 °C-ra történő emelése. Ismert a teljestestés lokális hipertermia is (9). A melegítés nyomán biofizikai eltérések alakulnak ki, amelyek vagy közvetlen metabolikus hatással járnak, vagy a közvetett immunmodulálás révén tumorölő hatásúak (8, 9). A lokális hipertermiás módszerek egyik legújabb vívmánya az úgynevezett modulált elektro-hipertermia (mEHT). Technikailag az mEHT egy precíziós, kapacitív csatolású, impedanciaillesztésű módszer, amely szelektíven célozza meg a tumorsejteket. A malignus sejtekre jellemző eltérő funkcionális és bioelektromos jellemzőknek köszönhető a szelektív folyamat. A részletekbe bocsátkozás nélkül a módszer azt használja ki, hogy a tumorsejtekben a membránraftokon való energiaelnyelés eltér az egészséges sejtekétől, és a kezelés hatására ún. károsodással összefüggő molekuláris mintázatok (DAMPs) alakulnak ki, melyek végül programozott vagy immunogén tumorsejthalálhoz vezetnek (10). Az mEHT módszer a refrakter esetekben képes a tumorsejteket újraérzékenyíteni, növelve ezzel a párhuzamosan alkalmazott kemoterápiák hatásosságát, a túlélési időt és az életminőséget is (11, 12).

Az elmúlt években több klinikai tanulmány is megjelent, melyben mEHT-vel kezeltek PDAC-betegeket (13–17). Az összes vizsgálat egyetértett abban, hogy az mEHT-nek pozitív hatása van a progressziómentes és a teljes túlélésre, valamint hosszabb ideig tartható fenn stabil betegség a kezelés alkalmazása mellett. Habár Fiorentini és munkatársai (13) felvetették, hogy a diagnózistól az első mEHT-kezelésig eltelt idő és az első mEHT-kezelés utáni túlélési idő között feltehetően szignifikáns összefüggés van, a kérdés máig nem tisztázott. A jelen retrospektív megfigyeléses vizsgálat célja volt, hogy megvizsgáljuk, előrehaladott PDAC-betegeknél a konkomittáns mEHT alkalmazása előnyös-e az önállóan alkalmazott hagyományos szisztémás terápiához képest. További kérdésünk volt, hogy mikor érdemes az mEHT-t bevezetni a betegek kezelése során?

ANYAG ÉS MÓDSZER

A vizsgálatot a Semmelweis Egyetem Regionális, Intézményi Tudományos és Kutatásetikai Bizottsága engedélyezte (SE TUKEB 8/2017 és SE TUKEB 8-1/2017). Az mEHT-kezelt betegek mind írásbeli hozzájárulásukat adták a vizsgálathoz, míg az utólagos retrospektív, anonim módon történt további adatgyűjtés során nem volt szükség beleegyező nyilatkozat aláírására. A kutatás és az adatok gyűjtése a WMA Helsinki Nyilatkozat és az Európai Unió Általános adatvédelmi rendelete (GDPR) előírásainak megfelelően zajlott.

BETEGEK

Retrospektív obszervációs vizsgálatunkban a Semmelweis Egyetem Belgyógyászati és Onkológiai Klinika Onkológiai Profilján 2016 és 2021 között kezelt 96 fő, előrehaladott stádiumú PDAC-beteg adatait dolgoztuk fel. Mind a 96 beteg konkomittáns mEHT-kezelésben részesült. Továbbá az intézetben ugyanezen időszak alatt megjelent (n = 416), de mEHT-kezelésben nem részesült betegek közül egy 86 fős, korban és nemben „propensity score" illesztett, előrehaladott stádiumú PDAC-kontrollcsoportot is bevontunk a vizsgálatba. Kizárási kritérium volt a 18 év alatti életkor, nem adenokarcinóma típusú pankreászdaganat, korai betegségstádium, korábbi tumoros megbetegedések, mentális megbetegedések, gyulladásos bélbetegségek (pl. colitis ulcerosa, Crohnbetegség), kezeletlen pajzsmirigybetegség, szisztémás autoimmun megbetegedések, és a ≥ 2 Eastern Cooperative Oncology Group (ECOG) státusz.

AZ mEHT-KEZELÉS

A konkomittáns mEHT-kezeléseket az Oncotherm EHY-2000 és EHY-2030 (Oncotherm Kft., Budaörs) készülékkel végeztük, amelyek technológiai elve azonos. A betegek kezelése hetente kétszer, fekvő helyzetben, kezelésenként 60 percig tartott. Az első kezelési héten alacsonyabb teljesítményen végeztük a kezeléseket, a készülék teljesítményét 30 perc alatt, 60-ról 100 W-ig fokozatosan növeltük, majd a fennmaradó 30 percben fenntartottunk a 100 W-os teljesítményt. A kezelés második hetétől kezdődően a készülék teljesítményét az első 30 percben 100-ról 150 W-ig emeltük, majd a kezelés befejezéséig tartottuk a 150 W teljesítményt (1. ábra). Amennyiben a beteg a készülék teljesítményének emelési szakaszában panaszt említett, a teljesítményt nem emeltük tovább, és a kezelést.
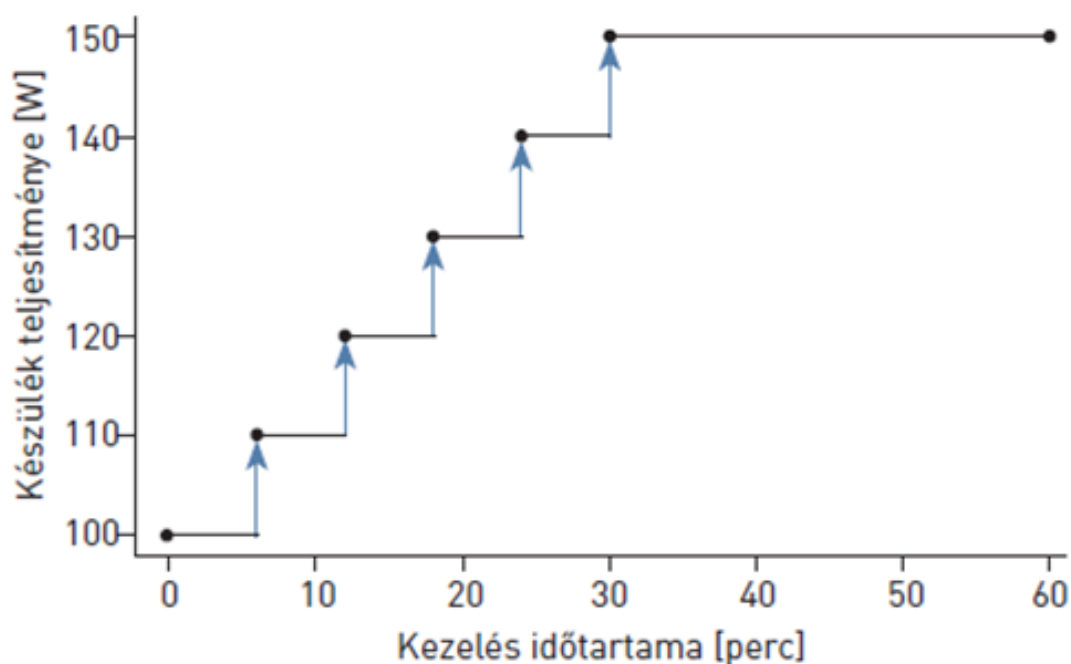
1. ÁBRA. Az EHY-2030 készülékkel végzett kezelés teljesítményének tervezett emelése 150 W-ig. A maximális teljesítmény a beteg egyéni toleranciájának függvényében csökkenthető

a beteg által tolerált legmagasabb teljesítményen folytattuk. Bármilyen panasz, tünet esetén (pl. bőrpír, túlzott hőhatás érzete) a kezelést meg kell szakítani. A jelen vizsgálat során az mEHT-kezelések folyamán nem figyeltünk meg nemkívánatos esemény(eke)t.

Klinikai adatok rögzítése

Az Egyetem kórházi informatikai rendszerének (e-MedSolution; Egészséginformatikai Szolgáltató és Fejlesztési Központ, Budapest) felhasználásával rögzítettük a vizsgálatban részt vevő mEHT-kezelt és kontroll PDAC-betegek alapvető onkológiai és kezelési adatait. A betegek kemoterápiás kezelése a hazai, európai és nemzetközi irányelvek szerint történt (6, 7). Rögzítettük továbbá az alkalmazott mEHT-kezelések számát, valamint a tumor diagnózisa és az első mEHT-kezelés között eltelt időt is. A betegek teljes túlélési idejét (OS) a daganat diagnózisától a beteg haláláig vagy az adatgyűjtés befejezéséig (2 fő; 2023. július 31-ig) eltelt időként definiáltuk. A túlélő betegek esetében jobbra cenzorálás történt.

Statisztikai elemzés

Statisztikai elemzéseinkhez az R for Windows version 4.3.1 (R Foundation for Statistical Computing, 2023, Bécs, Ausztria) programcsomagot használtuk. A csoportok összehasonlításait kétmintás Welch-féle t-teszttel és Fisher-féle egzakt próbával végeztük. A korban és nemben illesztett kontroll személyek kiválasztásához, illetve a további betegpárillesztésekhez úgynevezett „propensity score matching" technikát alkalmaztunk (Matching R csomag, version 4.10-8). A túléléselemzésekhez „egyszerű" és időfüggő együtthatóval kiterjesztett Cox-féle túlélési modelleket alkalmaztunk (survival R csomag, version 3.5-7). Az mEHT-kezelés időben történő hatásosságának elemzéséhez az időfüggő Cox-modelleket ún. lépcsőfüggvényekkel is kibővítettük (18). A túlélési görbéket a survminer R-csomaggal (version 0.4.9) rajzoltuk meg. Statisztikailag szignifikánsnak tekintettük, ha $p < 0{,}05$. A többszörös összehasonlítások esetében a p-értékek korrekciója Holm módszerével történt (19). A folytonos változókat átlag ± szórással, az előfordulási gyakoriságokat a megfigyelések számával (százalékos arány), a túlélési adatokat pedig hazárdrátákkal (HR) és azok 95%-os konfidenciaintervallumaival (95% CI) adtuk meg.

1.táblázat. A két vizsgálati csoport alaptulajdonságai (átlag ± szórás; esetszám [százalék])

Paraméter	Kontrollcsoport (n = 86)	mEHT-csoport (n = 96)	Korrigált p-érték
Életkor (év)	65,06 ± 9,52	64,78 ± 9,65	1,0000
Nem (férfi : nő)	42 : 44 (48,8% : 51,2%)	48 : 48 (50,0% : 50,0%)	1,0000
Tumor lokációja			
– Fej	63 (73,3%)	55 (57,3%)	
– Test	16 (18,6%)	24 (25,0%)	0,7369
– Farok	7 (8,1%)	17 (17,7%)	
Áttétek (szinkron : metakron)			
– Máj	34 : 16 (39,5% : 18,6%)	39 : 14 (40,6% : 14,6%)	1,0000
– Intraabdominális	17 : 6 (19,8% : 7,0%)	25 : 17 (26,0% : 17,7%)	1,0000
– Tüdő	8 : 8 (9,3% : 9,3%)	7 : 11 (7,3% : 11,5%)	1,0000
– Egyéb	7 : 7 (7,0% : 7,0%)	9 : 6 (9,4% : 6,2%)	1,0000
Aszcitesz	26 (30,2%)	35 (36,5%)	1,0000
Kezelés			
– Gemcitabin	74 (86,0%)	81 (84,4%)	1,0000
– Paklitaxel	30 (34,9%)	37 (38,5%)	1,0000
– FOLFIRINOX	21 (24,4%)	31 (32,3%)	1,0000
– Kapecitabin	9 (10,5%)	20 (20,8%)	0,7544
– Egyéb	23 (26,7%)	30 (31,2%)	1,0000
Az mEHT-kezelés adatai			
– Diagnózis és mEHT között eltelt idő (hónap)	–	4,53 ± 5,42	–
– Az mEHT-kezelések száma (db)	–	32,02 ± 25,05	–

FOLFIRINOX: leukovorin + 5-FU + irinotekán + oxaliplatin; mEHT: modulált elektro-hipertermia

EREDMÉNYEK

Retrospektív megfigyeléses vizsgálatunkba összesen 182, előrehaladott stádiumú PDAC-beteget vontunk be. A 182 betegből 96 fő kapott legalább 4 db mEHT-kezelést. A fennmaradó 86 pácienst egy 416 fős, mEHT-kezelést nem kapó kontroll poolból válogattuk ki „propensity score matching" technikával, életkorra és nemre illesztve. A két betegcsoport között nem tudtunk egyik vizsgált klinikai paraméterben sem különbséget igazolni. A két csoport betegeinek klinikai adatai az 1. táblázatban olvashatók. Vizsgálatunk során összesen 3080 db mEHT-kezelés történt. Ebből a legkevesebb 4, a legtöbb 119, a kezelések mediánja pedig 24 db volt. Az első mEHT-kezelést a betegek közel 80%-ánál (76 fő) a tumor diagnózisától számított első fél éven belül megkezdtük. A tumor diagnózisa és az első mEHT-kezelés között eltelt idő mediánja 72 nap volt. 12 beteg (12,5%) volt, akiknek a bevonása és első mEHT-kezelése több mint egy évvel a tumor diagnózisát követően történt.

AZ OPTIMÁLIS mEHT-KEZELÉSSZÁM MEGHATÁROZÁSA

Irodalmi adatok alapján ismert, hogy a konkomittáns mEHT-kezelés akkor jár a legnagyobb előnnyel, illetve akkor tud a tumorsejt-károsító hatása is a legjobban érvényesülni, ha minél több kezelés történik (8, 10, 12, 13, 15). E kérdést az alábbi módon vizsgáltuk meg. A 96 és 86 fős kiindulási csoportokból további, párosított alcsoportokat hoztunk létre. 76, 57, 38 és 33 beteg kapott legalább 10, 20, 30, illetve 40 mEHT-kezelést. Majd ezeket felhasználva, 1:1 arányú, korban, nemben és tumorlokációban illesztett mEHT-kontroll betegpárokat alakítottunk ki. A létrehozott csoportok

klinikai adatait az 1–4. kiegészítő táblázatokban foglaltuk össze. Az összes beteg vizsgálatakor nem lehetett szignifikáns eltérést igazolni az mEHT- és kontrollbetegek túlélése között (HR: 0,9432; 95% CI: 0,7028–1,2660; p = 0,6970). Hasonlóan, a legalább 10 (HR: 0,8366; 95% CI: 0,6067–1,1540; p = 0,2770; 2.a ábra) vagy 20 (HR: 0,7437; 95% CI: 0,5127–1,0790; p = 0,1190; 2.b ábra) mEHT-kezelésen átesett alkohorszoknál sem lehetett szignifikáns eltérést igazolni. Azonban fontos kiemelni, hogy a medián túlélés mind a 10, mind pedig a 20 mEHT-kezelésen átesett betegcsoportban magasabb volt. Számszerűen 3 és 6 hónappal volt hosszabb (2.a és 2.b ábra)

Ezzel szemben azoknak a betegeknek a túlélése, akik legalább 30 (HR: 0,5011; 95% CI: 0,3129–0,8027; p = 0,0041; 2.c ábra) vagy 40 (HR: 0,5048; 95% CI: 0,3035–0,8397; p = 0,0085; 2.d ábra) mEHT-kezelésben részesültek, szignifikánsan hosszabb volt a kontrollcsoporthoz képest. Az előbbiekhez hasonló eredményt kaptunk abban az esetben is, ha multivariáns túlélési modellekkel vizsgáltuk az mEHT túlélésre gyakorolt hatását. Míg az összes betegnél, és a legalább 10, illetve 20 mEHT-kezeléses alkohorszok esetében egyedül a különböző áttétek túlélést rontó, és az egyes kemoterápiák rizikócsökkentő hatását lehetett igazolni, addig a legalább 30 (HR: 0,4016; 0,209 – 0,7692; p = 0,0059), illetve 40 (HR: 0,3697; 0,1849–0,7393; p = 0,0049) mEHT-kezelésen átesett betegeknél az mEHT-kezelés is szignifikánsan javította a betegek túlélését. Az életkor, a nem, a tumorlokáció és a hasi folyadék egyik modellben sem befolyásolta szignifikánsan a betegek túlélését (2. táblázat).

Mikor érdemes bevezetni az mEHT-kezelést?

Kutatásunk további kérdése volt, hogy mikor érdemes bevezetni az mEHT-kezelést a PDAC-betegek terápiája során? E kérdés megválaszolására egy időfüggő együtthatóval és lépcsőfüggvénnyel kiterjesztett Cox-féle regressziós modellt alkalmaztunk. Ezzel a túlélési modellel lehetőségünk volt annak modellezésére, hogy az obszervációs időt több részre osztva kiderítsük, melyik időszakaszokban van szignifikáns hatása az mEHT-kezelésnek, illetve a kezelések számát is figyelembe véve. A túlélési modell eredménye (3. táblázat) alapján az mEHT-kezelés bevezetése PDAC-ben az első egy évben (0–6 hónap: p = 0,0056; 6–12 hónap: p = 0,0031) jár a legnagyobb előnnyel. Megjegyzendő, hogy habár a jelen vizsgálatban csupán a betegek kb. 20%-ánál lett az mEHT-kezelés a diagnózistól számított több mint egy évvel később inicializálva, a túlélési modell eredménye alapján hasonló tendencia volt megfigyelhető ezeknél a betegeknél is (p = 0,0788). A kezelések számában ugyanezt a tendenciát tudtuk igazolni. Minél korábban sikerül nagyobb számú mEHT-kezelést beütemezni a diagnózist követő első évben, annál hosszabb lesz a betegek várható élettartama (0–6 hónap: p = 0,0007; 6–12 hónap: p = 0,0037; 3. táblázat).

MEGBESZÉLÉS

Az előrehaladott PDAC kezelésére a mai napig is csupán korlátozott lehetőségek állnak rendelkezésre. A jelenleg is elérhető szisztémás onkológiai terápiák hatékonyságának növekedése ellenére a betegek életkilátásai továbbra is rosszak, a várható medián élettartam fél év körüli (1, 6). Az elmúlt évtizedekben számos módszeren dolgoztak, amely elősegítheti a betegek hosszabb túlélését, vagy a kezelések melletti jobb közérzetet, életminőséget teremt. Az egyik ilyen a lokálisan alkalmazott onkológiai hipertermia, mely a tumor és annak környezete mesterséges melegítésével próbálja felvenni a harcot a daganatokkal (8, 9). A hipertermiás kezelések egyik legújabb típusa az

mEHT (12). Az mEHT és a pankreásztumorok közötti összefüggéseket eddig csupán pár munkacsoport vizsgálta. A korábbi tanulmányok arra a következtetésre jutottak, hogy a hagyományos kemoterápia kiegészítéseként alkalmazott konkomittáns mEHT mellett a pankreászdaganatos betegek progressziómentes és általános túlélése javul, továbbá jobb betegségválasz érhető el (13–16, 20). Egy kínai tanulmány eredményei alapján az mEHT és a hagyományos kínai gyógynövény-terápia kombinált alkalmazása metasztatikus PDAC-ben az aszcitesz jobb felszívódásával, a kezelésre adott válasz és az életminőség javulásával járt együtt, szemben a hagyományos kemoterápiával és rendszeres perkután drenázzsal (20). Dani (16) és saját munkacsoportunk (15) korábbi eredményei alapján az mEHT-vel kezelt metasztatikus PDAC-s betegek szignifikánsan lassabb progresszióképződést mutatnak, a legjobb terápiás válasz azoknál a betegeknél érhető el, ahol nincs aszcitesz.
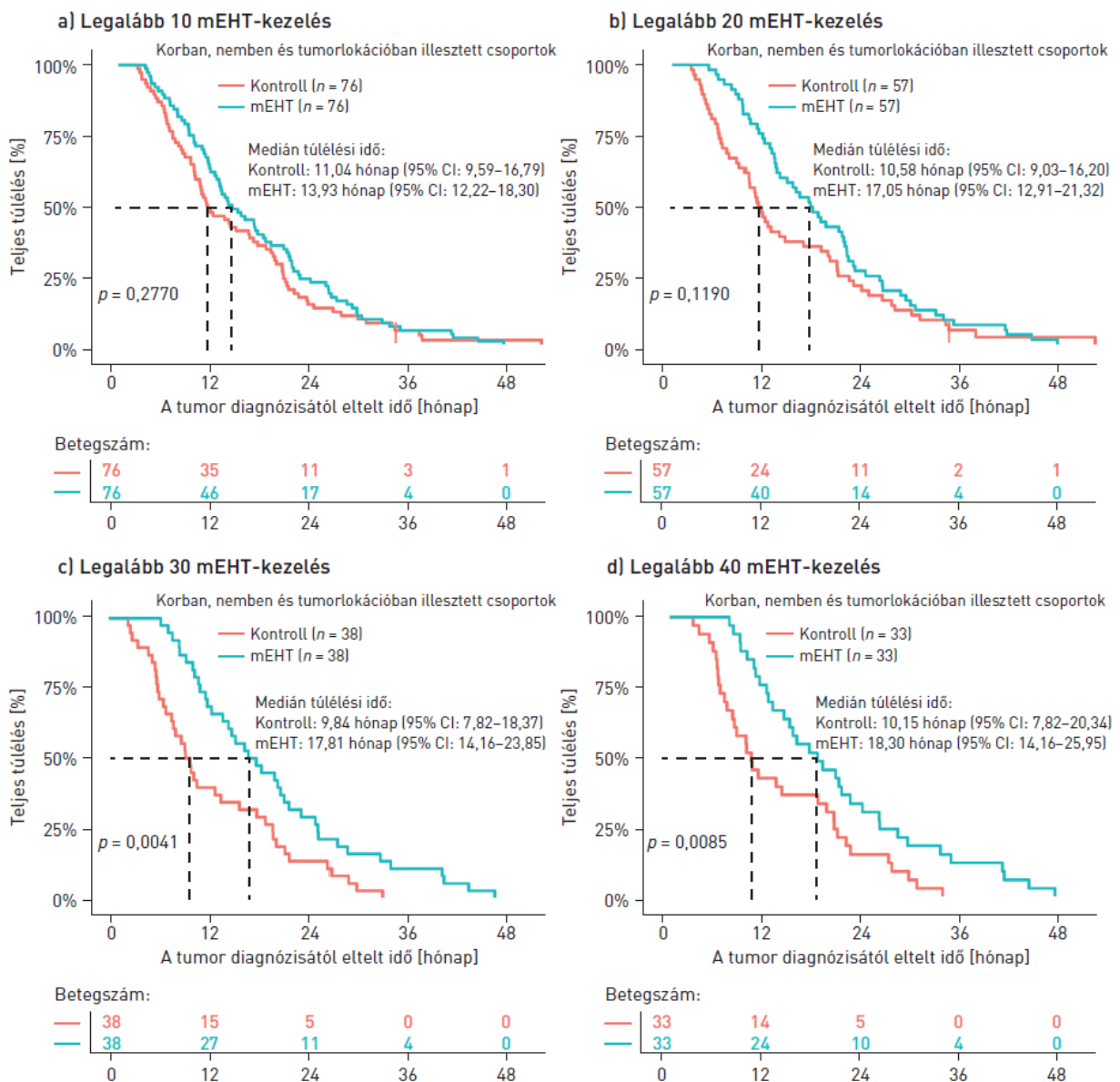
2.Ábra. A modulált elektro-hipertermia- (mEHT-) kezelések száma alapján létrehozott vizsgálati kohorszok túlélésének összehasonlításai a korban, nemben és tumorlokációban illesztett kontroll betegpárokéval. Míg a legalább 10 (a) és 20 (b) mEHT-kezelésen átesett betegek túlélése nem különbözik szignifikánsan a kontrollokétól, addig a legalább 30 (c) és 40 (d) mEHT-kezelésen

átesett betegek várható túlélése szignifikánsan hosszabb. A várható medián túlélés minden esetben az mEHT-csoportban volt hosszabb. A legalább 30 és 40 kezelésen átesett beteg esetében közel kétszer olyan hosszú volt, mint a kontrolloknál (10 vs. 18 hónap)

2.Táblázat. Multivariáns Cox-regressziós túlélési modellek p-értékei

Paraméter	Kontroll vs. mEHT ≥ 4	Kontroll vs. mEHT ≥ 10	Kontroll vs. mEHT ≥ 20	Kontroll vs. mEHT ≥ 30	Kontroll vs. mEHT ≥ 40
Kezelés: kontroll (ref.) vs. mEHT	0,7777	0,8435	0,4530	0,0059	0,0049
Életkor (év)	0,0900	0,3324	0,1138	0,4652	0,3550
Nem: férfi (ref.) vs. nő	0,8376	0,2340	0,8612	0,9856	0,6775
Tumor lokációja					
– Fej (ref.) vs. test	0,8394	0,2801	0,4463	0,2899	0,2150
– Fej (ref.) vs. farok	0,4529	0,4777	0,1646	0,5517	0,2233
– Farok (ref.) vs. test	0,7333	0,1438	0,0959	0,1682	0,0608
Májáttét					
– Nincs (ref.) vs. szinkron	< 0,0001	0,0046	0,0285	0,0043	0,0008
– Nincs (ref.) vs. metakron	0,1469	0,7917	0,7113	0,5259	0,7651
– Szinkron (ref.) vs. metakron	0,0480	0,0670	0,2178	0,2040	0,0233
Intraabdominális áttét					
– Nincs (ref.) vs. szinkron	0,0214	0,0509	0,1160	0,6534	0,4342
– Nincs (ref.) vs. metakron	0,4324	0,3812	0,1999	0,2145	0,0557
– Szinkron (ref.) vs. metakron	0,2594	0,3761	0,8877	0,5452	0,3584
Tüdőáttét					
– Nincs (ref.) vs. szinkron	0,0414	0,6522	0,6934	0,0554	0,1262
– Nincs (ref.) vs. metakron	0,2633	0,3876	0,4249	0,5180	0,5970
– Szinkron (ref.) vs. metakron	0,3708	0,8589	0,4295	0,0495	0,0972
Egyéb áttét					
– Nincs (ref.) vs. szinkron	0,0352	0,4551	0,2795	0,2534	0,1650
– Nincs (ref.) vs. metakron	0,0690	0,2013	0,3160	0,5574	0,2214
– Szinkron (ref.) vs. metakron	0,8180	0,7699	0,8391	0,1782	0,0558
Aszcitesz: nincs (ref.) vs. van	0,9010	0,6859	0,4803	0,7475	0,6552
Kezelés					
– Gemcitabin: nincs (ref.) vs. van	0,0313	0,0083	0,0495	0,0612	0,0366
– Paklitaxel: nincs (ref.) vs. van	0,0596	0,0838	0,0209	0,0012	0,0014
– FOLFIRINOX: nincs (ref.) vs. van	0,0106	0,0374	0,0076	0,0312	0,0292
– Kapecitabin: nincs (ref.) vs. van	0,0016	0,0121	0,0069	0,0981	0,0303
– Egyéb: nincs (ref.) vs. van	0,0115	0,0131	0,0068	0,0049	0,0166

FOLFIRINOX: leukovorin + 5-FU + irinotekán + oxaliplatin; mEHT: modulált elektro-hipertermia

Az mEHT-kezelések számával és az optimális terápiába illesztéssel kapcsolatos eddigi eredmények rendkívül hiányosak. Vizsgálatunkban ezért ezekre a kérdésekre kerestünk választ. Egy korábbi magyar vizsgálat eredménye alapján eddig az volt feltételezhető, hogy nincs összefüggés a kezelések száma és a betegek túlélése között (16). Ezzel szemben a jelen vizsgálatban sikerült ennek az ellentétét bebizonyítani. Amennyiben az előrehaladott stádiumú PDAC-betegek legalább 30 mEHT-kezelésen átestek, a várható medián túlélés közel kétszeresére, 10-ről 18 hónapra növekedett. Megjegyzendő, hogy habár az alacsonyabb mEHT-kezelésszámok esetében nem tudtunk szignifikáns eltérést igazolni a két kezelési csoport között, de már a kevesebb mEHT-kezelésen átesett betegeknél is tendenciózusan hosszabb túlélések voltak megfigyelhetők. Legalább 10 mEHT-kezelést követően 3 hónappal, míg 20 mEHT-kezelést követően 6 hónappal

hosszabb medián túlélés volt megfigyelhető. A jelen és Dani vizsgálatának (16) eredményei közötti különbségek hátterében feltehetően a szignifikánsan magasabb mEHT-kezelésszám állhat. Míg a mi vizsgálatunkban a kezelések számának mediánja 24 volt, addig a Dani-féle vizsgálatban részt vevő mindkét centrumban ugyanez 6 kezelés volt (16). Az előzőhöz hasonlóan szintén nem állt rendelkezésre korábban információ, hogy mikor érdemes bevezetni az mEHT-terápiát a PDAC-betegek onkológiai kezelésébe.

3.Táblázat. . Longitudinális együtthatókat is tartalmazó túlélési modell eredménye, amelynek segítségével a következő kérdésekre kerestük a választ: 1. A tumor diagnózisát követően milyen hatása van a túlélésre a modulált elektro-hipertermia- (mEHT-) kezelésnek, ha a diagnózishoz képest eltérő időpontokban inicializálják? 2. Mekkora rizikócsökkentéssel lehet számolni azoknál a pácienseknél, akiknél több mEHT-kezelés volt kivitelezhető?

Paraméter	HR	95% CI	p-érték
Az mEHT-kezelés bevezetése a tumor diagnózisát követően a(z)			
– első 6 hónapban (hónap)	0,5382	0,3472–0,8341	0,0056
– 6–12. hónap között (hónap)	0,7861	0,6700–0,9222	0,0031
– 12. hónapot követően (hónap)	0,9624	0,9222–1,0044	0,0788
Az mEHT-kezelések számának mekkora hatása van a túlélésre, ha a diagnózist követő			
– első 6 hónapban kezdtük meg (db)	0,9094	0,8611–0,9604	0,0007
– a 6–12. hónapban kezdtük meg (db)	0,9741	0,9569–0,9915	0,0037
– a 12. hónapot követően kezdtük meg (db)	0,9997	0,9883–1,0113	0,9652

HR: hazárdráta; CI: konfidenciaintervallum

Fiorentini és munkacsoportja (13) az eredményeik alapján már korábban is felvetették, hogy a diagnózistól az első mEHT-kezelésig eltelt idő és az első mEHT-kezeléstől és/vagy diagnózistól számított túlélési idő között szoros összefüggés lehet. E kérdés alaposabb vizsgálata volt kutatásunk másik fontos kérdése. Eredményeink alapján az előrehaladott stádiumú PDAC-betegek kezelésében az mEHT akkor jár a legnagyobb haszonnal, ha a diagnózistól számított első évben vezetik be. A korábbi halálozás rizikójának csökkentése a túlélési modellünk alapján az első 6 hónapban a legnagyobb, de a 6–12. hónapban történő mEHT-inicializáció is szignifikánsan javítja a betegek túlélését. A diagnózist követően több mint 12 hónappal történt első mEHT-kezelések esetén csak tendenciózus (statisztikailag marginális) eredményt tudtunk bemutatni. Megjegyzendő, hogy a diagnózishoz képest egy évvel később megkezdett mEHT-kezelések száma a vizsgált kohorsz mindössze 20%-ában fordult csak elő. Az elmúlt években több kohorsz konkomittáns terápiáját kíséreltük meg, legnagyobb esetszámban PDAC-pácienseket kezelve. Hasonló elven működő elektromágneses eljárások klinikai vizsgálata PDAC-ben, első vonalú (nab-paklitaxel és gemcitabin kombinációs) kezelésként folyamatban van hazánkban is: az ún. tumor treating fields technológiával (TTF; Novocure, Root, Svájc). A TTF számos daganatban bizonyítottan eredményes volt, és finanszírozást nyert pl. Németországban is (kifejezetten javasolt: glioblasztómában) (22).

Az mEHT-kezelések a TTF-fel ekvivalensnek bizonyultak (23), és számos más tumortípusban és stádiumban hatásosak lehetnek (24). Emlőtumoros betegek terápiája során látott eredményeink alapján nem szponzorált, prospektív, randomizált klinikai vizsgálatot (NeoHTerMa; ClinicalTrials.gov

azonosító: NCT05889390) indítottunk neoadjuváns sémában HER2-negatív (lumináris B és tripla-negatív) emlődaganatos betegeknek, ennek eredményeiről a későbbiekben kívánunk beszámolni. Vizsgálatunk eredményeit összefoglalva, az inoperábilis és/vagy előrehaladott stádiumú PDAC-betegek terápiájában az mEHT-kezelés minél korábbi bevezetése javasolható. Jelen vizsgálatunkban a betegek heti kétszeri mEHT-kezelésben részesültek, azonban más tumorokban nyert újabb eredmények alapján (21) a kezelésszám heti 3-ra emelése nagy valószínűséggel még tovább fokozhatja a kezelés hatékonyságát. Ennek a kérdésnek a tisztázásához azonban további, lehetőség szerint randomizált klinikai vizsgálatok szükségesek. Vizsgálatunk limitáló tényezői a viszonylag alacsony esetszám, a betegcsoport heterogenitása, a retrospektív elrendezés és a randomizáció hiánya. Utóbbit a „propensity score matching" technikával történt betegpár-kiválasztás valamennyire kompenzálta. A retrospektív elrendezés befolyásolta, hogy az esetleg felmerülő kérdéseket, az egyéb klinikai paraméterek hatásait jelen tanulmányunkban tovább tudjuk elemezni.

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KIEGÉSZÍTŐ TÁBLÁZATOK

1. KIEGÉSZÍTŐ TÁBLÁZAT. A legalább 10 modulált elektro-hipertermia- (mEHT-) kezelésben részesült, előrehaladott hasnyálmirigy-daganatos betegek, illetve a korban, nemben és tumorlokációban illesztett kontrollcsoport betegeinek összehasonlítása (átlag ± szórás; esetszám [százalék])

Paraméter	Kontrollcsoport (*n* = 76)	mEHT-csoport (*n* = 76)	Korrigált *p*-érték
Életkor [év]	63,59 ± 9,05	63,86 ± 10,14	1,0000
Nem (férfi : nő)	38 : 38 (50,0% : 50,0%)	37 : 39 (48,7% : 51,3%)	1,0000
Tumor lokációja			
– Fej	53 (69,7%)	43 (56,6%)	
– Test	16 (21,1%)	23 (30,3%)	1,0000
– Farok	7 (9,2%)	10 (13,2%)	
Áttétek (szinkron : metakron)			
– Máj	33 : 13 (43,2% : 17,1%)	26 : 13 (34,2% : 17,1%)	1,0000
– Intraabdominális	16 : 6 (21,1% : 7,9%)	18 : 14 (23,7% : 18,4%)	1,0000
– Tüdő	7 : 6 (9,2% : 7,9%)	4 : 9 (5,3% : 11,8%)	1,0000
– Egyéb	7 : 5 (9,2% : 6,6%)	5 : 6 (6,6% : 7,9%)	1,0000
Aszcitesz	25 (32,9%)	31 (40,8%)	1,0000
Kezelés			
– Gemcitabin	65 (85,5%)	67 (88,2%)	1,0000
– Paklitaxel	26 (3,2%)	33 (43,4%)	1,0000
– FOLFIRINOX	19 (25,0%)	28 (36,8%)	1,0000
– Kapecitabin	8 (10,5%)	19 (25,0%)	0,4223
– Egyéb	20 (26,3%)	25 (32,9%)	1,0000
Az mEHT-kezelés adatai			
– Diagnózis és mEHT között eltelt idő [hónap]	–	4,90 ± 5,87	–
– Az mEHT-kezelések száma [db]	–	38,79 ± 23,99	–

FOLFIRINOX: leukovorin + 5-FU + irinotekán + oxaliplatin

2. KIEGÉSZÍTŐ TÁBLÁZAT. A legalább 20 modulált elektro-hipertermia- (mEHT-) kezelésben részesült előrehaladott hasnyálmirigy-daganatos betegek, illetve a korban, nemben és tumorlokációban illesztett kontrollcsoport betegeinek összehasonlítása (átlag ± szórás; esetszám [százalék])

Paraméter	Kontrollcsoport (n = 57)	mEHT-csoport (n = 57)	Korrigált p-érték
Életkor [év]	65,83 ± 9,26	65,30 ± 9,42	1,0000
Nem (férfi : nő)	28 : 29 (49,1% : 50,9%)	28 : 29 (49,1% : 50,9%)	1,0000
Tumor lokációja			
– Fej	34 (59,6%)	31 (54,4%)	
– Test	16 (28,1%)	19 (33,3%)	1,0000
– Farok	7 (12,3%)	7 (12,3%)	
Áttétek (szinkron : metakron)			
– Máj	25 : 11 (43,9% : 19,3%)	19 : 11 (33,3% : 19,3%)	1,0000
– Intraabdominális	14 : 4 (24,6% : 7,0%)	13 : 9 (22,8% : 15,8%)	1,0000
– Tüdő	6 : 7 (10,5% : 12,3%)	3 : 9 (5,3% : 15,8%)	1,0000
– Egyéb	6 : 4 (10,5% : 7,0%)	4 : 6 (7,0% : 10,5%)	1,0000
Aszcitesz	19 (33,3%)	21 (36,8%)	1,0000
Kezelés			
– Gemcitabin	51 (89,5%)	52 (91,2%)	1,0000
– Paklitaxel	20 (35,1%)	26 (45,6%)	1,0000
– FOLFIRINOX	13 (22,8%)	19 (33,3%)	1,0000
– Kapecitabin	5 (8,8%)	17 (29,8%)	0,1040
– Egyéb	18 (31,6%)	21 (36,8%)	1,0000
Az mEHT-kezelés adatai			
– Diagnózis és mEHT között eltelt idő [hónap]	–	4,52 ± 5,33	–
– Az mEHT-kezelések száma [db]	–	47,11 ± 22,07	–

FOLFIRINOX: leukovorin + 5-FU + irinotekán + oxaliplatin

3. KIEGÉSZÍTŐ TÁBLÁZAT. A legalább 30 modulált elektro-hipertermia- (mEHT-) kezelésben részesült előrehaladott hasnyálmirigy-daganatos betegek, illetve a korban, nemben és tumorlokációban illesztett kontrollcsoport betegeinek összehasonlítása (átlag ± szórás; esetszám [százalék])

Paraméter	Kontrollcsoport (n = 38)	mEHT-csoport (n = 38)	Korrigált p-érték
Életkor [év]	65,31 ± 8,35	64,82 ± 9,28	1,0000
Nem (férfi : nő)	19 : 19 (50,0% : 50,0%)	21 : 17 (55,3% : 44.7%)	1,0000
Tumor lokációja			
– Fej	23 (60.5%)	23 (60.5%)	
– Test	12 (31,6%)	12	1,0000
– Farok	3 (7,9%)	3 (7,9%)	
Áttétek (szinkron : metakron)			
– Máj	17 : 7 (44,7% : 18,4%)	10 : 7 (26,3% : 18,4%)	1,0000
– Intraabdominális	10 : 2 (26,3% : 5,3%)	10 : 7 (26,3% : 18,4%)	1,0000
– Tüdő	3 : 5 (7,9% : 13,2%)	2 : 4 (5,3% : 10,5%)	1,0000
– Egyéb	6 : 2 (15,8% : 5,3%)	4 : 2 (10,5% : 5,3%)	1,0000
Aszcitesz	14 (36,8%)	13 (34,2%)	1,0000
Kezelés			
– Gemcitabin	36 (94,7%)	38 (100%)	1,0000
– Paklitaxel	16 (42,1%)	20 (52,6%)	1,0000
– FOLFIRINOX	5 (13,2%)	11 (28,9%)	1,0000
– Kapecitabin	4 (10,5%)	14 (36,8%)	0,1787
– Egyéb	13 (34,2%)	16 (42,1%)	1,0000
Az mEHT-kezelés adatai			
– Diagnózis és mEHT között eltelt idő [hónap]	–	4,21 ± 5,66	–
– Az mEHT-kezelések száma [db]	–	58,79 ± 17,71	–

FOLFIRINOX: leukovorin + 5-FU + irinotekán + oxaliplatin

4. KIEGÉSZÍTŐ TÁBLÁZAT. A legalább 40 modulált elektro-hipertermia- (mEHT–) kezelésben részesült előrehaladott hasnyálmirigy-daganatos betegek, illetve a korban, nemben és tumorlokációban illesztett kontrollcsoport betegeinek összehasonlítása (átlag ± szórás; esetszám [százalék])

Paraméter	Kontrollcsoport (*n* = 33)	mEHT-csoport (n = 33)	Korrigált *p*-érték
Életkor [év]	65,10 ± 8,93	65,03 ± 9,79	1,0000
Nem (férfi : nő)	16 : 17 (48,5% : 51,5%)	17 : 16 (51,5% : 48,5%)	1,0000
Tumor lokációja			
– Fej	19 (57,6%)	19 (57,6%)	
– Test	11 (33,3%)	11 (33,3%)	1,0000
– Farok	3 (9,1%)	3 (9,1%)	
Áttétek (szinkron : metakron)			
– Máj	15 : 5 (45,5% : 15,2%)	9 : 4 (27,3% : 12,1%)	1,0000
– Intraabdominális	9 : 2 (27,3% : 6,1%)	8 : 6 (24,2% : 18,2%)	1,0000
– Tüdő	3 : 5 (9,1% : 15,2%)	1 : 3 (3,0% : 9,1%)	1,0000
– Egyéb	5 : 1 (15,2% : 3,0%)	3 : 2 (9,1% : 6,1%)	1,0000
Aszcitesz	12 (36,4%)	11 (33,3%)	1,0000
Kezelés			
– Gemcitabin	31 (93,9%)	33 (100%)	1,0000
– Paklitaxel	16 (48,5%)	17 (51,5%)	1,0000
– FOLFIRINOX	4 (12,1%)	10 (30,3%)	1,0000
– Kapecitabin	3 (9,1%)	13 (39,4%)	0,1093
– Egyéb	10 (30,3%)	13 (39,4%)	1,0000
Az mEHT-kezelés adatai			
– Diagnózis és mEHT között eltelt idő [hónap]	–	3,82 ± 5,44	–
– Az mEHT-kezelések száma [db]	–	62,24 ± 16,35	–

FOLFIRINOX: leukovorin + 5-FU + irinotekán + oxaliplatin

Memristor Hypothesis in Malignant Charge Distribution

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Abstract

Tissues in biological objects from the point of view of electromagnetic effects must be modeled not only for their conductivity. The ionic double layer induced by the electric field, built by electrolytic diffusion, must be counted. The micro (frequency dispersion phenomena) and macro (interfacial polarization), as well as more generalized by Nernst-Planck cells describe the biophysical aspects of this phenomena. The charge distribution depends on the processes and produces charge gradients in space. The dynamic feasibility of the-charge transition layer has memory and adaptability, working like a memristor in cancerous development. The memristor processes may complete the adaptation mechanisms of cancer cells to extremely stressful conditions. Our objective is to show the distribution and redistribution of space charges that generate memristors and internal currents like injury current (IC) in the development of cancer. We show some connected aspects of the modulated electrohyperthermia (mEHT) limiting the proliferation process in the micro-range like the macro-range electrochemotherapy (ECT) processes do. The internal polarization effects form space-charge, which characteristically differ in malignant and healthy environments. The electrical resistivity of the electrolytes depends on the distribution of the charges and concentrations of ions in the electrolytes, consequently the space-charge differences appear in the conductivity parameters too. The polarization heterogeneities caused by the irregularities of the healthy tissue induce a current (called injury current), which appears in the cancerous tumor as well. Due to the nonlinearity of the space-charge production and the differences of the relaxation time of the processes in various subunits. The tumor develops the space-charge which appears as an inductive component in the otherwise capacitive setting and forms a memristive behavior of the tumorous tissue. This continuously developing space-charge accommodates the tumor to the permanently changing conditions and helps the adopting the malignant cells in the new environment. Applying external radiofrequency electric field, the disturbance of the space-charge may change the conditions, and seek to reestablish the healthy homeostatic equilibrium, blocking the pathologic injury current components. The hypothetical memristive behavior of the tumor microenvironment and the tumor mass may be a biophysical addition to the adaption mechanisms of tumor cell and could provide a way to block the pathogen biophysical processes. An electric field in the direction of the place of disturbance from the healthy neighborhood appears, starting a current, which promotes cell migrations and wound healing, re-establishing homeostatic equilibrium. In pathological disturbance, the same process starts, which supports further proliferation, so its blocking is desired.

Keywords

Malignancy, Tumors, Memristor, Imperfect Dielectrics, Heterogeneity, Charge Distribution, Injury Current, Nernst-Planck Cell

Introduction

Bioelectromagnetism refers to the study of electromagnetic phenomena within living organisms. It encompasses the electrical and magnetic properties of cells, tissues, and organs, as well as it could be used to treat diseases by repairing faulty processes [1]. Endogenous physiological electric fields

are an accessible source of physical stimulation in cancer therapy [2] [3]. Electrical stimulation may affect apoptosis and cell proliferation [4] [5], it may induce immune-modulative processes [6], and abscopal effect [7]. While research has explored the effects of electromagnetic fields on cancer cells, the relationship between bioelectromagnetism and cancer cell adaptability is not yet fully understood. Understanding bioelectromagnetic interactions is at the forefront of many biophysical problems and their clinical applications. Some active electromagnetic treatments as well as diagnostic applications, intensively apply bioelectric phenomena. Complexity is the biggest challenge in bioelectromagnetism. Understanding requires a combined analysis of numerous fields of physics, biology, and medicine. Biomaterials are highly complex. The role of electric fields in living materials is a fascinating and complex area of study that encompasses various biological processes and phenomena. Electric fields are generated by the movement of charged particles, such as ions, within and around living organisms. These fields play a significant role in several aspects of biological systems:

- Forming cellular membrane potential by electro-diffusive selection forms an intensive electric field. Having in average ~70 mV membrane potential of healthy cells, the electric field in the membrane could be as high as 1 mV/nm = 1 MV/m.

- Electric fields are involved in cellular communication, both within individual cells and between neighboring cells. Neurons, for instance, transmit signalsthrough the generation and propagation of electric impulses, known as action potentials. These electrical signals enable the nervous system to transmit information rapidly across long distances.

- Many cellular processes are governed by ion movements and electric potentials across cell membranes. These processes include the regulation of ion channels, which are responsible for maintaining the proper balance of ions within cells. Ion gradients and electric potentials are crucial for processes like muscle contraction, cell division, and cellular signaling.

- Electric fields are known to influence wound healing. When tissues are injured, they generate electric fields that guide cellular migration and tissue regeneration.

- Electric fields have been found to play a role in tissue regeneration in various organisms. Certain animals, like salamanders, can regenerate lost body parts, and electric fields are believed to contribute to the process by guiding cell movement and tissue growth.

- Electric fields are being explored for their potential in cancer treatment. Electric fields can interfere with cell division, potentially inhibiting the growth of cancerous cells.

- Electric fields can act as signaling cues for cells to change their behavior. For example, during development, electric fields guide the patterning of tissues and organs. These bioelectric signals are believed to work in conjunction with biochemical signals to orchestrate complex processes.

- Electric fields have been shown to influence cell differentiation, the process by which cells become specialized for specific functions. Electric fields can influence the fate of stem cells and direct their differentiation into specific cell types.

- Cells can sense and respond to electric fields by migrating towards or away from them. This phenomenon, known as galvanotaxis or electrotaxis, is observed in various cell types and plays a role in processes like wound healing and development.

- Polarization interactions are one of the crucial factors in the structure of tissues and organs. These are not only crucial in cellular level, but are also active in many electrolytes of the living system, where the structure of water is polarizable, and semi-crystalline. The cancer disorders the electrolyte structure in the cellular vicinity, facilitating its detection. It is likely that the ordered water bound to the membrane is also oriented by the membrane potential and the polarized epithelial plates.

Overall, electric fields are an integral part of living materials, influencing a wide range of biological processes from cellular communication to tissue regeneration. The field of bioelectricity continues to be an area of active research, with ongoing efforts to understand the underlying mechanisms and potential applications in medicine and regenerative biology. The aqueous multicomponent electrolyte (extracellular matrix, ECM) is anessential constructional component of living objects is. ECM has ionic species presenting free charges and their flows. Here the polarization effects are accompanied by free-charge conductivity.

Cells, tissues, and organs have internal polarization, which is a crucial factor in the interactions in the living system [8]. All cells have various membranes defining the fundamental functions of the cells. The membrane structures are strictly polarized layers, separating the various electrolytes and governing the selective ionic exchanges. The capacitive electric part is the membrane, which is a selective barrier mixing the various electrolytes, selects the ionic and molecular transports and controls the "deliveries" into the cell. Furthermore, membrane sensors and bonds may profoundly influence the cellular processes with structural and signaling effects and play a decisional role in its fate. Well-known polarization characterizes many tissues, like epithelial separating adjacent tissues from each other and having a specific role in the homeostasis of organs. Epithelia form a well-structured layer; it is a permanently polarized sheet fixing the post-developed complete organism for its entire life. The human body has definite polarization measured on the skin on the whole body surface [9]. Polarization is fundamental not only in epithelial cells but active in many tissues in the organisms. It arranges the water structure to polarize too, which shows semi-crystalline behavior in this way [10]. It is likely that the ordered water bound to the membrane is oriented by the membrane potential (ϕ_m), and by the polarized epithelial sheets as well. The actual polarization modifies the dielectric permittivity of the tissues. The biological cell membrane can filter the external current flow. It critically limits the penetration of the low-frequency currents into the cell but allows high-frequency signals to pass through [11]. The growing frequency increases the capacitive conduction of the membrane. When the frequency is as high as f > 15 MHz the capacity becomes a good conductor and practically shortcuts the resistor, while in low-frequencies (f < 10 Hz) it appears as a high resistive barrier, the current flows through the resistor. In the intermediate frequency interval, both electric parts actively contribute to the complete resistivity of the tissue. Due to this mechanism, the radio-frequency (RF) current does not flow through the tissue homogeneously, and when the frequency does not exceed 15 MHz the current density differs in the extracellular and intracellular electrolytes **(Figure 1).**

The absorbed power depends on the σ conductivity $(SAR = \frac{1}{2}\sigma E^2)$, while the reactive absorption (Ω) periodically goes in and out of the system by double frequency, and depends on the dielectric permittivity $(\mathcal{W} = -\frac{1}{2}\omega\varepsilon E^2)$, and changes by its heterogeneity.

The cell–membrane representing an essential part of the overall permittivity combines the barrier function between aqueous solutions and the selective conduction between them, controlling the ionic and molecular exchange between the electrolytes. The control could be passive, driven by the diffusional forces between the different electrolyte concentrations; however there are numerous active transmembrane transports by other gradients like the temperature, voltage, electric and magnetic fields, chemical potential and concentration inhomogeneities (Figure 2). The thermal effect certainly appears in the thermally sensitive channels [12] (Figure 2(A)), but most of the bioelectromagnetic activities are nonthermal. The voltage change activates the voltage gated channels [13] or the voltage sensitive molecules like VSPs [14] (Figure 2(B)). The magnetic field [15] and electric field [16] proceed ion cyclotron resonance (Figure 2(C)). The diffusion is driven with the concentration gradient, which could be thermal, or field created by charge separation [17] (Figure 2(D)).
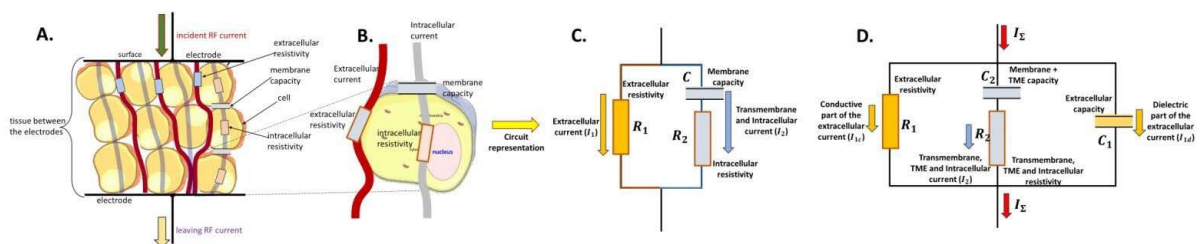
Figure 1. The RF current flow in tissues. The intracellular and membrane resistance are connected in serial mode, so they may be added together. (A) It selects between the extracellular and intracellular electrolytes in the range of radiofrequency < 15 MHz. (B) The enlarged single cell in the tissue. (C) The microscopic situation could be modeled by the shown electric circuit. (D) Usually we must consider a parallel capacitive factor in the impedance of ECM, due to the high concentration of non–ionized molecules in the microenvironment of the cells.
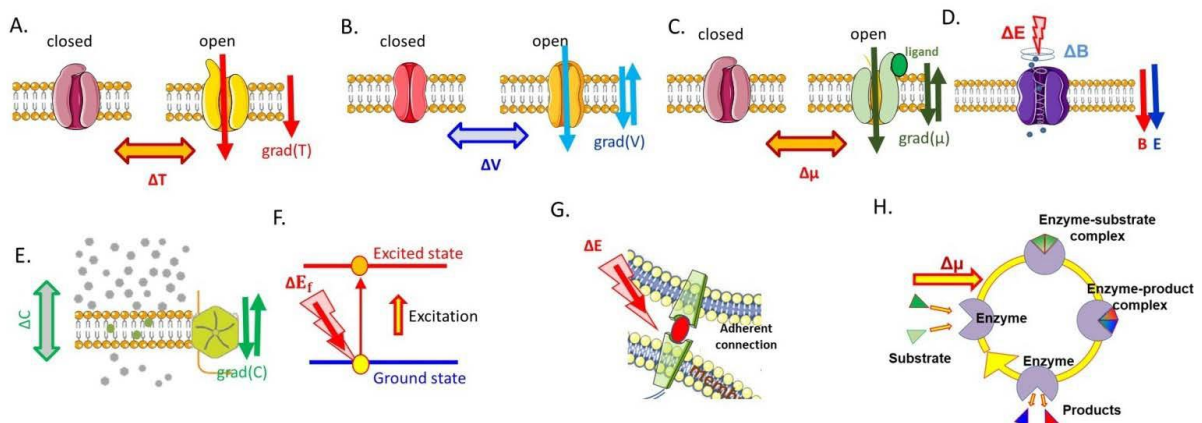
Figure 2. A few processes ignited by bioelectromagnetic interactions and their consequences. The transmembrane gradients of the temperature (T), potential (V), chemical potential (μ), and concentration of the ions, molecules, and other particles (C) are the driving forces in many the processes. They are combined in practice. The direction of the transmembrane gradients is mostly perpendicular on the membrane and may change by the direction of the external field and the given condition of the TME. (A) The thermally sensitive (temperature sensor as TRPV) channels are activated by temperature gradient. The gradient usually points from TME of the cells

to its cytosol. (B) The voltage-gated channels are activated by the voltage gradient. (C) Ligand-gated channels are controlled by the fitting chemical potential of the reaction. (D) The ionic exchange may be through ion cyclotron resonance ignited by electric and magnetic fields pointing the cytosol from external excitation. (E) Diffusion processes ignited by the concentration gradient assisted by the electromagnetic charge separation. (F) Electron (atom & molecule) excitation by the electric field. (G) The adherent connections could be changed by the electric field. (H) Enzymatic processes are ignited by the gradient of the chemical potential assisted by the electric field.

The chemically controlled (ligand gated) channel senses the ligand bond which driven by the chemical potential (μ) of the reaction (Figure 2(E)). The quantum-mechanical processes by electric field excite electrons (in molecular bonds) [18] (Figure 2(F)). The electromagnetic field may modify (break or unite) the adherent connections [19] (Figure 2(G)). The electric field influences the enzymatic processes and may modify their outcome [20] (Figure 2(H)). The electric structure of the cells is principally involved in the chemical reactions and their absorbed energy acts selectively through different gradients. This active channelling controls the membrane potential and has critical role in all life processes. The membrane potential may control the permeability of the ion channels between extra and intracellular electrolytes (Figure 3(A)). Nevertheless, this ion-selective rectification has two directions, depending on the sign of the ionic charge. The membrane furthermore rectifies the external signals, working like a gate controlled field-effect (FET) transistor gaining the signal amplitude (Figure 3(B)) with two basic processes:

- Normal rectification by the highly polarized cell-membrane, [21] [22] [23].
- Stochastic resonance that makes the rectification, [24].

The applied electromagnetic fields could make structural reorganization [25] [26], and may influence the various kinds of transports. The electric field drives transport on proton-flow [27] and Na/K ion pump [28] and changes the messengers [29]. The transmembrane potential also could be changed by an external electric field. The Schwan equation of electric field effect on the transmembrane potential [30] based on dispersion relation does not contain temperature. Only the field acts. Its validity is experimentally proven [31]. The static picture could be modified by the correlation between the amplitude of plasma-membrane fluctuation (fluctuates the Cm) and the applied electromagnetic effects [32].
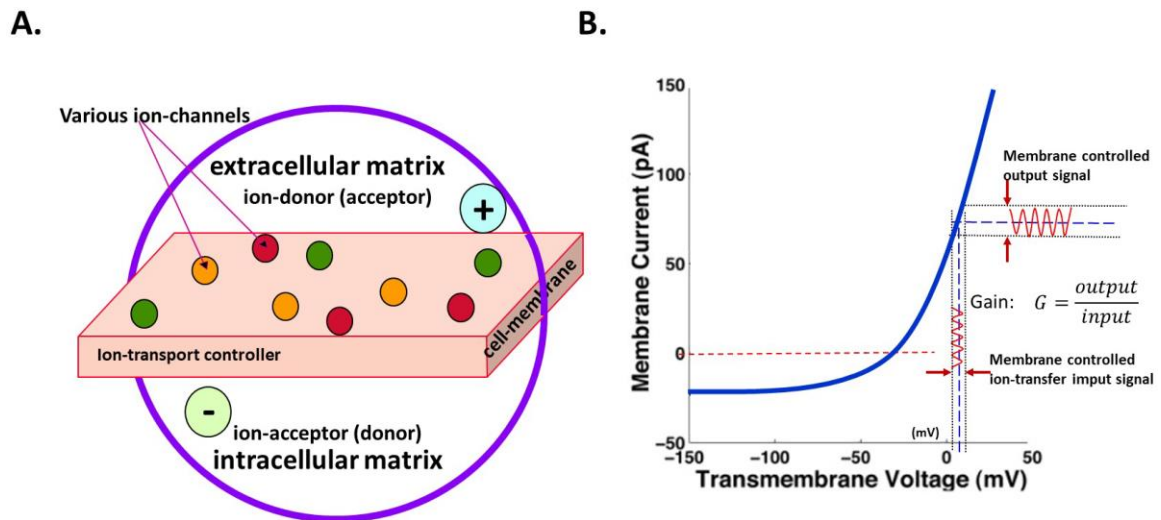
Figure 3. The membrane controls the ion flow between the electrolytes. (A) The direction of the flow depends on the charge and concentration of the species going through, changing the donor–acceptor situation by conditions. (B) The non–linear membrane current can gain the incoming signal.

Cancer cells have numerous challenges in a competitive, individual "fight" for their high energy demand. Cancer cells developed numerous tools to improve their adaptability in stressful environments. Cancer cells adapt and evolve in stressful conditions. As the disease progresses, cancer cells can undergo various adaptations that allow them to survive, proliferate, and evade the body's immune system and anticancer treatments. Adaptability is a complex process influenced by various factors, including genetic mutations, microenvironmental conditions, immune responses, and therapeutic interventions. The tumor microenvironment (TME) is the complex cellular and non–cellular environment surrounding a tumor, which consists of various leucocytes, chemical components, soluble factors of the extracellular matrix (ECM), and signaling molecules and vehicles. The increased metabolism of cancer cells profoundly changes the distribution of the electric charge in TME, also associated with a decrease in the cell membrane potential of the cancer cells. TME consists of various cellular and non–cellular components surrounding a tumor. The TME plays a decisional role in many functions of the tumor cells, including their adaptability to environmental stresses [33]. The key mechanisms by which cancer cells adapt are:

- The genetic mutations, which can occur spontaneously or be induced by external factors, enabling them to acquire new characteristics that promote their survival and growth.

- Phenotypic plasticity, which means they can change their characteristics in response to environmental cues. This plasticity allows them to adapt to different microenvironments.

- Epithelial–mesenchymal and opposite transitions allow the cancer cells to invade nearby tissues or metastasize to distant places in the body.

- Develop resistance to anticancer therapies through various mechanisms.

The adaptability of cancer cells poses significant challenges in the treatment of cancer and highlights the importance of developing new therapeutic strategies to overcome resistance and improve patient outcomes. TME has an essential role in the adaptation mechanisms of the tumor-cells. Due to the TME specialties and the intensive proliferation, tumors have unique electromagnetic properties and due to the permanent proliferation require more energy than healthy cells. Cancerous tissue relies on glycolytic (fermentative) ATP production, while healthy cells use the Krebs cycle phosphorylation in mitochondria. Although the fermentative process delivers 18 times less ATP [34], glycolysis is simpler and more intensive to supply cells with ATPs than the Krebs cycle does. The simplicity of glycolysis favors the massive ATP demand of malignant cells. Transport processes, such as glucose, sodium, pyruvate, lactate, and hydrogen ion transporters, are crucial for energy production and are simpler in the case of glycolytic procedures. The cancer cells act autonomously, competing for energy sources with the neighboring cells, they do not cooperate, but all together the individual actions makes the tumor like a cooperative organ [35] [36].

Warburg defined the metabolic deviation of malignant cells, originated from mitochondrial dysfunction [37] [38] [39] or at least the mitochondria are not able to produce enough ATPs in time for cancerous proliferation. The high glucose influx of most cancers appears in the composition of the TME and the whole extracellular environment, allowing to distinguish the malignant cells from normal. The positron emission tomography, (PET) [40] detects the metabolic difference. The transport processes are also crucial in glycolysis: the function of the glucose, sodium, pyruvate, and lactate, as well as the hydrogen ion transporters, must serve in time the actual energy-production processes. These forms of transport are also simpler in the glycolytic case than in the mitochondrial routes. Note, the situation is comparable to the well-known change of glucose metabolism in the sport-medicine when the muscles are overloaded, and the oxygen is not enough to supply the ATP demand by mitochondria. The ionic and molecular concentration of the ECM changes robustly by the highly concentrated metabolites and waste of the processes. The increased electrolyte volume in the tumor [41] additionally improves the higher conductivity, and so the selection of the malignancy, which may increase the enhancing conductivity with the necrosis. Furthermore, the growing glucose concentration keeps the real but lowers the imaginary part of complex permittivity. Considering a healthy concentration of glucose 100 mg/dL, and the tumor tissue has double of that, the conductivity decrease is less than 10% [42]. According to (3), the decrease of conductivity with constant permittivity is also < 10%. The reprogrammed metabolic process is a strong dysregulation of the electrolyte balance in malignant volume [43].

The composition change, the high ion concentration, is measurable by the electric conduction of the various electrolytes, directing the electric current to the more conductive path (Figure 4). This process clearly selects between the cells of different metabolic forms and automatically focuses the RF-current on the close TME electrolyte of malignant cells. In time-domain spectroscopy of breast tumor tissues of patients also shows the much larger conductivity of tumors than normal tissue [44] [45] [46] [47] [48] and liver tumors [49] [50].
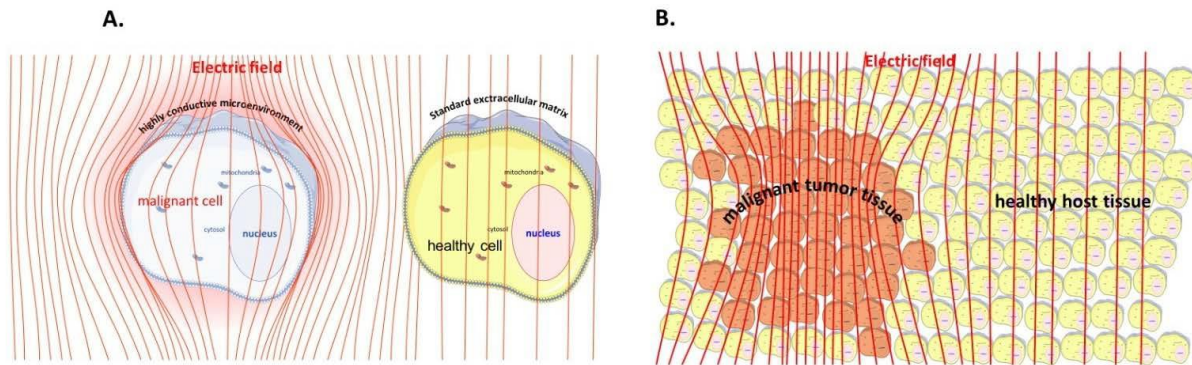
Figure 4. The conductivity selection. (A) The high electric conductivity of TME focuses the RF–current on the tumor cells (micro selection). (B) The overall higher conductivity of ECM in tumor mass concentrates the current to the cancer lesion.

The in vivo measurement of SMT–2A tumors in rats shows a significant difference (<6 times) in the conductivity of tumorous and normal tissues [51].This irregular behavior of electric conduction can be imaged by Electric Impedance Tomography (EIT), [52] [53]. Also, this effect could be applied in prophylactics like mammography [54] [55] too. Another impedance method, the MRI Electrical Impedance Tomography (MREIT) also clearly shows the conductance selection of tumors in theoretical description and in vivo [56]. The increase of the current density in the tumor could be visualized by the measurements of real processes by radiofrequency current density image (RF–CDI) [57][58] [59] [60].

The high conductivity also characterizes the intracellular electrolytes of malignant cells [61], growing by the progression rate of malignancy [62]. The intracellular networks of the cytoskeleton and the collective excitation (soliton [63]) of microtubules increase intracellular conductivity. The progression increases the TME and Tumor EMC conductivity too, so the precision of the selection by conduction differences growths by the development of malignancy. The living matter exists in an aqueous solution, which is partly ordered, [10] [64]. The ordered electrolyte states were suggested as much as 50% of the total amount of the aqueous solution in membrane living systems [65]. The membrane potential forms the dominant electrolyte order [66] [67] in its near vicinity. Carcinogenesis has drastic changes in the membrane of the cell uncoupling it from the neighbors, and producing membrane defects and numerous outside unconnected transmembrane proteins and their clusters (rafts). This process induces a rearranging (disordering) of the electrolyte structure which utilizes energy [68], similar, to the melting of ice with latent heat. This drastic change (phase transition) modifies the actual physical properties (like the dielectric constant) of the material without changing the composition of the medium itself. The TME alternates the electron/proton homeostasis increases the pH intracellularly and decreases it in the TME [69]. The cell membrane of the malignant cells is lowered compared to the healthy equivalents [70], and together with the lower pH of the TME the membrane thickness grows the inner side of the bilayer considerably changes, and the mixture of the lipid molecules replaced by the dominant phosphatidylcholine [71]. Decrease of the membrane potential in malignant cells disorients a part of the ordered electrolyte [72] [73] [74], which increases the electric permeability, [75], and decreases the cell–cell adhesion, [76]. The order–disorder phase–transition indicates the development of the disordered autonomy (α–state) and the disappearance of the collective networking (β–state), [77].

The significantly larger permittivity in tumor tissue in vitro is explained on this basis, [78]. The ordered structure makes it possible to "channel" the energy flow in the direction of the polarization order, while the outside electric field can spin the disordered polar molecules, whose movements absorb the energy functioning like "friction" in the medium. In this way, the high dielectric constant allows the additional selection by its higher energy absorption from the optimally applied RF energy. The malignity increases both the conductivity and dielectric permittivity (capacity) of the membrane of cancer cells [62], which promotes higher selectivity in severe malignant cases. The specific capacitance of the membrane and the cytoplasmic conductivity growths with increasing malignancy supports the focusing process in severe malignant cases [62]. The actual dielectric properties are also distinguishable by the water content of the malignant tissue, which is higher than that of their healthy counterparts. The proliferating cells control their cell volume by their water content, in the malignant growth [79], and by making more biophysical distinctions for properly applied electromagnetic treatment. The developing malignancy of the cancer cells of the mouse ovarian surface epithelial cell line increases the cytoplasmic and membrane electric permittivity with swelling in a low degree of malignancy [62]. The conductivity and relative permittivity do not considerably change by time and temperature in the MHz region for healthy ex-vivo muscle samples [80], so we also expect a relatively small variation of the other tissues ex-vivo. However, in vivo the change is considerable [44] [45] [46] [47] [49] [50] [51] [55], the transporting electrolytes (mainly the blood perfusion) change of the electric parameters by time and temperature. These processes are dominantly physiologically regulated, so homeostatic control has substantial importance in the changing electromagnetic properties [81]. The higher dielectric permittivity of the tumor cells and mass gives an additional selection factor [82] to the higher conductivity discussed above. The missing bonds and disordered TME definitely changes the electric behavior of the malignancy [83]. The RF current can recognize the altered structure [75] [78], useful in practice [52] [54]. The membranes may have phase transitions, causing restructuralization of the lipid layers.

The temperature may cause a chain melting transition which increases the membrane fluctuations and the dielectric permeability near the transition temperature [84]. The penetration of the membrane also rises near transition temperature increasing the likelihood of spontaneous lipid pore formation [84]. The chemical and electric gradients promote ligand connections and receptor activity, respectively. These processes may cause chemo- and electro-taxis [85], which promotes the drift movement of the cells, and could have a role in invasion of the malignancy. The electrolytes, which are charge neutral on the two opposite sides of the membrane lipid layers have a diffusion driving force due to different concentrations of the ions in the electrolyte. However, the diffusion of charged ions changes the charge distribution in both electrolytes, which affects the membrane. An ion present in a higher concentration on one side of the membrane crosses the membrane as a result of the diffusion driven by the concentration gradient.

This process creates an electric field by the carried charge of the ion opposing the driving force of diffusion. The ion-selecting process actively moves both anions and cations gaining the electric field between the two sides of the membrane. In the same time the membrane is not permeable for some ions and large units. The exchange of ionic species on this cannot form electric or diffusion equilibrium alone. The penetration processes last until the concentration gradients and the electric field are balanced, forming the observed membrane potential. The Goldman-Hodgkin-Katz equations modelized the electro-diffusion process [86], with conditions:

- A constant concentration difference between outer and inner side of the membrane ⇒ constant transport rate through membrane.

- Migration of ions through membrane ⇒ electric bilayer on both sides of the membrane.

- All kinds of ions on both sides of the membrane are considered simultaneously.

- Membrane is neither fully permeable nor fully non-permeable for any ion.

- Different ions have different permeability.

The ionic exchange processes which create the membrane potential fluctuate adding a noise to the resting potential which is in fact an average of the actual potentials. Ion channels, which are responsible for the movement of ions across the cell membrane, can exhibit stochastic behaviour. The opening and closing of ion channels can introduce variability in the resting potential. A single ion or its groups driven by concentration gradient may be overloaded and create electric contra force against the diffusion restores the balance. The same thing happens when the electric field prevails against the diffusion. It works like a promoter-suppressor pair which precisely tunes the necessary value defined by the given conditions [87] [88]. The potential oscillations dramatically improve action potential precision [89]. While bioelectromagnetic fields may potentially interact with cancer cells, it is currently challenging to draw definitive conclusions about their impact on cancer cell adaptability. Our present objective is to describe the charge distribution/redistribution in malignant tissues with the aim of understanding one of the electrodynamic aspects of the high adaptability of malignant cells. We give a proposal on how to decrease the detrimental effect of charge distribution in tumors.

2. METHODS

The biological material is imperfect dielectrics, having displacement current and conductive current as well. Two parameters are used to characterize tissues from an electric point of view: conductivity (σ) and dielectric permittivity (ε). These physical properties could be used in diagnosis [90] and in treatments [91].

The current density (j) induced by E field vector and ρ charge density:

$$divE = \rho, \quad j = \sigma E,$$

and the charge conservation.

$$\frac{\partial \rho}{\partial t} + divj = 0$$

Having f frequency in harmonic signal the j became complex $(i = \sqrt{-1})$:

$$j = (i\omega\varepsilon + \sigma)E \qquad (1)$$

where $\omega = 2\pi f$ is the circular frequency.

When there is no free charge density

$$div(i\omega\varepsilon + \sigma)E = div(\sigma^* E) = \rho \qquad (2)$$

The superscript * denotes the complex numbers:

$$\sigma^* = \sigma + i\omega\varepsilon \quad \varepsilon^* = \varepsilon - i\frac{\sigma}{\omega} \qquad (3)$$

Hence:

$$grad\,\varepsilon \cdot E + \varepsilon \cdot divE = \rho$$
$$grad\,\sigma^* \cdot E + \sigma^* \cdot divE = 0 \qquad (4)$$

where ρ charge density appears only by the polarization (space–charge). With further simplification of (4), we get:

$$\left[grad(\varepsilon) + grad(\ln \sigma^*) \right] \cdot E = \rho \qquad (5)$$

The gradient of permittivity determines the behavior, which linearly depends on the E field. The logarithm of the conductivity smooths its changes, having only a minor effect. The (5) has no structural components but the parameters (ε and σ) are frequency dependent. According to (5) the inhomogeneous dielectric material of the living tissues, and their higher organizations, the permittivity gradient creates space charge. The accumulated space charge increases capacity with frequency dependence, without structural modification. The simplest arrangement to show interfacial polarization is a condenser with two layers of permittivity between its electrodes. The dielectric heterogeneity is a cube having two different materials, representing the inhomogeneity of the material with d1 and d2 thickness, placed in a serial arrangement in the condenser The space charge will be created at the boundaries of the permittivity blocks, which are parallel to the electrodes (Figure 5).

We define the conductivity of this composite dielectric block by:

$$\frac{d_1 + d_2}{\sigma^*} = Z = \frac{d_1}{\sigma_1^*} + \frac{d_2}{\sigma_2^*} \qquad (6)$$

From this definition, we receive the independent geometry conductivity and complex permittivity depending only on the ratio of volumes:

$$\varepsilon^* = \frac{\sigma^*}{j\omega} = \frac{\sigma_1^*\sigma_2^*}{i\omega\left[a(\sigma_2^* - \sigma_1^*) + \sigma_1^* \right]} \qquad (7)$$

where $a = \dfrac{d_1}{d_1 + d_2}$ represents the geometry. The solution depends just on the layer thicknesses. The parallel blocks are irrelevant, because the gradient and the field are perpendiculars, and their scalar product is zero. The dielectric permittivity depends on the frequency, causing dispersion. The simple theory of dispersion was laid out by Debye [92]. According to Debye theory the

complex dielectric material has a breaking-point circular frequency $(\omega_c = 2\pi f_c)$, characterizing the transition between the two states, and the relaxation time (τ) is $\tau = 1/\omega_c$. The physical meaning of this relaxation time is the time during the displacement vector oriented by the switched-on unit electric field. The complex dielectric material has different permittivity at high frequencies (ε_∞), and at low frequencies (ε_s) (Figure 6), and according to Debye considerations:

$$\varepsilon^*(\omega) = \varepsilon_\infty + \frac{\varepsilon_s - \varepsilon_\infty}{1 + i\omega\tau} + \frac{\sigma_s}{i\omega} \qquad (8)$$

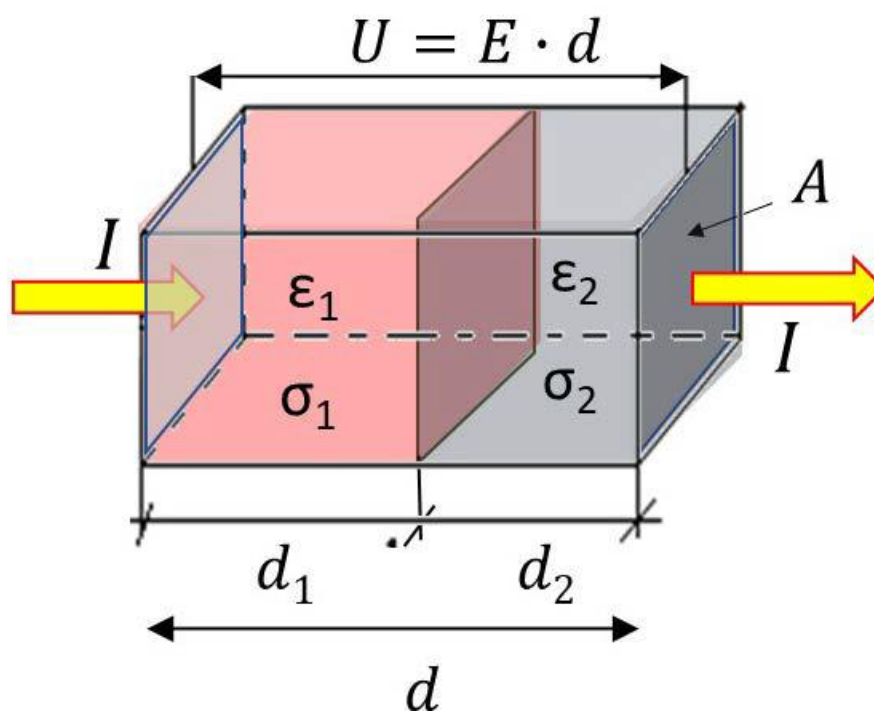Considering the heterogeneity form Figure 5. We obtain:

Figure 5. Arrangement of two dielectric materials in a condenser. U is the potential, d the length and A the cross section of the block, which has I current through.
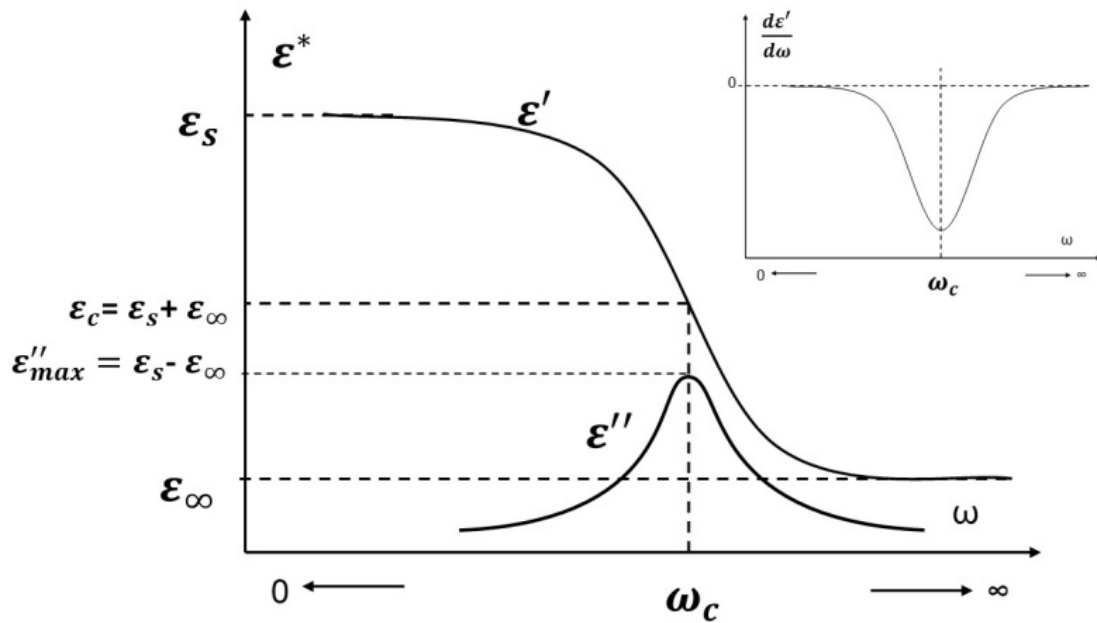
Figure 6. The complex dielectric permittivity $(\varepsilon^*(\omega))$ in Debye scheme. The derivative of the real

part $(\dfrac{d\varepsilon'}{d\omega})$ has a minimum in ωc . It is shown in the insert.

$$\varepsilon^* = \frac{\varepsilon_1\varepsilon_2}{a(\varepsilon_2-\varepsilon_1)+\varepsilon_1}+\frac{1}{i\omega}\cdot\frac{\sigma_1\sigma_2}{a(\sigma_2-\sigma_1)+\sigma_1}+\frac{\mathfrak{E}}{1+i\omega\tau}$$

$$\varepsilon_\infty = \frac{\varepsilon_1\varepsilon_2}{a(\varepsilon_2-\varepsilon_1)+\varepsilon_1}$$

$$\varepsilon_s = \varepsilon_\infty + \mathfrak{E} \tag{9}$$

$$\mathfrak{E} = \frac{\left(\sigma_1\varepsilon_2-\sigma_2\varepsilon_1\right)^2 a(1-a)}{\left[a(\varepsilon_2-\varepsilon_1)+\varepsilon_1\right]\left[a(\sigma_2-\sigma_1)+\sigma_1\right]^2}$$

$$\tau = \frac{a(\varepsilon_2-\varepsilon_1)+\varepsilon_1}{a(\sigma_2-\sigma_1)+\sigma_1}$$

This result is remarkable: the inhomogeneous dielectric arrangement could have larger dielectric permittivity than the individual components. The permittivity, in this case, is a complex value, which is frequency-dependent and can be approximated by the Debay principles. The complex

dielectric material has different permittivity at high frequencies (ε_∞), and at low frequencies (ε_s). Note that when the gradients of permittivity and conductivity are perpendicular on the penetrated external field, space-charge is not formed. The parallel blocks are two independent imperfect condensers, calculated as a parallel circuit of two condensers.

The overall frequency-dependent conductivity (σ Σ) of a unit cube (d=1, A=1) of the tissue is shown with discrete electric elements on Figure 1(B), is:

$$\sigma_\Sigma = \frac{R_1 + R_C + R_2}{R_1(R_C + R_2)} = \frac{1}{\sigma_1} + \frac{1}{\frac{1}{i\omega\varepsilon} + \sigma_2} \qquad (10)$$

The high and low–frequency current differently depend on the frequency (Figure 7(A)) slope of σ Σ , and have changed in f = 10 –100 MHz , (Figure 7(B)) at the range of β/δ frequency dispersion identified by Schwan and colleagues, [93] [94],
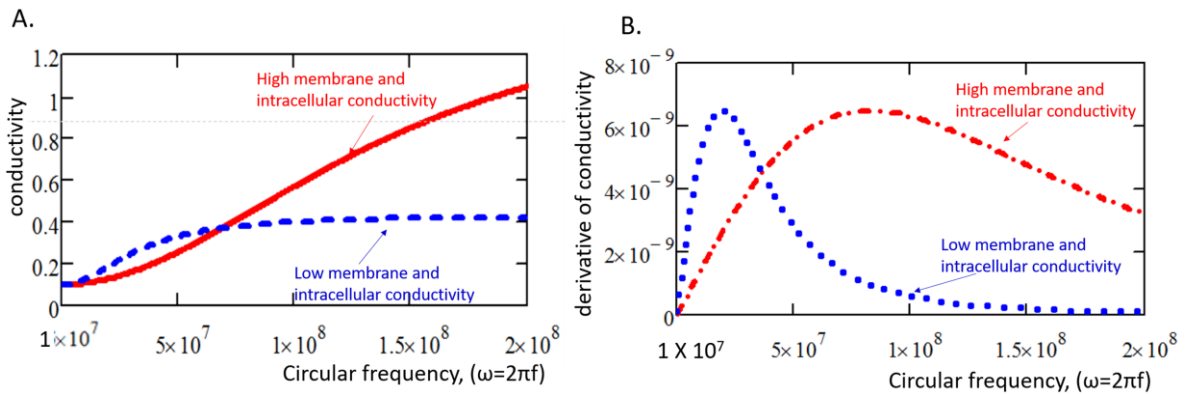

Figure 7. The conductivity vs. circular frequency ω = π2 f of a unit cube of the tissue at the low $(\sigma_2 = \frac{1}{3} \, S/m)$ and high $(\sigma_2 = 1 \, S/m)$ membrane and intracellular resistivity, $(C = 10^{-8} \, F; \ \sigma_1 = 0.1 \, S/m)$. (A) The conductivity; (B) The derivative (slope) of the conductivity function. The numeration of conductivity corresponds to Figure 1(B).

The charge distribution, forming considerable space charge within the TME (Figure 8) can arise from several factors:

- Charge: Cells within the TME, including cancer cells and immune cells, possess an overall net charge due to the presence of charged molecules, such as proteins and nucleic acids. The charge on the cell membrane arises from the distribution of charged phospholipids and membrane proteins. These effects can lead to imbalances in ion concentrations within the TME. The increased metabolism of cancer cells profoundly changes the distribution of the electric charge. The change is principally associated with a decrease in the cell membrane potential of the cancer cells and an increase in the charge of the TME. The TME can exhibit altered electrical conductivity due to changes in ion concentrations and the presence of charged molecules. These conductivity variations can affect the distribution of electric fields and influence electrodiffusion.

- Extracellular Matrix: The ECM, which provides structural and electrolyte support to tissues, is composed of a complex network of proteins, proteoglycans, and glycosaminoglycans. Proteoglycans and glycosaminoglycans have negatively charged sulfate and carboxyl groups, contributing to the overall charge distribution within the ECM. Alterations in the ECM composition and stiffness [95] can affect the diffusion properties of ions in the TME.

- Soluble Factors: Soluble factors present in the TME, such as cytokines, growth factors, and chemokines, can also possess charged regions. These factors can be secreted by various cells within the TME and may interact with cells or components through electrostatic interactions.

- It's important to note that the charge distribution within the TME is a dynamic and complex system, influenced by various cellular and molecular interactions. The overall charge distribution can impact cellular behaviors, signaling pathways, and interactions between cells and the ECM. Understanding the charge distribution within the TME is a part of the broader study of the tumor microenvironment, which is crucial for unraveling the mechanisms of tumor growth, invasion, and metastasis.

The membrane potential (ϕ_m) is usually 30% smaller in cancer cells than the normal ones [96], however its TME is richer in ionic and inert molecular species. The high proliferation rate, the high intracellular Na+ concentration and changes on the transmembrane proteins lower ϕ_m, which may induce the Ca2+ efflux. The reduced membrane potential makes the cell outside relatively negative compared to the constant outside positive membrane potential of healthy cells. The difference rearranges the charge distribution between healthy and tumor cells, and as a result of active cancer proliferation, the charge distribution changes over time. The charge redistribution makes strong heterogeneity in the electrical conductivity for transmitted RF current and so the electrical resistance depends on the charge distribution. A depolarized membrane is considered a driving force for the intracellular increase of Ca2+ which is partly a bioelectronic cancer regulator that affects proliferation, migration, invasion and metastasis of cancer cells.
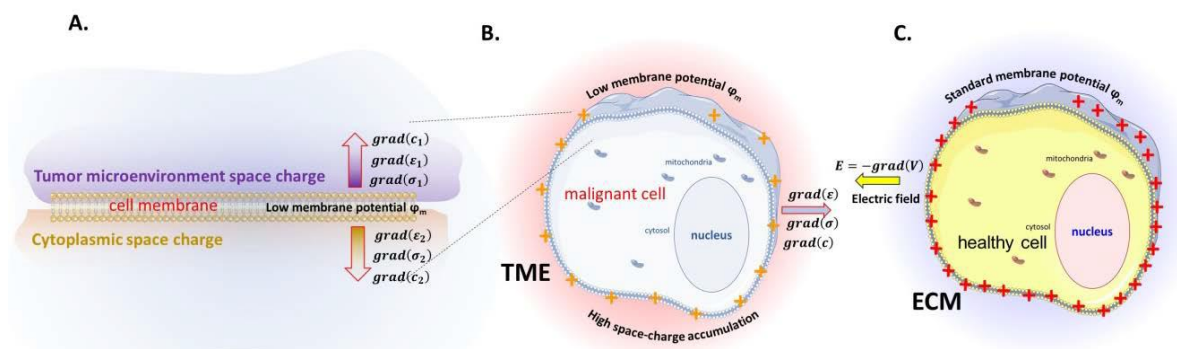
Figure 8. The cell membrane polarization is outside positive in resting case. The directions of the concentration (c), dielectric permittivity (ε) and conductivity (σ) gradients are perpendicular on the membrane. (A) A part of the cell–membrane of malignant cell with its environment. (B) The malignant cell has low membrane potential. (C) The healthy cell has normal membrane potential, and an E–field appears to the direction of lower potential of malignant cells.

Furthermore, changes in ϕ_m are related to the modulation of local concentrations of signaling molecules and ions, the spatiotemporal regulation of morphogenesis, the interaction with heterogeneous networks (that combines conventional gene regulatory network) is controlled by spatiotemporal bioelectrical patterns based on electric potentials and currents from steady and oscillatory multicellular states, among others. In turn, these spatiotemporal bioelectrical patterns influence on the spatiotemporal distributions of signaling ions and molecules that modulate biochemical pathways in cancer cells, and therefore in growth and regeneration. Several studies have investigated the potential impact of electromagnetic fields on cancer cells and their proliferation [97]. A special electromagnetic oncotherapy combines the thermal and nonthermal

effects to improve the efficacy of the electromagnetic effects [98] [99]. This method uses the modulated electric field impact to space-charge and promotes the Ca2+ influx to the cancer cells [100], blocks the tumor-supporting injury current [101] and reestablishes the E-cadherin + β-catenin intercellular adherent connections to repair the lost collectivity of the cells [102]. The method uses a strong synergy between the thermal and nonthermal effects [103], using the thermal component as a environmental factor gaining the chemical reaction rate [91]. The specific mechanisms underlying any potential effects of bioelectromagnetic treatments on cancer cell adaptability presently are not well-established [104]. The ionic structure and density of ions differ in the microenvironment of cells from the average ECM.

The Nernst-Planck equation is used to describe the flux of ions due to diffusion and electrostatic forces. It considers the concentration gradient, the electrical potential gradient, and the ion mobility [105]. The Nernst-Planck equation describes electrodiffusion, refers to the combined effect of diffusion under influence of electrical forces. This movement rearranges the charges in the medium, and forms heterogenic distribution developing internal electric field induced by the various space charges. These fields interact even in the relative large distances. A local arrangement of the charges embedded in the material structure (in electric meaning impedances in the electric fields) is regarded as a "cell" in the complex structure. These cells overlap and interact in a fractal-like behavior. The Nernst-Planck type space charge [106] can be formed in every non-perfect dielectric material, including the biological tissues. The basic process is driven by the complex interaction of the diffusion and electromagnetic charge transfer. The driving force of the diffusion is the concentration gradient, while the charge-transport made by electric field. In aqueous solutions like the electrolytes in the living objects, ions represent the charges interaction with the electric field. The electrodiffusion in the TME is important as it can impact various tumor-related processes. In the context of tumor development, electrodiffusion can have several influences.

- Tumor Microenvironment: Electrodiffusion can affect the composition and properties of the tumor microenvironment. The electrical gradients within tissues can influence the distribution and movement of ions, nutrients, and signaling molecules. Alterations in the ionic concentrations and pH due to electrodiffusion can create a microenvironment that supports tumor growth and survival.

- Cell Proliferation and Migration: Electrical gradients and ion movements can affect the behavior of cells within a tumor. Studies have shown that electric fields can influence cell proliferation, migration, and invasion. Certain ion channels and transporters play crucial roles in these processes, and their dysregulation can contribute to tumor progression.

- Angiogenesis: Electrodiffusion can also impact angiogenesis, the process of new blood vessel formation. Electrical signals can guide endothelial cells, which line the blood vessels, and influence their migration and organization. Electrodiffusion-mediated changes in ion concentrations and pH can modulate the expression of angiogenic factors, thereby affecting the formation of blood vessels within tumors.

- Drug Delivery: Electrodiffusion can influence the delivery and distribution of drugs within tumors. The presence of electrical gradients can affect the transport of charged molecules, such as chemotherapeutic agents, into tumor tissues. This can impact the efficacy of treatments and contribute to drug resistance.

- Electric Field-Based Therapies: In recent years, electric field-based therapies, such as electroporation and electrochemotherapy, have emerged as potential treatment modalities for cancer. These therapies utilize externally applied electric fields to enhance drug delivery or induce cell death. The principles behind these therapies rely on the effects of electrodiffusion on tumor cells and their microenvironment.

Studying electrodiffusion in the TME can help uncover potential targets for therapeutic interventions aimed at modulating ion concentrations and restoring normal electrochemical gradients within the TME. It's important to note that the influence of electrodiffusion on tumor development is a complex phenomenon, and the specific effects can vary depending on tumor type, location, and other factors. Ongoing research in this field aims to further elucidate the underlying mechanisms and explore potential therapeutic applications. The ionic electric conductions naturally carry chemical mass transport too, synergizing the electric conduction and the diffusion- and drift-like material transport as well. According to Fick's Law, the diffusive current density is:

$$j_{diff} = -D\frac{dC(x)}{dx}, \tag{11}$$

where D is the diffusion constant of the given chemical component and the ionic chemical component at x has a concentration C(x). Denote the ionizing level of the given chemical component by Z, and its ion mobility by β. Then at the applied E field-strength, the drift velocity is:

$$v_{drift} = -\beta E . \tag{12}$$

which (using the Einstein relation) could also be written in the form:

$$v_{drift} = -\beta eE = -\frac{D}{kT}ZeE \tag{13}$$

where e is the elementary charge (electron charge), k is the Boltzmann constant, and T is the tissue temperature and Z is the valence of the moving charge particle. Hence, the drift-current density in the transition layer is:

$$j_{drift} = -C(x)\frac{D}{kT}ZeE \tag{14}$$

Therefore, the particle current density of the chemical component is:

$$j = -D\frac{dC(x)}{dx} - C(x)\frac{D}{kT}ZeE \tag{15}$$

From this the jointly transported electric current density is:

$$j_e = Zej = -ZeD\frac{dC(x)}{dx} - C(x)\frac{D}{kT}Z^2e^2E \tag{16}$$

which looks in Ohm's Law–form like:

$$j_e = \sigma \left(E + E^{(i)} \right),$$

$$\sigma = C(x) \frac{D}{kT} Z^2 e^2 \tag{17}$$

$$E^{(i)} = -\frac{ZeD}{\sigma} \frac{\mathrm{d}C(x)}{\mathrm{d}x}$$

where E(i) is the field created by charge density. For numerical investigation we normalize these values:

$$j = \frac{j_e}{\dfrac{ZeDC_2}{\delta}} = \frac{\dfrac{C_1}{C_2} e^{-\frac{ZeU}{kT}} - 1}{e^{-\frac{ZeU}{kT}} - 1} \frac{ZeU}{kT} = \frac{Qe^{-\theta} - 1}{e^{-\theta} - 1} \theta \tag{18}$$

where C_1 and C_2 are the concentrations of the interface layer incident and emergent sides, and δ is the thickness of the transition layer by U potential-drop on it; the $\theta = \dfrac{ZeU}{kT}$ and the $Q = \dfrac{C_1}{C_2}$ is the concentration ratio. This result shows that the "foreign" field strength appears only where the particle concentration has a gradient (e.g. in the phase boundaries).

Suppose a linear change of the potential in the transient layer; from (18) we get:

$$j_e = \left(\frac{Z^2 e^2 D}{kT\delta} \right) \frac{C_1 e^{-\theta} - C_2}{e^{-\theta} - 1} U \tag{19}$$

The tissue boundaries are inhomogeneous interfaces, where external field strengths $E^{(ex)}$ may be a perturbative addition to (17), causing a charge redistribution. The charge conservation at the perturbation:

$$\frac{\partial \rho}{\partial t} = -\frac{\sigma}{\varepsilon} \rho - \sigma \frac{\partial E^{(ex)}}{\partial x} = -\frac{\sigma}{\varepsilon} \left(\rho - \rho^{(s)} \right),$$

$$\rho^{(i)} = \varepsilon \frac{\partial E^{(ex)}}{\partial x} \tag{20}$$

Its solution is:

$$\rho = \rho^{(s)} + \rho_0 e^{-\frac{t}{\tau}} \qquad (21)$$

The perturbation exponentially decays in (21), but space charge $\rho^{(s)}$ remains due to the inhomogeneity. The time constant differs from the previous one, and it is the same order of magnitude as the periodic time of the external signal.

The charge of the ith and k^{th} condensers in the interface is:

$$Q_i = -C_i U_i = -C_i R_i I_{iR} = -\tau_i I_{iR}$$
$$Q_k = C_k U_k = C_k R_k I_{kR} = \tau_k I_{kR} \qquad (22)$$

where U_i, U_k are the potentials, I_{iR}, I_{kR} the resistivity dependent currents in the condensers, $\tau_i = C_i R_i$ and $\tau_k = C_k R_k$ are the time constances of the given circuits. Moreover, the resultant surface charge:

$$q_{i,k} = \frac{Q_k + Q_i}{A} = \tau_k j_{kR} - \tau_i j_{iR} \qquad (23)$$

where A is the surface of the electrodes which provides the external field (i.e. a plate condenser model). The surface charges drastically differ in the case of networked healthy and autonomic cancer cells. The current flow in the presence of external fields is well-known in conductors. We have shown above the polarization effect by external electric fields in imperfect conductors in the macro and micro range even in "porous" conditions like the cellular structure. This polarization differs from that that induces injury current when its stability is disturbed. Contrary to the internal polarization, the external triggering has no stable (current free) state, when the external field is time-varying (i.e. alternating). In the cases of electric currents, irrespective of the internal or external sources, the conductivity and the electric field-induced processes by the polarized layers constructed by electrolytic diffusion has to be counted (Figure 9).

These electric current are called bio-currents. We use conditions in which time constants (k τ and i τ) are commensurable with the quarter of the periodic time. The electric circuit schematic of this model is shown in Figure 10.

This model has Nernst-Planck characteristics, so we refer to it as a Nernst-Planck cell. The interconnected cells produce a variant of the Nernst-Planck cells, having a heterogenic distribution of the space-charges (Figure 11)

3. RESULTS

Due to the concentration variance of charges the conductivities differ, depending on the values of the potentials. The approximations of (19) at an external signal with U potential:

$$j_e \cong \frac{Z^2 e^2 D C_1}{kT\delta} U \tag{24}$$

2) If $U \gg 0$ is a large positive potential, then from (19):

$$j_e = \frac{Z^2 e^2 D C_2}{kT\delta} U \tag{25}$$
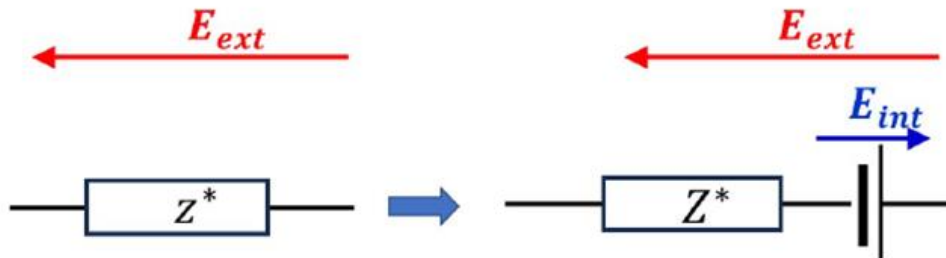
Figure 9. Model of the effect of the Nernst–Planck principle on a material having z ∗ impedance developing Z int ∗ + E by forming Nernst–Planck space charge. The non–perfect dielectric material develops an internal electric field by a double layer influenced by the external electric field. The newly formed electric field can be modelled by an additional opposite field caused by the internal rearrangement of charges.

A.
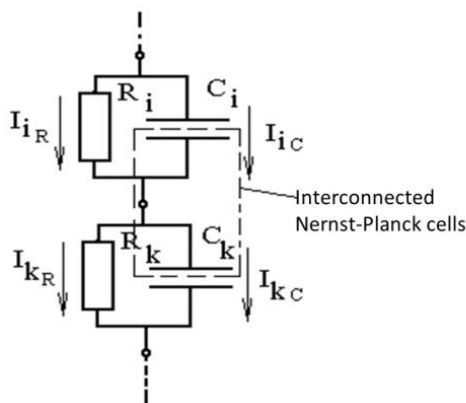
B.
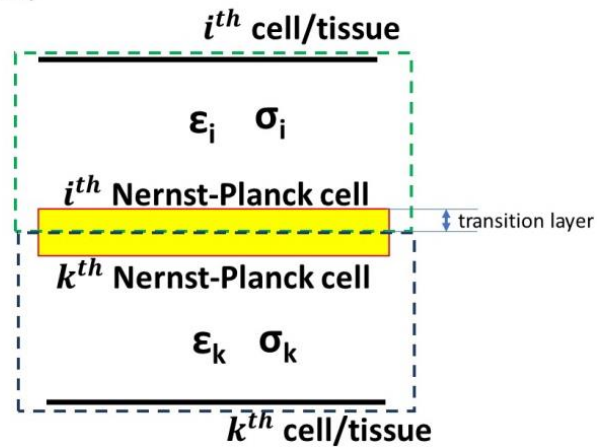
Figure 10. Transition layer schematic of the i th Nernst–Planck cell of the corrected model.
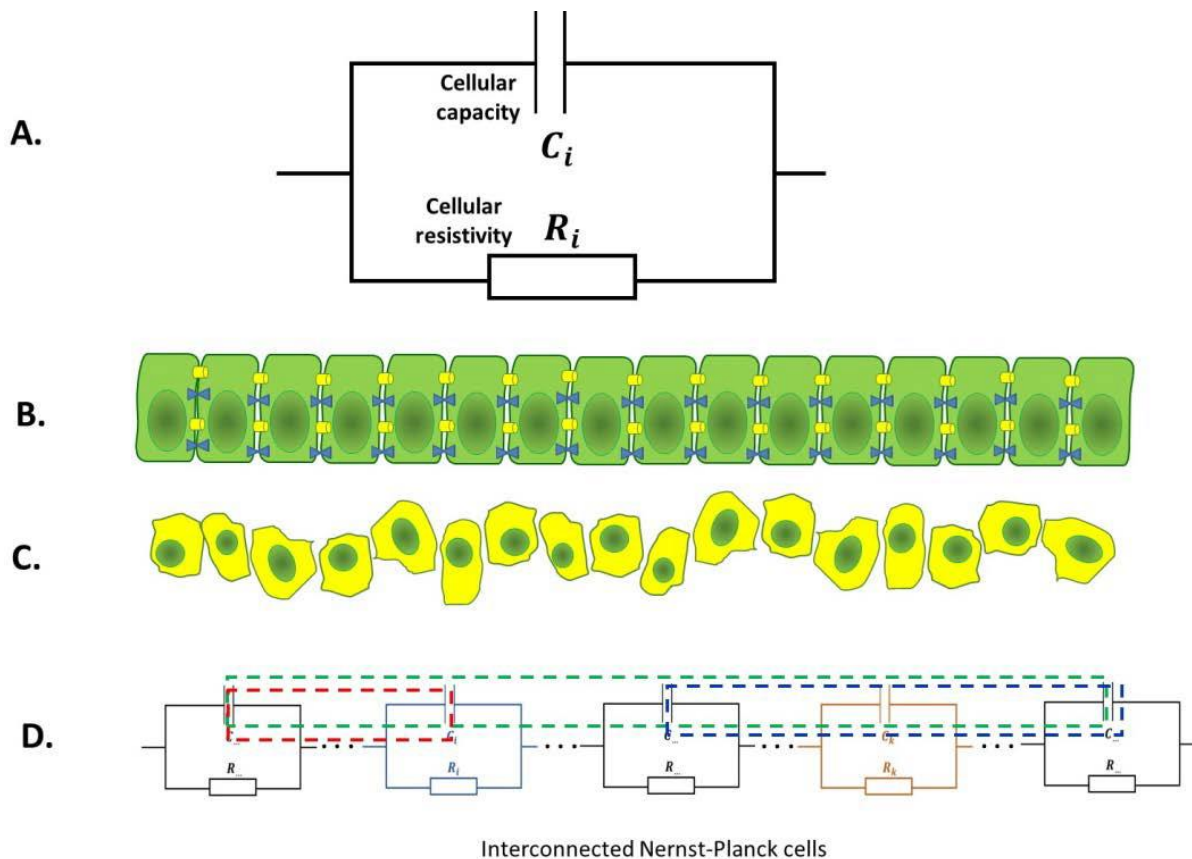
Interconnected Nernst-Planck cells

Figure 11. A schematic of space–charge model in serial connection shown in one dimension. (A) The cellular electric analogue of the i th cell in the line. (B) A line of healthy cells, connected network. (C) Line of malignant cells. Broken connections, changed cellular morphology, seeking to more spherical shape. The space–charge between them essentially differs from the healthy situation. (D) The electric analogue of the cellular line. The transition layer for Nernst–Planck cell is shown between the two condenser components of the cells in the line. The dashed rectangles are examples of the formed Nernst–Planck cells. These are embedded, interacting with distant cells in long range, not only with the next neighbor.

3) If $U \ll 0$ is a large negative potential, then from (19):

$$j_e = \frac{Z^2 e^2 D C_1}{kT\delta} U \qquad (26)$$

The results (24)-(26) apparently forms Ohm-law in extremes of the U potential having apparent conductivity $\sigma_a = \frac{Z^2 e^2 D}{kT} C_i$, where C_i is the concentration of given chemical component in the electrolyte. Due to the multi-ionic behavior (n components) of the TME the Ohm law has further generalization. When the kth component has specific Z_k valence, D_k diffusion constant and C_k concentration, the generalized apparent conductivity would be.

$$\sigma_a^{(S)} = \sum_{k=0}^{n} \frac{Z_k^2 e^2 D_k}{kT} C_{i,k} \qquad (27)$$

As a result, the Nernst-Plank cell is a potential dependent two–pole with nonlinear characteristics, as its conductivity depends on the direction of the current. Hence, the cell rectifies and distorts

(Figure 12), so, for example, the supplied sinusoidal potential will gain a non-sinusoidal current containing upper harmonics. It is vice versa valid: if we have over harmonics on a harmonic potential excitation. The harmonic alternating potential shows the rectification of the signal.

The frequency spectrum of the current density by Fourrier transformation of the alternating potential is shown in Figure 13. The time-derivative of current density shows that the slope of the current development depends on the direction of the external field, making some-kind of hysteresis. Practical clinical applications frequently use 13.56 MHz RF radiation, which frequency is reserved for medical and industrial applications. This RF shows a particularly good relative difference in the dielectric permittivity of normal and tumorous tissues. The imaginary part of the conductivity, which is proportional to the relative permittivity ($\operatorname{Im}(\sigma) = \omega\varepsilon$) is ~15 times higher in the tumor than in the connective and the adipose tissues in breast cancer (Figure 14). The same minimal complex resistance was obtained in two model calculations for ellipsoidal cells [107], so the selection at the 13.56 MHz frequency is optimal.

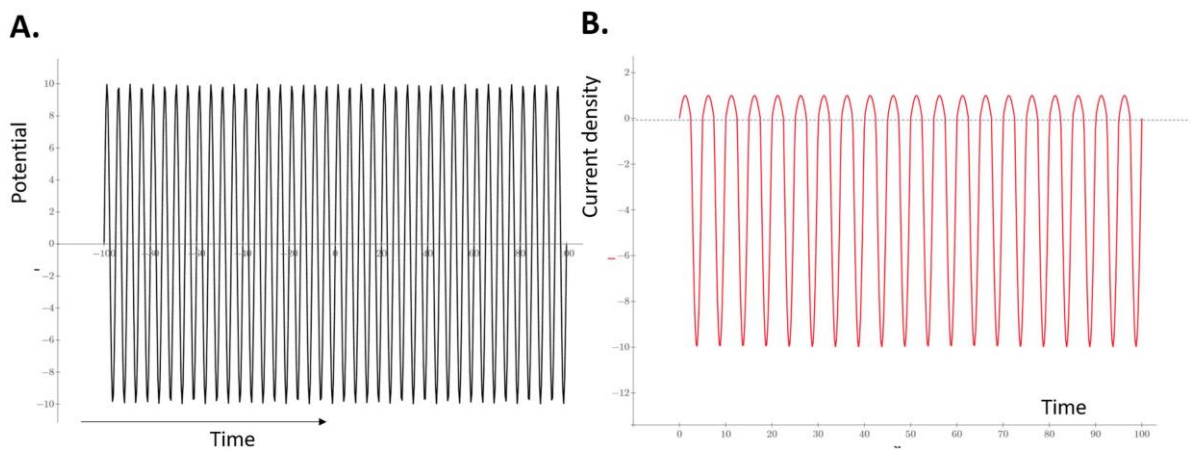**Figure 12.** Nernst-Plank cell characteristics. (A) The harmonic external signal. (B) The current density having C_1 C_2 =10 . The rectification modifies the time-dependence.
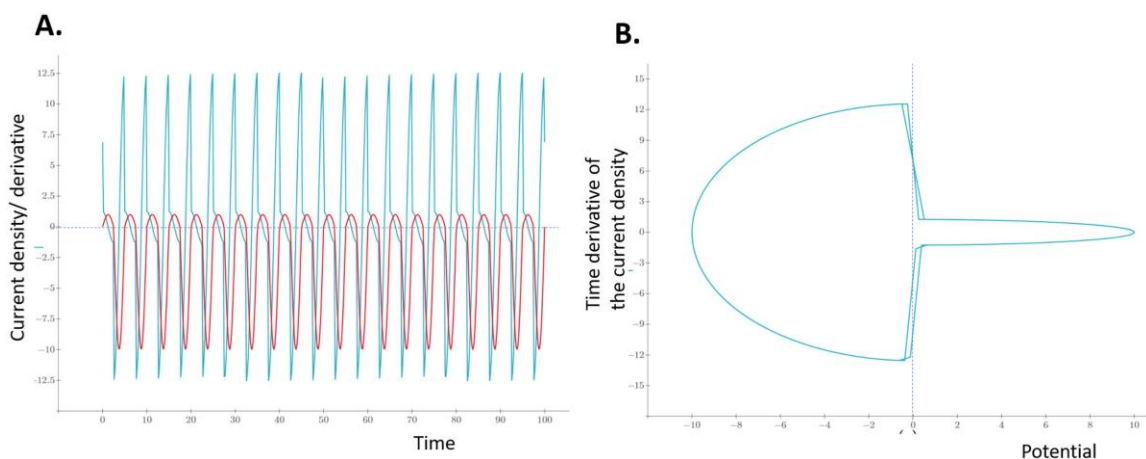
Figure 13. The time-derivative of the current density shows non-linear behavior. (A) The current density and its derivative. (B) The time derivative of current density has hysteresis by the changing potential.
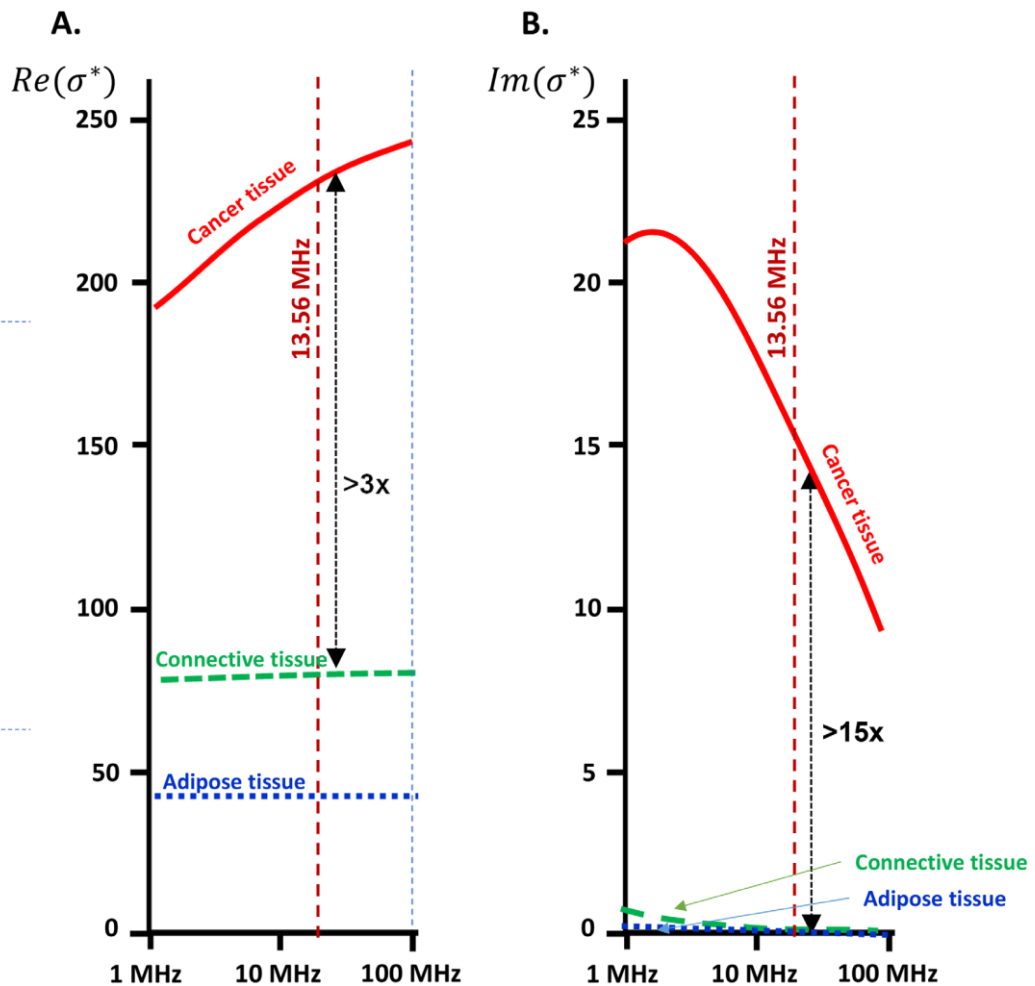
Figure 14. The electric parameters of breast tissues vs. frequency [55]. The 13.56 MHz shows high selectivity in both parts of the electric conductivity. (A) The real part; (B) The imaginary part ($\omega \varepsilon \cdot$) of the conductivity.

The selective energy–absorption is promoted by a characteristic frequency dispersion in the applied frequency range (β/δ dispersion [108]). The active nonthermal excitation effects also happen in this dispersion range [109], which targets the lipid–protein interactions and selects water-bound states [110] at the membrane, effectively focusing the energy on the target [111]. This high frequency promotes dipolar processes of proteins and other large molecules (like cellular organelles, biopolymers) [112], and is active on the suspended particles surrounded by cells [113], as well as may modify the protein–bound water, and cell organelles such as mitochondria [114] [115]. The excitation of the transmembrane proteins, charging intercellular structures and electrochemical changes mostly happen in lower frequencies (α-dispersion). To achieve the complete effect of selecting and modifying the TME the combination of the high and low frequencies offers the optimum. The high frequency component is used as a carrier frequency of the low one which modulates the signal [116] [117]. The current density of the modulated signal has special nonlinear properties, similar to the unmodulated applications (Figure 15).

The charge redistribution and its changing of the applied RF current allow a special memory. In the presence of an alternating current electric field, the behavior of the electrodiffusion becomes time dependent. The time dependent electric field exhibits a memory effect depending on the specific

conditions and assumptions. In general, the memory effect refers to a system's response being dependent on its previous states or history. In the case of electrodiffusion, if the system exhibits memory, it means that the transport properties of the charged species at a given time depend not only on the current electric field but also on its previous history. The presence of memory can arise if there are mechanisms that introduce temporal dependencies in the transport coefficients, like relaxation processes. The memory element of the discrete electric circuit is the memristor [118] [119]. Memristors are devices that can exhibit electric charge redistribution, known as the memristor effect. As the potential difference changes, so does the resistance. The change in time is determined up to the time constant of the process: the diffusion and the dielectric polarization relaxation. Memristors exhibit a memory-like behaviour by changing their resistance based on the magnitude and direction of the applied electric current. The memristor's memory depends on the charge distribution w ≈ grad (ρ) in the resistive volume, and the resistivity Rm depends on the gradient (w), the current (I) and the time (t), and so the Ohm-law: $V = R_m(w, I, t)I$ memresistive system [120]. Memristors can indeed exhibit the electric charge redistribution effect.
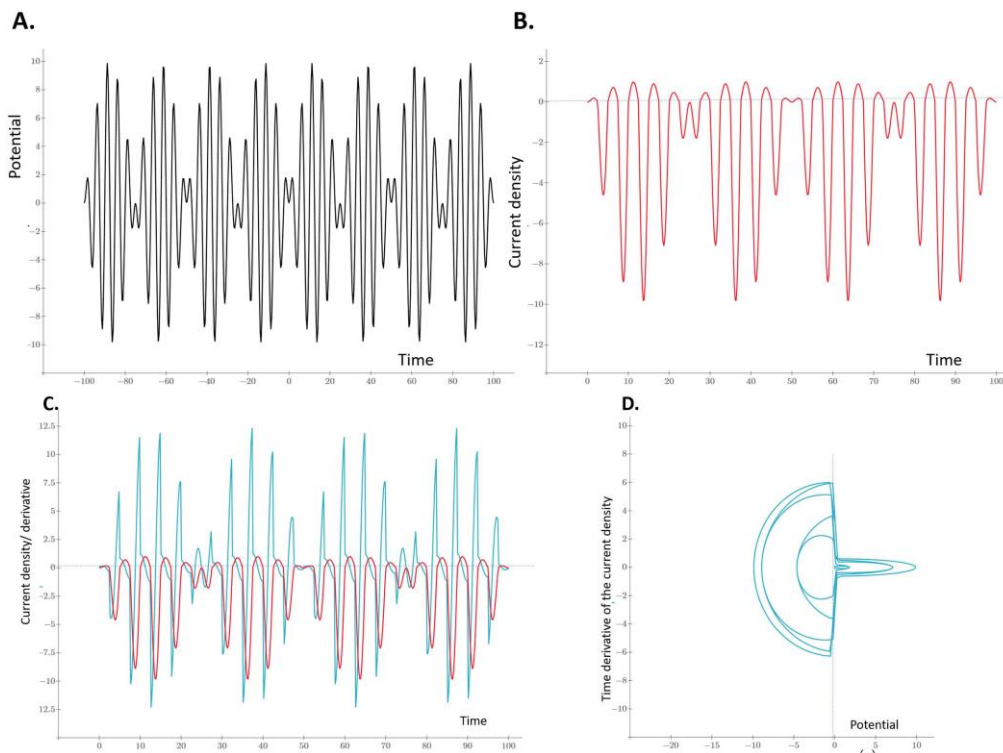
Figure 15. The modulated field makes similar but more complicated behavior tan Figure 12 and Figure 13. (A) The modulated external signal. (B) The current density having $C_1/C_2 = 10$. The rectification modifies the time-dependence. (C) The current density and its derivative. (D) The time derivative of current density has hysteresis by the changing potential.

Memristors are a type of electronic device that can "remember" the amount of charge that has flowed through them in the past states through which the system has evolved. The memristor is a two-terminal electronic component that can change its resistance in response to the magnitude and direction of the applied voltage or current. This change in resistance is a result of the redistribution of electric charge within the device. The resistivity of memristor depends on the charge (ρ) distribution. The Ohm-law in differential form with V potential, I current and R resistivity:

$$dV(\rho) = R(\rho)dI(\rho) \tag{28}$$

and the voltage depends on the magnetic flux (φ).

$$dV(\rho) = \frac{d\varphi(\rho)}{dt} \quad \text{and} \quad dI(\rho) = \frac{d\rho}{dt} \tag{29}$$

Consequently, the memristive effect, when the flux depends on the charge:

$$M[\rho(t)] = R(\rho) = \frac{d\varphi(\rho)}{d\rho} \tag{30}$$

which was the basic idea of the memristor as a missing circuit element [118 115]. On this way, the biological systems with memristive behaviour, when the relaxation time of space-charge comparable with the one quarter of the exciting electric field time constant, otherwise the quick relaxation does not interact with the field. Then opposite change of the current must happen during the space-charge relaxation processes for the memristive interaction. The charge dependence of the current-density causes an apparent inductive behaviour, the current left behind the voltage, due to the rearranging space charge. The time-lag changes by time, the system "learns". According (9) the characteristic relaxation time of the space-charge (sc) is $\tau_{sc} \cong 2.5 \times 10^{-9}$ s, , which is directly influenced by the quarter of the time-constant of 13.56 MHz carrier frequency ($\frac{\tau_{13.56}}{4} \cong 2.9 \times 10^{-9}$ s), causing the memristor behaviour. The induced space charge by polarization at the boundaries does not complete the rearrangement of the charges. The charge separation at the boundaries introduces non-linearity, having a rectification effect that makes the low-frequency modulation active on the carrier in deeper tissues as well. The application of memristor principles in living objects is an emerging field of research known as "memristive biology" or "biomemristors." The application of memristor principles in living objects is a complex and interdisciplinary field. The changing membrane conductance was observed as early as 1940, introducing a variable resistance of the membrane [121], and observing the emphasized rectification [122] an inductive element was introduced [123] [124] (Figure 16). The inductivity directly connects the process to the memristor behavior. The inductive behavior was used in neuronal models [125]. Memristive behaviour of neuronal system was measured with in impedance spectroscopy [126] [127].

Characteristic behaviour of the memristors their charge gradient, which varies by the current density flowing through of it. The heterogenic dropping of the solid materials were the first realizing of the memristive idea [128] (Figure 17). The capacitance has similar arrangement where the charge and the potential are connected: $q = C_m(w, V, t)V$. In all cases the time derivative of the charge. gradient is a function with the parameters of the memristor [120]. Memristors can be applied in the synaptic contacts [129] [130], and other memory applications like neuronal calculations [131], perspiration processes [132] and biosensors [133]. Microtubules composed of tubulin dimers are show also memristor effects [134]. Memristors can play a role in developing neuromorphic systems for cancer treatment. Neuromorphic systems mimic the structure and function of the human brain, allowing for intelligent and adaptive treatment strategies. By incorporating memristors into these

systems, it is possible to create efficient and dynamic treatment protocols that can adapt to the evolving characteristics of tumors.
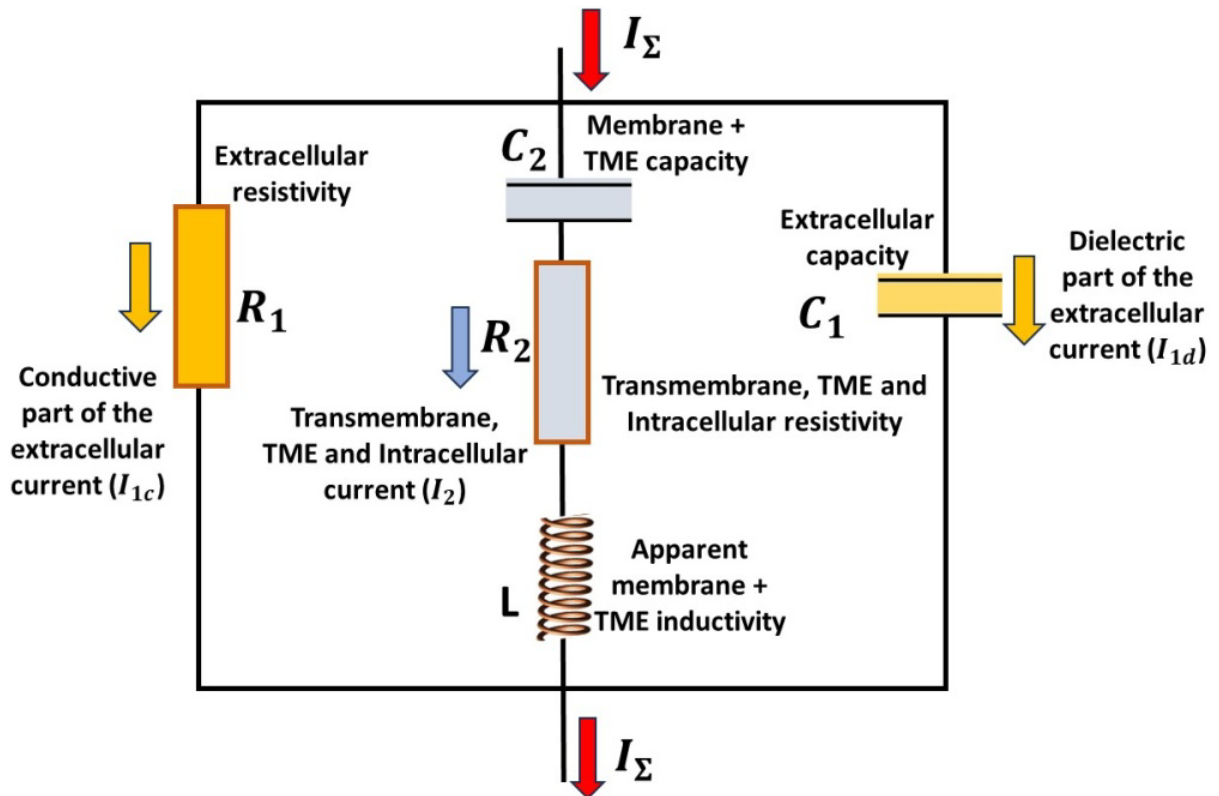

Figure 16. The electro–impedance spectroscopy observed an apparent inductivity in the system, which is shown in the discrete elements of the electric circuit model. The central part of the circuit contains the membrane and its nearest neighborhood as shown in Figure 8(A).
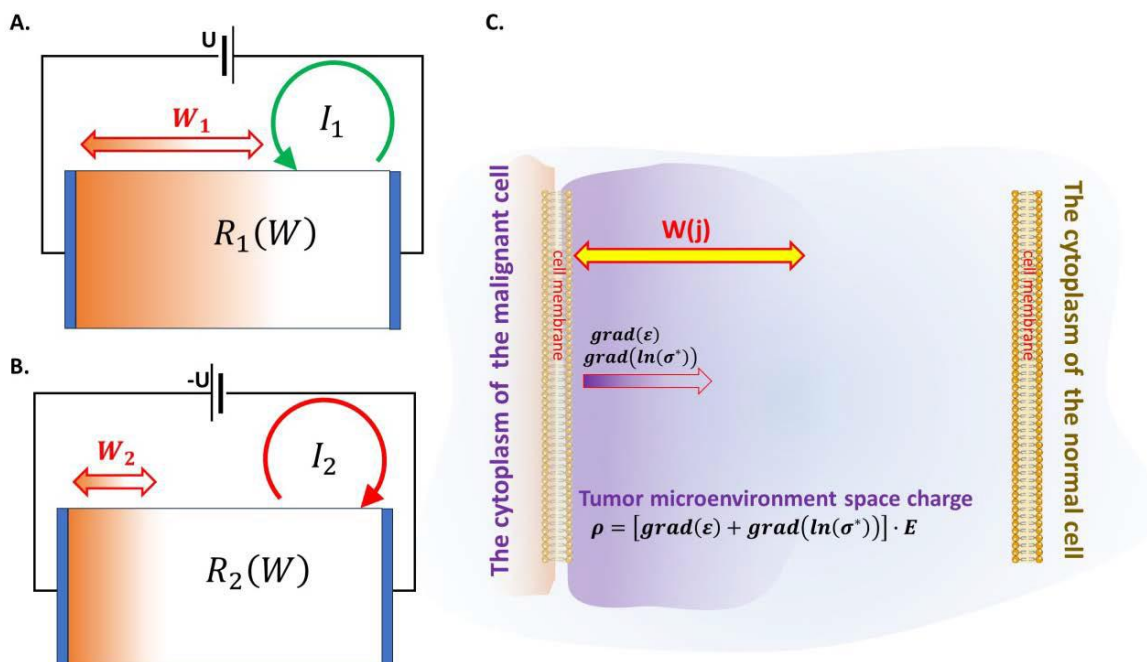
Figure 17. The memristor idea. The resistivity depends on the current direction and intensity, and due to the relaxation times it could be tuned as a memory element. (A) The R1 resistivity of the memristor depends on the inhomogeneous charge distribution, which widens by a current flowing in the right direction. (B) The current suppress the charge distribution, and the resistivity increases. (C) The space–charge between two cells may behave as a memristor.

The metabolic differences fundamentally deviate the microenvironments of the malignant and healthy cells. The fundamental request to generate sufficient ATP and biosynthetic precursors to maintain the intensive cellular fission and proliferation. The ionic distribution at the tumor/normal cellular interface can vary depending on various factors, including the type and stage of the tumor, the specific location within the body, and individual variations among patients. The altered ionic distributions in TME compared to normal ECM can affect several ions, including potassium (K^+), sodium (Na^+), calcium (Ca^{2+}), and chloride (Cl^-). The changes in ion distribution are attributed to the dysregulation of ion channels and transporters in tumor cells. A concentration gradient of the ions and consequently their charge define the interface between the cancer and normal cells. The resistivity of this transition layer changes by charge redistribution which the current flow causes. The conductivity of the layer follows the changes of the electric field. On this way the charge gradient serves as a typical memristor layer (Figure 18). The memristive charge effect appears not only extracellularly, but appears in the ionic channels revising the Hodgkin–Huxley membrane model [135]. The entire body has space–charge connections, electrically networking the structures (Figure 19). The body and all of its subunits have heterogenic space–charge distribution embedding each other in a complex network (Figure 20).

Figure 18. The memristive behaviour of the tissue. The resistivity of this connection depends on the transition charge distribution between the cells and the RF current changes it. (A) The memristive connection between the cells. (B) Memristive chain connection. Space charges form the memristors.

4. DISCUSSION

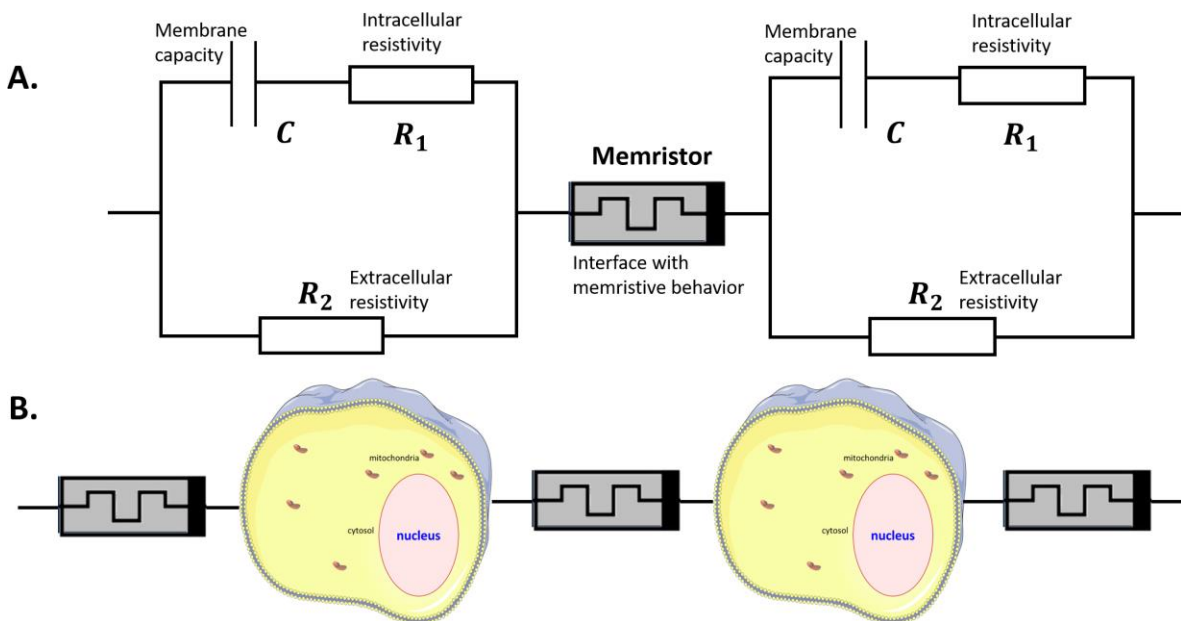The generalized Ohm's law (27) for inhomogeneous media approximates the current density versus field strength. In real cases, various chemical species are transported through tissue. The charge transport is connected to the mass transfer, which could be described by the generalized current densities. Principally, it has two current density components: the drift forced by field strength and the diffusion current driven by the concentration gradients. The uncharged particles (some molecules, cells and other TME compartments) have only diffusion driving force, but in many cases they're accompanied with charged particles to penetrate to the cell over the membrane barrier. Such happens with inert glucose molecule, which has a Na+ cotransport allowing their membrane penetration [136].
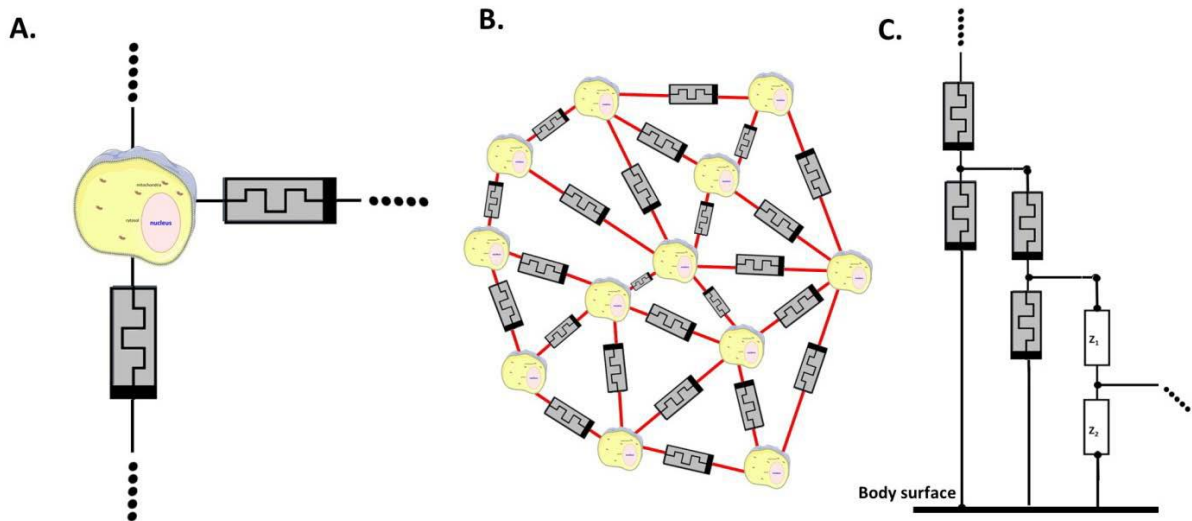
Figure 19. The network forming of the memristors caused by the space charges. (A) cellular space charge connection to the next neighbour cell. (B) Example of the memristor network in a tissue. (C) The subunits of the organism (like organs, tissues) are also connected with space–charge. The body surface is regarded as a common electric reference.
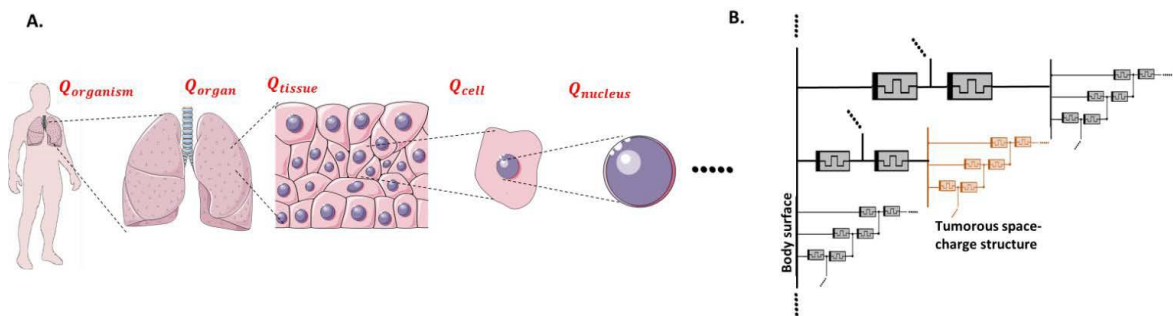
Figure 20. The charge (Q_i) distribution heterogeneity from organism to cells and beyond. (A) The charge distribution follows the structure of the human body. (B) The tumor represents a deformed charge distribution compared to the healthy structures.

On this way the inert molecules participate in the electrodiffusion processes. Hysteresis in the context of electrodiffusion in the tumor microenvironment refers to a phenomenon where the transport of ions or charged particles within the tumor tissue exhibits a time–dependent and non–linear behavior. This behavior is characterized by a lag or delay in the response of ion transport to changes in the applied electrical field or concentration gradients. In the tumor microenvironment, electrodiffusion involves the movement of charged particles, such as ions or drugs, in response to electric fields or concentration gradients. This process is influenced by various factors, including

the tumor's unique physical and biochemical properties. Hysteresis arises due to the complex interplay of these factors and manifests as a disparity between the forward and reverse processes of ion transport. The hysteresis effect can be explained by the presence of barriers or hindrances within the tumor microenvironment. These barriers may include cellular membranes, extracellular matrix components, or irregularities in tissue structure. As ions or charged particles encounter these barriers, their movement becomes restricted or delayed, leading to a time lag in their transport. Additionally, the hysteresis effect can be influenced by dynamic changes in the tumor microenvironment, such as alterations in pH, oxygen levels, or the presence of specific molecules. These factors can modulate the properties of cellular membranes, affect the hydration state of the tumor tissue, or induce conformational changes in proteins, further contributing to the hysteresis observed in electrodiffusion. Understanding and characterizing hysteresis in electrodiffusion within the tumor microenvironment is crucial for the development of effective strategies for targeted drug delivery and electrotherapy. By comprehending the complex dynamics of ion transport and hysteresis, researchers can optimize treatment approaches and enhance the efficiency of drug delivery systems in combating tumors.

The currents driven by the electric heterogeneity of the tissues affect the internal polarization structure. The membrane potential does not induce extra charge flow by its high electric field in normal functions of the body. The polarization forms an internal field opposing the external effect. An internal charge redistribution causes current density between the different imperfect dielectrics. When the integrity of living tissue is perturbed, injury of other disturbances rearranges the actual state, and current is generated due to the potential difference in the conductive media. In the case of an injury, the wound in the epithelium provides a shortcut: its potential tends to zero in this localization. The injury disorders the arrangement of the tissue, and critically change properties, and the charge balances. The difference in electric field induces an electric current directed to the wound. The current, powered by this process of endogenous field strength, is called the injury current [137]. It is the consequence of the internal rebalancing of the charge distribution without being triggered by an external electric field. The injury current promotes cellular migration (centripetal migration [138]) and proliferation to heal the wound [139] [140]. The frequency of the cell division and space orientation of the cells is determined by the electric field [141].

The injury current certainly plays a central role in wound healing [142]. Injury currents are physiological [143], and their typical value is around 100 µA/cm2 on the physiological potential gradient drops ~100 mV/cm and may be extended to the 0.5 - 1 mm distance from the wound [144]. This very weak power (~0.01 mW/g) does not increase the local temperature [145]. It can be measured using high-tech methods during the wound-healing process [146] [147] [148]. The induced electric field in the tissue is oriented to the wounded area (Figure 21). The current has an electric circuit loop through the surface of the epithelium, where the electric current travels to the surface from the depth of the wound itself. This electrically controls the wound-healing process and persists as long as the wound exists. Spontaneous biological charge transfers have a significant role, being one of the basic phenomena of tissue repair [149] [150], and especially control the cells and heal the wound by electrical manipulations [151]. The injury current concept is well proven [26] [139] [152] [153]. It needs sensitive experimental setups to measure, but many invasives [85] [145], and noninvasive [146] [148] [154] measurements have been performed to prove the current experiment. The malignant cell membrane potential is markedly lower [70] [155] than for normal cells and so its outside surface is less positive (relative negative) than their healthy

counterparts [156]. A certain potential gradient between malignant tissue and its healthy neighborhood exists [157]. The gradient acts to promote and direct the cancer-cell migration [158]. There is an argument on the cancerous process as wound repairs [159]. The bio-system falsely recognizes a tumor as a wound and stimulates its environment to heal the irregularity, cure the wound. The injury currents produced by the potential gradients actively support the wound-healing mechanism (Figure 22).
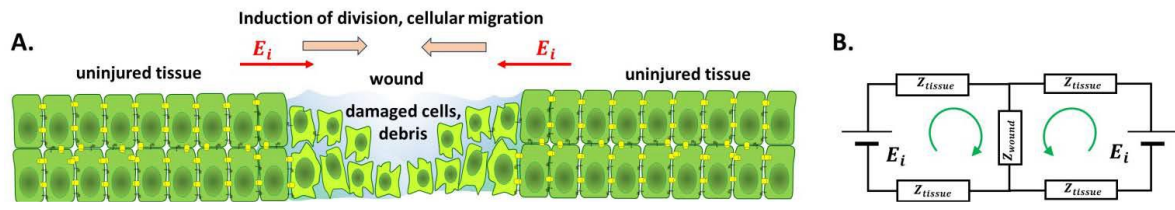
Figure 21. The injury current. (A) The dielectric permittivity and conductivity changes in the damaged tissue. The differences induce an electric field, which promotes the cell proliferation and migration. (B) The electric field drives a current (injury current) from all borders of the wound gaining each other in the damaged volume.
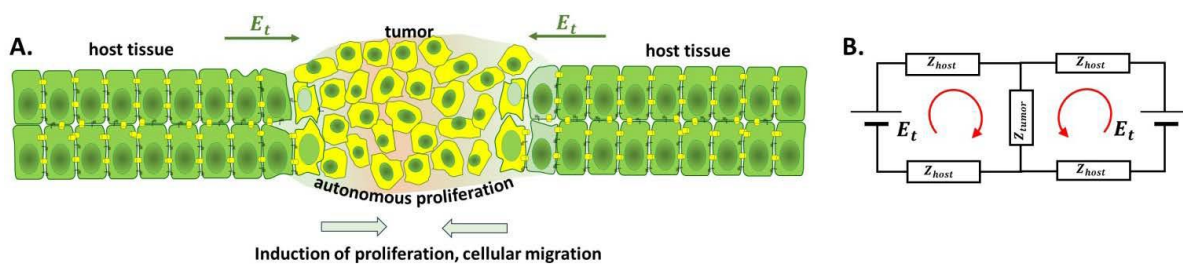
Figure 22. The tumor electronically represents similar conditions than the wound presents. The starting injury current intend to "heal the wound" which supports the tumor-growth.

The adaptation of cancer cells depends on many factors [95] including the special vehicles of information [160], and the main driving originated from the TME as the closest environmental condition [116]. The memristor processes arrange the new charge distribution fit to the actual conditions. The formed space-charge promotes the growing adaptation of cancer cells in a healthy environment. Due to the relative negative charge of cancer, the compensating spacecharge constraints electric current to the cancer-disk is formed, starting an injury current between the cancerous and healthy parts. This current could differentiate between the healthy cells and the multipotent ones, which became autonomic and redifferentiated to cancerous. The challenge is the high metabolic rate of the cancerous cells which are in the permanent division and perpetually produce the negative space-charge. The mechanism creates the "precancerous cells" measured by Loewenstein [161]. The dynamic change of the space-charge keeps the injury current active. The process is a self-gaining positive feedback mechanism, while the injury current promotes the cancer proliferation, which promotes the injury current further. The memristor effects between the cells and rapidly "learns" their role, and dynamically adapt the space-charges in the individual TMEs. Therefore the natural mechanisms of the bio-system are not able to block the cancerous development after a definite size. Artificial intervention to reprogramming the spacecharge and block the proliferation mechanisms of the injury current we deliver external field. The near 10 MHz carrier frequency causes the higher elecytric impact on the malignant cell

membrane [162]. However, the effect of body electrolytes on the electrode surface in the chemically reactive biomaterial develops Warburg impedance [163] in the low frequency region, which would be active to rearrange the developing charges. The low frequency modulated high carrier could help to overcome this challenge. The measured impedance spectroscopy showed significantly higher relaxation time (lower relaxation frequency, where the imaginary part of the impedance is the highest) in malignant tissues than in normal [82] [164]. The higher relaxation time allows also further selection possibilities of malignant cells, to block their proliferative processes.

5. CONCLUSIONS

The internal polarization effects form space-charge, which characteristically differ in malignant and healthy environments. The electrical resistivity of the electrolytes depends on the distribution of the charges and concentrations of ions in the electrolytes, consequently the space-charge differences appear in the conductivity parameters too. The polarization heterogeneities caused by the irregularities of the healthy tissue induce a current (called injury current), which appears in the cancerous tumor as well. Due to the nonlinearity of the space-charge production and the differences of the relaxation time of the processes in various subunits. The tumor develops the space-charge which appears as an inductive component in the otherwise capacitive setting and forms a memristive behavior of the tumorous tissue. This continuously developing space-charge accommodates the tumor to the permanently changing conditions and helps the adopting the malignant cells in the new environment. Applying external radiofrequency electric field, the disturbance of the space-charge may change the conditions, and seek to reestablish the healthy homeostatic equilibrium, blocking the pathologic injury current components. The hypothetical memristive behavior of the tumor microenvironment and the tumor mass may be a biophysical addition to the adaption mechanisms of tumor cells and could provide a way to block the pathogen biophysical processes.

CONFLICTS OF INTEREST
The author declares no conflicts of interest regarding the publication of this paper.

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The Clinical Validation of Modulated Electro-Hyperthermia (mEHT)

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Citation

Simple Summary

Modulated electro-hyperthermia (mEHT) is a heating therapy that uses synergized thermal and nonthermal effects to heat and destroy malignant cells selectively without damaging healthy cells. This article presents the clinical validation of mEHT. The therapy is dominantly applied for such advanced malignancies when the conventional oncotherapies fail to apply. Survival results of mEHT were collected and compared with other methods. The results demonstrate the superiority of the mEHT method.

Abstract

The mEHT method uses tissues' thermal and bioelectromagnetic heterogeneity for the selective mechanisms. The success of the therapy for advanced, relapsed, and metastatic aggressive tumors can only be demonstrated by measuring survival time and quality of life (QoL). The complication is that mEHT-treated patients cannot be curatively treated any longer with "gold standards", where the permanent progression of the disease, the refractory, relapsing situation, the organ failure, the worsening of blood counts, etc., block them. Collecting a cohort of these patients is frequently impossible. Only an intent-to-treat (ITT) patient group was available. Due to the above limitations, many studies have single-arm data collection. The Phase III trial of advanced cervix tumors subgrouping of HIV-negative and -positive patients showed the stable efficacy of mEHT in all patients' subgroups. The single-arm represents lower-level evidence, which can be improved by comparing the survival data of various studies from different institutes. The Kaplan–Meier probability comparison had no significant differences, so pooled data were compared to other methods. Following this approach, we demonstrate the feasibility and superiority of mEHT in the cases of glioblastoma multiform, pancreas carcinomas, lung tumors, and colorectal tumors.

Keywords

heterogenic heating; cellular selection; thermal processes; nonthermal actions; clinical studies; clinical evidence; survival time; quality of life

1. Introduction

The history of clinical hyperthermia can be traced back to the past. Ancient Greek medicine already used the method to treat oncological cases without detailed knowledge about the physiological feedback of the human body. Knowing the nature of the febrile condition, which in many cases was the guarantee of recovery, it was believed that proper heating would solve most medical problems [1]. They trusted that the body's reactions would lead to the heating effect, inducing self-healing reactions of the organism. They essentially took advantage of the system's striving for homeostatic balance, which is facilitated by heat stimuli. This principle is still appropriate in modern medicine, considering the limits of complex natural regulation.

Homeostatic surveillance controls the system's stability and adaptability. The local or systemic heating interrupts the regulatory processes of stability, reacting with non-linear physiological responses to correct the inconsistency [2]. Theoretical biology often ignores this complex control facing a tragicomedy challenge [3]. The homeostatic control tries to re-establish the unheated conditions by non-linear feedback, increasing the cooling blood flow (BF) [4,5] as an effective heat exchanger. This complex dynamic behavior guarantees the robust stability of health conditions, so the reactive BF challenges the heating processes. Homeostatic control tries to restore healthy regulation by increasing blood flow and vasodilatation. However, the risk of invasion of tumor cells is enhanced by these corrective effects and may promote malignant dissemination. Modulated electro-hyperthermia (mEHT) aims at a harmonic solution to this contradiction [6]. It applies electromagnetic interactions to deliver energy to the tumor. The energy is realized in the synergy of two basic effects:

- Thermal effects occur in the form of heat and temperature increase. Thermal effects are mostly unselective; the heat spreads all over the volume seeking thermal equilibrium. The temperature characterizes the homogeneous distribution as average energy of the heat-absorbers.

- Nonthermal processes are electron excitations, generating chemical reactions. The nonthermal impact may change the intercellular membrane, and intracellular processes select them by the dielectric and conductive heterogeneity of the target.

The mEHT applies a precise, personalized theranostic selection and treatment of malignancy, supporting natural homeostatic processes such as apoptosis, immune reactions, conditional effects, etc. [7]. The selection of the malignant cells uses the microscopic natural heterogeneities of the tumor. The applied electric field has different interactions with the cancerous and healthy cells in four basic characteristics, Figure 1, discussed in multiple publications [8–12]:

1. Due to the intensive metabolism of the malignant cells, the ionic species of the nutrients and waste molecules (such as lactate) have high concentrations in the tumor microenvironment (TME), and together with the extended volume of the extracellular matrix (ECM), create a significantly higher electric conductivity of the microenvironment of malignant cells and the entire tumor [13,14]. This conduction difference drives the RF current to the area [15].

2. The malignant cells break their networking connections (e.g., adherent connections and junctions [16]), and became autonomic. This cellular individualism makes the tumor microenvironment different, causing a higher dielectric permittivity of the tumor microenvironment (TME) than it was in the networking conditions [17,18]. The high dielectric permittivity favors conducting the radiofrequency (RF), making an additional selective factor for tumor cells.

3. The broken connections leave numerous transmembrane proteins on the membrane of the malignant cells. These membrane-embedded proteins and their lipid-enriched clusters (membrane rafts) have significantly higher energy absorption from the RF current than

their surrounding lipid layer [19]. This makes these proteins particularly heatable and chemically excitable.

4. The malignancy had lost its healthy homeostatic control, and so it has locally modified physiologic regulations [20]. The arising structural and pathological modifications appear as an additional selectivity factor.
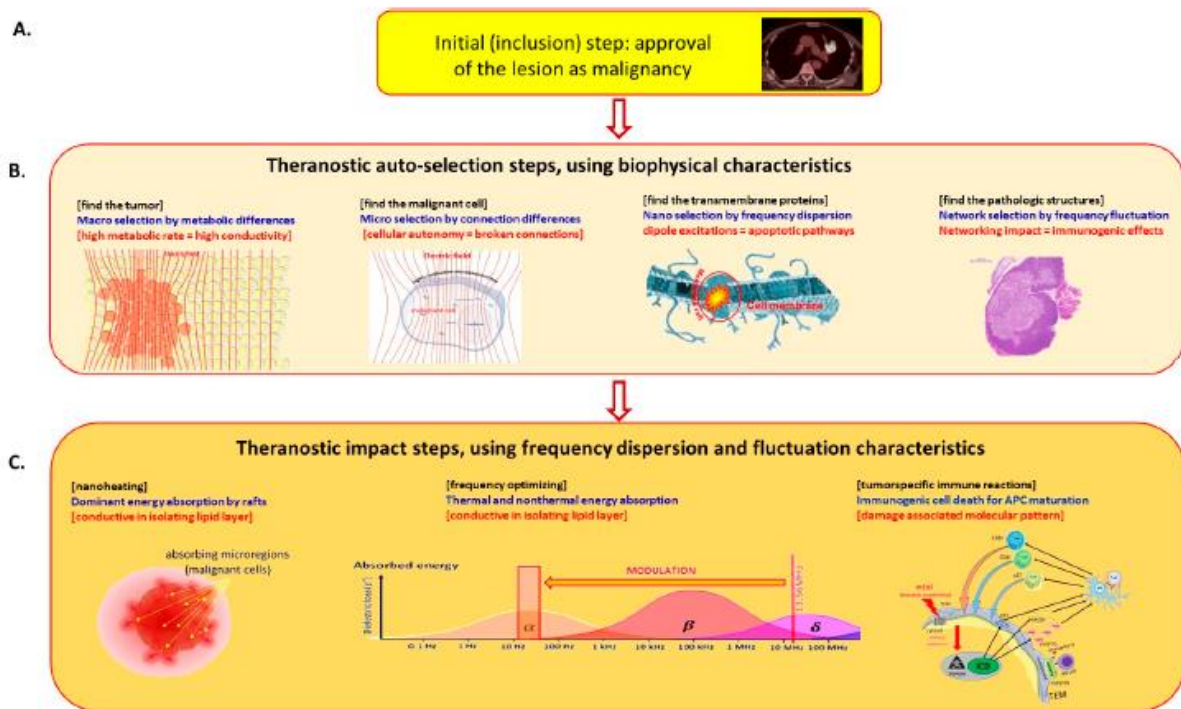
Figure 1. Selective and theranostic behaviour of mEHT. (A) The malignancy is localized and proven with conventional methods. (B) The RF current macroscopically selects the tumor by electric conductivity and microscopically by dielectric permittivity. The transmembrane proteins thermally and nonthermally absorb the energy in the malignant cells. The pathologic irregularities further increase the selection by applying RF current. (C) The nanoscopic membrane rafts locally heat the malignant cells, and the optimized modulated RF current makes the desired molecular changes for DAMP and ICD. The DAMP molecules induce antigen presentation and, as a consequence, antitumoral killer and helper T-cells appear, working as a tumor-specific vaccination.

The auto-selection Is theranostic, finding and treating the malignancy In macro-and micro-regions. The theranostic Impact has special enhancing factors:

1. The particular energy intake of membrane rafts of malignant cells selectively heats them, working similar to natural absorbing nanoparticles [21]. This makes effective micro- and macro-localization of the heat effect [22].

2. The frequency dispersion has an optimal range of RF application for the above selection. However, the requested optimal frequency range of the membrane energy absorption/excitation and the driving of the molecular changes during the excitation need

different frequencies which must be coordinated. The selection absorption optimum is near 10 MHz [23], while the desired molecular changes happen with a frequency less than 10 kHz. This 1/1000 ratio may be solved by modulation. The carrier is the approved medical frequency 13.56 MHz, and the modulation is a spectrum in the 10 Hz–10 kHz region [24]. The modulation spectrum is the physiologic noise of healthy homeostasis (its power density depends on the reciprocal value of the frequency) [25], and so forces the homeostatic control.

3. The applied modulated RF current kills the malignant cells in an apoptotic way, producing a damage-associated molecular pattern (DAMP) [26], realizing an immunogenic cell death (ICD) [27]. The ICD secrete calreticulin (CRT) [28] and heat-shock protein (HSP) on the malignant membrane [29] and attract the natural killer cells [30]; this is proven with mEHT too [31]. The ICD liberates the high mobility group box 1 (HMGB1) molecules [32] together with HSP70, HSP90 [33], and ATP [34] into the ECM. The membrane HSP-s activate the natural killer cells, and the other DAMP molecules maturate the dendritic cells, producing antigen presentation which creates immune reactions. The rising tumor-specific killer and helper T-cells activate antitumoral processes all over the body, acting on distant micro- and macro-metastases (abscopal effect) [35].

The preclinically proven selective mEHT processes [36] have numerous clinical studies [37]. The clinical efficacy of these trials is focused on patient-centered values: survival time and quality of life. A broad spectrum of cancers shows the practical applicability of mEHT in human oncotherapies [37], which validates the preclinical molecular results [36] and supports the method's feasibility [9]. The applied low incident energy is enough to detect, select, and treat the tumors in a theranostic way, irrespective of their form and size, and reach significant clinical achievements through the selected cellular heating [38]. The mEHT application in human clinical practice showed typical thermally enhanced BF measured in cervical carcinoma [39]. The produced mild 38.5°C temperature in cervical cancer looks optimal for the complementary treatments because it provides enough blood support but is not too high to increase the dissemination of the malignant cells. Furthermore, the synergy of the thermal and nonthermal processes improves the pharmacokinetics measured in healthy volunteers [40,41].

2. CLINICAL VALIDATION

2.1. CROSSROADS OF CLINICAL APPLICATIONS

The general cancer curative strategy is based on the direct distortion of the detected cancer cells by surgery, chemo, and radiotherapy. The distortion strategy offers a proper solution when the method is effective and selective enough. Selectivity means that it destroys all cancer cells, does not dangerously affect its healthy neighborhood, and does not escalate to paralyze essential body functions. The accuracy of the detection of malignant cells, including the disseminated ones, limits the destruction strategy because the remaining cancer cells may redevelop the disease and even could build resistance to the applied chemo and radio methods. These challenges create a new

approach, using the system's defense and protective procedures against malignancy and mobilizing the body's immune system through immuno-oncology.

2.1.1. CHANGE OF PARADIGM

We are in a war against cancer. The old military rule requests the attack of the enemy's weakest point, but the conventional oncotherapies, including hyperthermia, attack the strongest malignancy side: proliferation. Change is necessary. Attack the weakest side: the missing networking, and consequently, the cancer is out from the overall regulation of the system. The cancer cells lost their collective connections and became autonomic. Furthermore, the tumor-oriented curative approach has to be changed to the patientoriented strategy to cure the patient, taking care of their complex health issues. Therapy must concentrate on human complexity and turn the product-oriented focus to the processoriented one. This approach means that instead of concentrating on some molecular products, such as heat shock proteins, angiogenesis blockers, proliferation blockers, ionic blockers, blood-flow blockers, cell-poisoning, etc., we must focus on the dynamism of the cancer evolution process considering the immune effects, the physiologic feedback, and the overall homeostatic surveillance.

2.1.2. CLINICAL CHALLENGES

Unlike conventional hyperthermia, the mEHT treatment is recommended for:

1. Patients who cannot receive either surgery, chemo, or radiotherapy (conventional gold standards) according to various contraindicated aspects such as:
   a. They have a comorbidity that contraindicates the conventional oncotherapy procedures.
   b. There is no effective conventional procedure for the given tumor.

2. Conventional curative procedures are no longer available and usually get a palliative setting only.

   a. Patients with relapsed locally far-advanced tumors and no alternative standard curative therapy exists.
   b. Conventional therapy cannot be continued due to organ failure or low blood count.
   c. Despite standard treatments, patients show intense progression, relapse, and broad malignant dissemination.

3. Severe metastatic activity does not allow conventional treatment, salvage, or terminal state. Due to the above challenges, most of the clinical trials conducted with mEHT are single-arm prospective or retrospective observational studies, meaning that it does not differ from other studies of medical devices [42]. The frequently applied single-arm studies have ethical and statistical reasons. Patients in this stage have no other treatments possible, and a cohort reference frequently does not exist for complete study statistics.

2.1.3. STUDY CHALLENGES

The evidence from single-arm observational studies is usually less convincing than that from randomized double-arm studies due to the huge variation of personal situations for patients with severe advanced stages of the disease. The personalized protocol cannot be rigidly fixed in preliminary planning. Having personal variations of the protocol makes the single-arm prospective trial difficult because the patient cohort and its protocol are not homogeneous. In these cases, only an observational study is available to indicate the efficacy of the treatment with an intention-to-treat (ITT) schedule on a carefully chosen personal basis. Despite the diverse cohort, the principal self-similarity allows the selforganized approach [43] which fits the allometric scaling by the fractal structure of the tumor [44]. In consequence, self-organization data mining could prove that the results have the necessary information to measure and evaluate survival [45]. Medical evaluations of survival conventionally apply Kaplan–Meier (KM) non-parametric estimator [46] for incomplete observations. KM is useful to examine the probability of lifetime and effectivity of the chosen treatment for such lethal diseases such as cancer. Taking the self-similarity into consideration, the hazard function must be a self-similar time function [47] and in consequence, the KM could be approached with Weibull distribution [48] The invariance of magnification (scale invariance, when the up- or down-magnification show similar structures) is the form of self-similarity, which is a typical consequence of the self-organizing processes [49].

No "average" patient exists; the cohort is widely mixed. While in randomized studies, the randomization enables unbiased estimation of treatment effects; observational studies are typically not random. Propensity score matching (PSM) is a method of statistical analysis to estimate the effect of a treatment by accounting for the covariates that predict receiving the treatment. PSM is a conditional probability of being exposed given a set of covariates attempts to reduce the bias by the confounding variables [50]. The PSM improves the evidence level of the observation study, intending to reduce the treatment assignment bias by matching and mimicking randomization, by samples receiving the treatment that is comparable on all observed covariates without receiving the treatment. The possible reference solutions apply proper historical control from the same clinic/hospital where the observational study is performed, retrospectively choosing the same conditions. In the case of mEHT, the PSM was chosen from the patients from the same hospital with the same diseases and stages. The PSM increases the trustworthiness of the obtained results [50,51] by combining them with an available database, selecting similar cases to be used as a control [52]. Selecting a comparative group of patients uses data mining in large and representative databases, defining the disease's relevant and characteristic properties and the patients' conditions.

The expectation that selecting the independent parameters from the actual therapy does not change during the complete curative or palliative process drives the propensity score comparison. The propensity score method gives statistical proof if the confounding variables are chosen well [53]. Advanced cancerous cases may limit the selection because of the large variety of previously failed treatments, so the applied database has to be large enough to mine the appropriate data. Mathematical/statistical estimates may increase the single-arm's strength of evidence. The single-arm has complete information about the patients [45,54], but their evaluation is difficult due to the missing comparison cohort. The self-organizing behavior of tumors provides a satisfactory accuracy of evaluating the single-arm, statistically deducting a reference group from the measured data [55]. Repeating the single-arm trial in different research places at different times for the same

stage of the disease provides more realistic confidence supporting the evidence. The data pool of the different single-arm studies may increase the evidence level significantly. The most convincing statistical result is the similar, statistically equivalent survival curves of the studies performed at various times in various clinics and countries.

2.2. CLINICAL RESULTS

The definite primary endpoint of the mEHT studies is the synergy of overall survival (OS) time with quality of life (QoL). Secondary endpoints are the local effects (response, remission, and local control). While the secondary endpoints are popular, they do not provide enough information about the patient's overall status.

2.2.1. SAFETY

The Phase 1 clinical trial safety measure was made with patients having advanced glioblastoma multiforme (GBM). This safety study approved the applicability of mEHT to such sensitive organs as the brain without remarkable side effects, even with drastic transcranial dose escalation [56], Table 1. Alkylating chemotherapy (ACNU, nimustin) was administered at a dose of 90 mg/m2 on day 1 of 42 days for up to 6 cycles or until tumor progression Additional adverse effects of mEHT were not observed.

Group	Number of Patients	mEHT/Week (6 Cycles)
1	4	2
2	4	3
3	4	4
4	3	5

Table 1. The dose escalation of the GBM treatment with mEHT. Advanced GBM 3rd and 4th line treatment was studied. The complementary chemotherapy was nitrosourea drug Nimustine (ACNU) 90 mg/m2 . Patients were grouped in 4 disjunct study arms by dosing for 6 weeks cycles.

The GBM is a very fast-growing tumor that spreads rapidly to nearby normal brain tissue, but rarely forms extraneuronal metastases [57], so the distant dissemination to other organs is practically excluded. The selective behavior of the mEHT, and so the strict locality of the heating process, concentrates the energy on the malignant regions and the healthy tissues are unlikely to have harmful doses. Due to the nanoscopic heating, the common problem of the localization of the heat effect [58] is automatically controlled. The adverse effects were measured by dose escalation of mEHT complementary to the ACNU chemotherapy in four groups, increasing the weekly treatment dose from standard two/week to five/week. The results showed convincing safety of the method, even with extra-large (practically not applied) doses [56]. An essential consequence of this safety trial is when a safe treatment of such a sensitive organ as the brain with such advanced disease as GBM could be performed, we may also expect safety for various other organs. The radiotherapy combined mEHT trial (n = 20) was safe; no edema appeared with good local control of advanced GBM WHO Grade III–IV. The optimal dose was determined by dose escalation of mEHT in the Phase

I clinical study (n = 19) for patients with relapsed, refractory, or progressive heavily pretreated ovarian cancer. The dose optimum in this disease was 150 W/60 min [59].

Dose escalation of intravenous vitamin C (ivC) together with mEHT was measured with a Phase I safety study, which showed that 1.5 mg/kg ivC is safe for in Stage III–IV non-small cell lung cancer (NSCLC) patients [60]. A metabolically controlled complex therapy package of treatments could be effective in most advanced metastatic cases [61–64]. The monotherapy application of mEHT also presented promising results for patients with advanced disease when other therapies had failed [65,66].

2.2.2. SURVIVAL TIME

The clinical results of overall survival (OS) with the synergy of the quality of life (QoL) show the feasibility of complementary applications of mEHT with all standard adjuvant and neoadjuvant oncotherapies, including immuno-oncologic and integrative therapies. The cervix cancers were studied in a Phase III double-arm randomized prospective controlled trial involving two–two groups in both arms, patients ±HIV [67,68]. Metabolic response (MR) was measured by PET [69]. The advantages of mEHT appeared in the higher complete metabolic response (CMR) of the HIV groups compared to the control groups. The continuation of a Phase III randomized controlled study for advanced cervical cancer patients provides new insights through the strong evidence of mEHT's efficacy to improve the three-year overall survival [70], Table 2. The OS for patients with FIGO Stage III disease had a significant (p = 0.04) increase with mEHT addition compared to without it. The disease-free survival (DFS) was also significantly longer (p = 0.04) for these patients.

Groups		Number of Patients	%	Average Age (y)	3 y Overall Survival (%)	p-Value
All		210	100			
RT + ChT alone	HIV positive	55	52.9	50.6	33.7	
	HiV negative	49	47.1			0.04
RT + ChT + mEHT	HIV positive	52	49.1	49.2	44	
	HiV negative	54	50.9			

Table 2. Survival results of the Phase III uterus/cervix cancer study [67,68].

A Phase II randomized double-arm study compared platinum-based chemotherapy to additional complementary mEHT for patients with recurrent cervical cancer, dominantly disseminated squamous cell carcinoma, in a broad range of FIGO statuses [71]. The obtained overall remission rate at seven months of follow-up was significantly better in the active mEHT arm (p = 0.02), and despite of great difference, the overall survival had not reached the significance level (p = 0.24) [72], probably due to the small number (n = 20 + 18) of participants. FIGO Stage 2 locally advanced cervical cancer with lymph node metastases in more than half of the treated patients was studied in retrospective observational studies in a double-arm comparison of standard chemoradiotherapy and its extension with mEHT [73]. The results are convincing (Table 3). A comparison of the mEHT-treated and nontreated patients with the same stage brain tumors in the same research group in Italy [74] showed a highly significant (p = 0.0006) response rate compared to the conventional control and also for GBM (p = 0.026). The details of the study are shown in Table 4. The theoretical self-similar reference arm corresponds well with the KM plot of the

nontreated group of patients [55]. Retrospective observational study with mEHT–treated and untreated arms for advanced pancreatic cancer showed that mEHT was successfully applicable for various pancreatic tumors [75], and also for the specially nonresectable tumors compared to PSM [76], as well as relative long overall survival; Table 5.

Groups	Number of Patients	%	OS after 5 y		CR with Lymph Node Mets.		CR (NED)		DFS after 5 y		DFS with Lymph Node Mets. after 5 y	
			%	p-value	%	p-value	%	p-value	%	p-value	%	p-value
All	95	100										
RT + ChT alone	50	53	79.5	0.079	45	0.0377	58	0.0315	73	0.166	73	0.166
RT + ChT + mEHT	40	42	81		71		82		80		80	

Table 3. Results of retrospective double–arm observational study for cervix carcinoma. (CR–complete remission, DFS disease–free survival, NED—no evidence of disease.

	Astrocytoma				Glioblastoma			
Response	without mEHT (n, %)		with mEHT (n, %)		without mEHT (n, %)		with mEHT (n, %)	
	n	%	n	%	n	%	n	%
CR	6	28.6	2	6.9	2	2.4	1	3.4
PR	1	4.8	10	34.5	2	2.4	6	20.7
SD	5	23.8	9	31.0	13	15.3	11	37.9
PD	8	38.1	6	20.7	63	74.1	11	37.9
No data	1	4.8	2	6.9	5	5.9	0	0.0
OS median (months)	17		72		12		15	
OS range	3–120		3–156		2–84		2–108	
p-value	0.0006				0.026			

Table 4. The response data of the treatments. AST—astrocytoma (Grade III), CR—complete remission, PR—partial remission, SD—stable disease, PD—progressive disease, OS—overall survival.

	Fiorentini et al.				Petenyi et al.			
Response	without mEHT (n, %)		with mEHT (n, %)		without mEHT (n, %)		with mEHT (n, %)	
	n	%	n	%	n	%	n	%
Patients no.	67		39		39		39	
Males	38	56.7	24	61.5	19	46.2	18	46.2
Females	29	43.3	15	38.5	20	53.8	21	55.8
Age (mean, y)	66		61.8		66.02		65.9	
Distant metastasis *	37	55.2	25	64.1	24	61.5	20	51.3
Non metastatic **	30	44.8	14	35.9	15	38.5	19	48.7
Gemcitabine combination	64	95.5	27	69.2	31	79.5	31	79.5
Other complementary	3	4.5	12	30.8	8	20.5	8	20.5
OS median (months)	10.9		18		10.58		17.02	
OS range	0.4–55.4		1.5–68		2.4–48.8		4.4–47.1	
p	0.00165				0.0301			

Table 5. The main parameters of two survival studies [77,78] (*—macro metastases, ** possible micro metastases.).

A randomized double–arm Phase II study (n = 49 + 48) showed significant improvement in survival and quality of life for patients with Stage III–IV NSCLC treated with mEHT in combination with high–

dose vitamin C infusion and providing the best supportive care (BSC) in both arms [79]. The three months follow-up remission rate was significantly better (p = 0.0073) in the active mEHT arm than without it, and simultaneously the survival was also significantly (p < 0.0001) improved. A randomized two arms Phase II clinical trial for advanced non-small-cell lung cancer (NSCLC) patients also showed a clear advantage of mEHT application, showing a significant increase (p < 0.001) in the patients' OS [79].

The mEHT method also demonstrates the feasibility of treating advanced small-cell lung cancer (SCLC) patients [80], where a significant (p = 0.02) increase in overall survival was observed compared to the control arm of the study. The mEHT can also be used advantageously in gastrointestinal cases [62,64,81]. The first-line, single-arm, retrospective clinical study (n = 40) of metastatic colon cancer complementary to Bevacizumab+FOLFOX [82] observed progression-free survival (PFS) of 12.1 months and OS 21.4 months, which are remarkably good results. Neoadjuvant (preoperative) mEHT treatment for locally advanced rectal cancer was studied in a double-arm trial (n = 62 + 58) [83]. The tumor regression was significantly (p = 0.0086) decreased by the tumor volume in the control arm, while in active mEHT treatments, the regression grade was uniform (p = 0.91) and independent of the tumor size. Despite lower radiation doses in the mEHT group, the clinical measures were comparable to the control group; the proportion of downstaging (80.7% vs. 67.2%) and pathologically complete response (pCR, not only for imaging) (17.7% vs. 8.6%) was higher with mEHT than without it. The pathological T-stage (ypT) was significantly (p = 0.049) better with mEHT, and also the rejection margin was significantly (p = 0.013) improved by mEHT application. The survival measures (overall, disease-free, local recurrence-free, metastatic recurrence-free) were all improved by the mEHT, but the differences did not reach a significant level (p = 0.05). Another Phase II single-arm (n = 60) clinical trial for neoadjuvant mEHT was performed for rectal cancer in cT3-4 or cT2N+ stages [77]. The therapy showed T- and Ndownstaging in 40 patients (66.7%) and 53 patients (88.3%), respectively. In total, 15% of patients had complete pathologic response in the T-stage, and 76.7% in the N-stage. The treatment of peritoneal carcinomatosis with malignant ascites with mEHT combined with traditional Chinese medicine compared to intraperitoneal chemoinfusion (IPCI) in a Phase II randomized double-arm trial [78] observed a better overall response (77.7% in the study arm, while 63.8% in control). Notably, for cholangiocarcinoma [84,85] and tumors of the hepatopancreatobiliary system [38], treatments with mEHT show the feasibility of the method on presently low-success curing-rate tumor localizations. Two Phase II studies proved the successful applicability of mEHT in hepatocellular carcinoma [86,87]. The mEHT was successfully applied to advanced breast cancer [88], including triplenegative cases [89,90], and leiomyosarcoma of the breast [91]. The study of thirteen patients with complicated, advanced invasive ductal breast carcinoma, mostly triple-negative immunohistochemical status and multiple metastases, showed more than two months of median survival [92]. A study of advanced ovarian cancer treated with mEHT complementary combined with paclitaxel and cisplatin chemotherapy showed less toxicity and adverse effects than cisplatin [93]. The combined two drugs showed no Grade III or IV toxicity and a 57% remission rate during 30 months follow-up, together with 85.7% survival in the same period [94]. A Phase II study of heavily pretreated, mostly platinum-resistant ovarian cancer with relapsed, refractory, or progressive stages treated with mEHT, showed a remarkable 7.5 months median survival in the same treatment period [59].

2.2.3. COMPARISON OF SURVIVAL CURVES

Glioblastoma treatments with mEHT have numerous single-arm prospective and retrospective clinical studies [74,95–102]. The studies had the same treatment protocol in Figure 2.

1. Session		2. Session		3. Session		4. Session		5. Session		6. Session		7. Session		8. Session		9. Session		10. Session		11. Session		12. Session		Further	
min	Watt	min	Watt	min	Watt	min	Watt	min	Watt	min	Watt	min	Watt	min	Watt	min	Watt	min	Watt	min	Watt	min	Watt	min	Watt
10	40	10	50	20	60	20	60	20	60	20	60	20	60	20	60	20	60	20	60	20	60	20	60	20	60
10	50	10	60	10	80	20	80	10	80	10	80	10	80	10	80	10	80	10	80	10	80	10	80	10	80
				10	70	10	90	10	100	20	100	10	100	10	100	10	100	10	100	10	100	10	100	10	100
								10	120	10	120	10	120	10	120	10	120	10	120	10	120	10	120	10	120
										10	140	10	140	10	140	10	140	10	140	10	140	10	140	10	140
20		30		40		50		60		60		60		60		60		60		60		60		60	

Figure 2. The protocol for the GBM treatment. The modulation of the carrier frequency, and the energy load had to be adapted by the patient (green columns).

Multiple single–arm studies are appropriate for comparison. Evaluating the comparison of these results showed an excellent match with each other, Figure 3. The similarity of the curves may mean strong evidence of the mEHT success.

Figure 3. The survival results at different times and various clinics. The Kaplan–Meier probability comparison showed no statistical difference between the different clinical studies, so the data may be pooled containing 325 patients altogether. The averag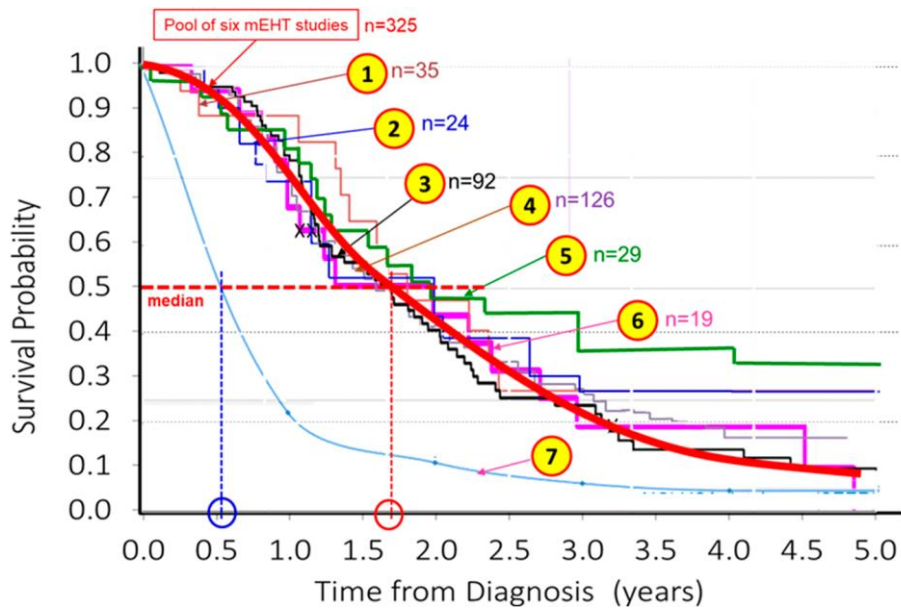e median value was significantly higher Figure 3. The survival results at different times and various clinics. The Kaplan–Meier probability comparison showed no statistical difference between the different clinical studies, so the data may be pooled containing 325 patients altogether. The average median value was significantly higher than the SEER database (data from [61,99]). (1 = [99], 2 = [96] 3 = [95] 4 = [101] 5 = [103], 6 = [97], 7 = SEER (NCI, USA) data from [99].).

The various GBM studies following the same general protocol are comparable, Table 6.

No.	Number of Patients	Treatments	OS Median (Months)	Reference
1	35	mEHT + RT + ChT + BST	26.4	Parmar, et al. 2020 [99]
2	28	mEHT + RT + ChT + BSC, (palliative)	14	Fiorentini, et al. 2018 [96]
3	92	mEHT + RT + ChT	20.4	Sahinbas, et al. 2007 [95]
4	126	mEHT + RT + ChT	20.3	Hager, et al. 2008 [101]
5	29	mEHT + RT + ChT	14	Szasz, et al. 2010 [103]
6	19	ChT (ACNU)	21.8	Douwes, et al. 2006 [97]

Table 6. The GBM studies which were used for KM comparison in comparison Figure 1.

A meta-analysis also showed the superiority of the mEHT treatment [104]. Furthermore, the results could be compared with the effective update chemotherapy of GBM, the temozolomide (TMZ) [105], Figure 4. The comparison with the pooled data shows the advantage of mEHT again.
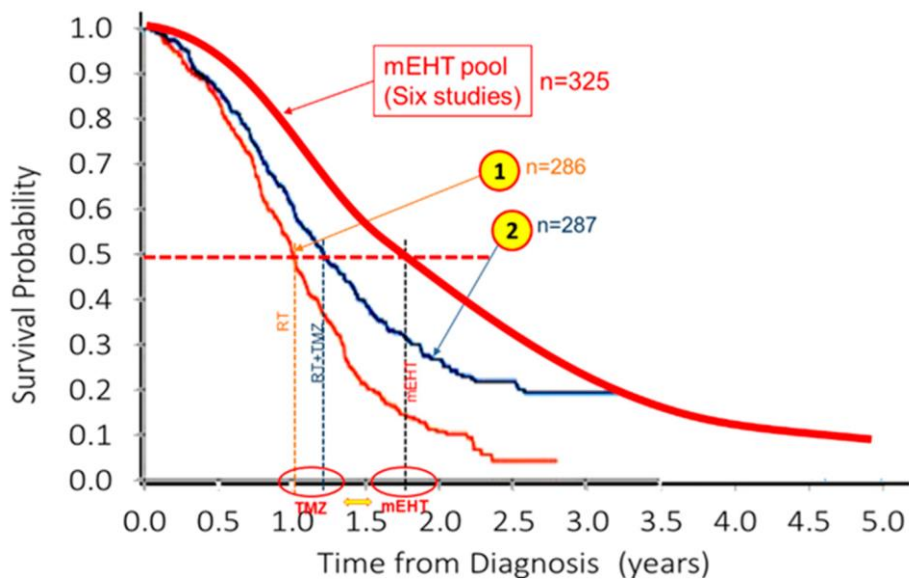
Figure 4. Comparison of mEHT pooled GBM survival probability with the literature [105]. 1 is the reference arm (radiotherapy (RT) alone) and 2 is the active arm, RT + TMZ.

Furthermore, the pool of survival rates of the GBM patients (n = 325) in various singlearm studies showed good agreement with the invasive transcranial brachytherapy ± invasive hyperthermia of the same disease [106], Figure 5. The invasive method (brachytherapy alone or combined with invasive hyperthermia (iHT)) do not differ from the survival results of noninvasive mEHT.
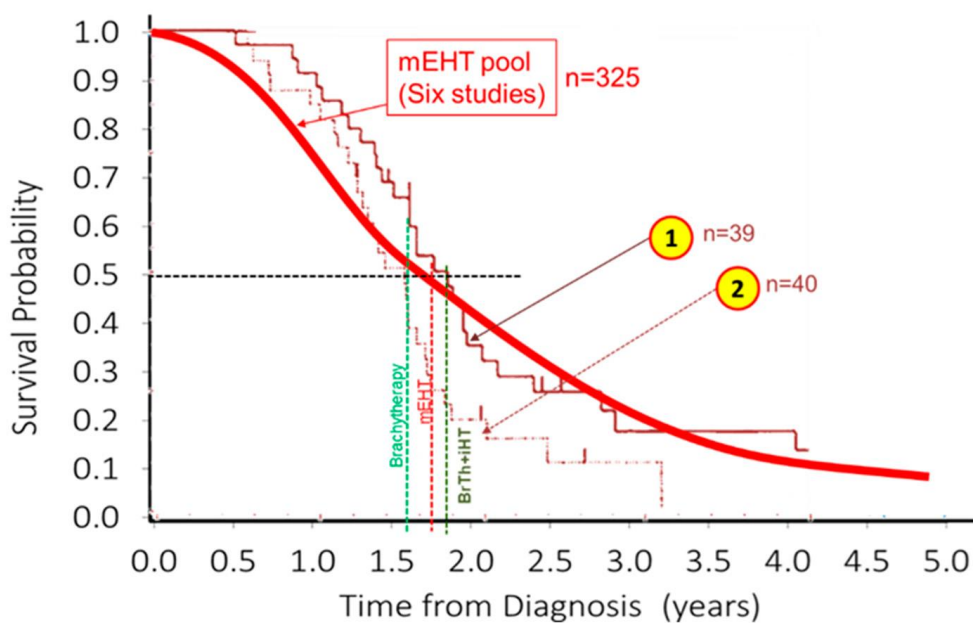
Figure 5. The invasive brachytherapy and invasive hyperthermia (iHT) in brain for GBM [106] do not differ from the mEHT pooled data. 1 = brachytherapy (bTh) alone and 2 = bTh + iHT.

The tumor treating field (TTF) is an emerging electromagnetic therapy for GBM, showing the electric field's efficacy for this disease [107]. Its focal point is the cytokinetic "neck" at the end of the mitotic spindle, applying nonthermal effects with capacitive coupling [108]. The electric field of TTF reorients the highly polarizable microtubules and actin fibers, and it may arrest the cytoskeleton's polymerization process and inhibit the assembling of the mitotic spindle [109]. Impressive clinical results were achieved with TTF, proving the feasibility of the nonthermal application of bioelectromagnetic processes against malignant proliferation. A comparative meta-analysis of TTF and mEHT [110] establish that the use of both mEHT and TTF in the treatment of glioblastomas can improve overall survival. A comparison of the mEHT pool and TTF results [111] showed no difference in the clinical results of the two electromagnetic therapies, Figure 6. The differences between TTF and mEHT are basically in the practical application and length to complete the therapy. TTF fixes a hat with multiple electrodes which the patient has to wear 18 h/day for several months, while mEHT makes intensive 60 min treatments every second days in 12 sessions.
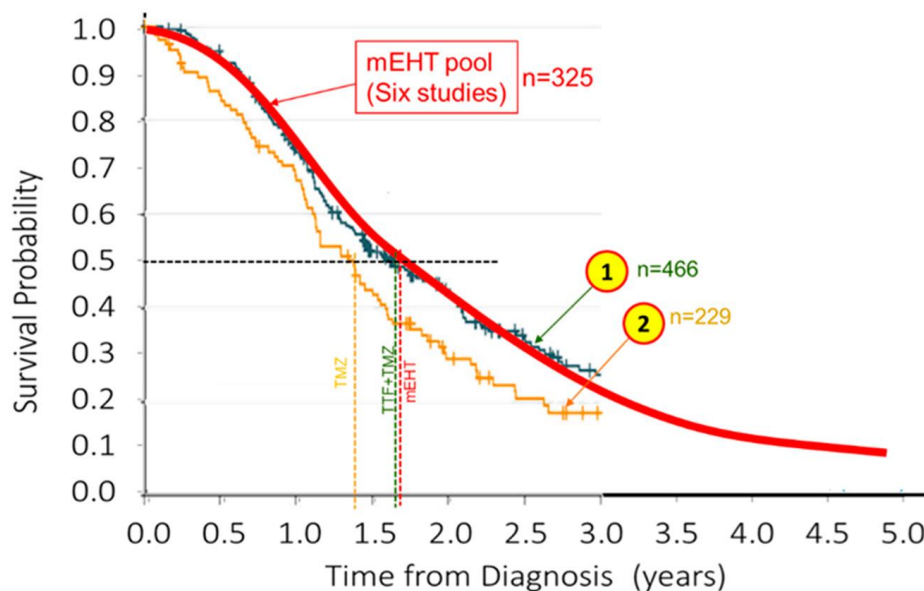
Figure 6. Comparison of the pooled data of six mEHT studies to TTF+TMZ survival data [72,111], where 1 shows the result of the active study arm TTF+TMZ and 2 is the reference with TMZ alone.

The successes of the complex therapy involving mEHT appear as some challenges for randomized studies of GMB [112]. An intensive naturopathic-oncotherapy (Bevacizumab + Boswellia serrata + Curcumin) combination with mEHT looks feasible for GBM patients in a terminal state [113]. This was measured earlier also using high-dose IV Vitamin C (30 g) combined with Thalidomide (50 mg) and Boswellia serrata (400 mg) plus fortecortin (0.5 mg) [114] in cases for patients for whom the chemotherapy is contraindicated. The GBM has only rarely metastases regardless of the ability of GBM cells to be disseminated over the blood–brain barrier (BBB) [115]. This specialty makes the disease mostly localized, and the various metastases only rarely influence the survival time, which is otherwise the drastic limiting factor of survival in other malignancies. Due to these conditions, we do not expect such unified survival probabilities in other tumors as we observed in GBM. There are protocols and guidelines not only for the therapy of central nervous system, but those available

for various other applied therapies as well [116,117]. We compared the various pancreas studies collected in Table 7. The treatment success of advanced pancreatic cancer has shown relatively little development recently. The applied protocols are that most pancreatic malignancies are non-resectable or can only be partially excised (R1) and frequently make liver metastases, increasing the mortality of this disease. Intensive clinical research with mEHT is underway in this area [61,118–121]. A comparison of the Kaplan–Meier survival curves shows a definite similarity in the survival probabilities, Figure 7, without such unified curves as we observed in GBM.

No.	Number of Patients	Treatments	OS Median (Months)	Reference
1	39	GMZ combination with mEHT	18	Fiorentini et al. 2019, [75]
2	27	GMZ combination with mEHT	13.2	Parmar et al. 2020 [99]
3	99	GMZ combination with mEHT	12	Dani et al. 2012 [120]
4	39	GMZ combination with mEHT	17	Petenyi et al. 2021 [76]
5	34	GMZ combination without mEHT	6.5	Dani et al. 2012 [120]

Table 7. The pancreas carcinoma studies which are used for KM comparison in comparison in Figure 5.

Another massively present malignancy in the world is advanced non-small-cell lung cancer (NSCLC). Application of mEHT on advanced NSCLCs also has numerous case studies [63,122–126] and trials [60,79,127–130]. The comparison of the survival distributions shows similarities again, and the data pool remarkably differs from the propensity score data by mining in USA and EU databases [130], Figure 8. The comparison of the survival plots from different NSCLC studies Figure 9, Table 8, is not as unified as the GBM comparison in Figure 1, because of the multiple variations of metastases and tumor locations in the lung. However, no significant differences appeared in the observed survival probabilities, so the pooling of data was also possible.
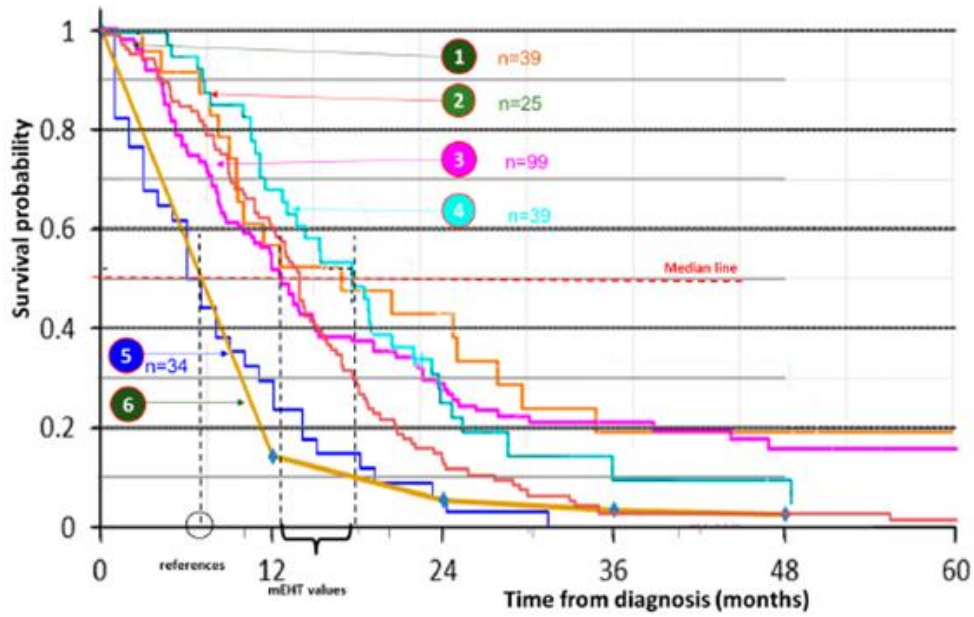
Figure 7. Comparison of the survival studies of advanced pancreas carcinomas 1 [75] (n = 106), 2 [99] (n = 25), 3 [120] (n = 99), 4 [76] (n = 78), 5 [120] (n = 34), 6 SEER (NCI, USA) data from [99].
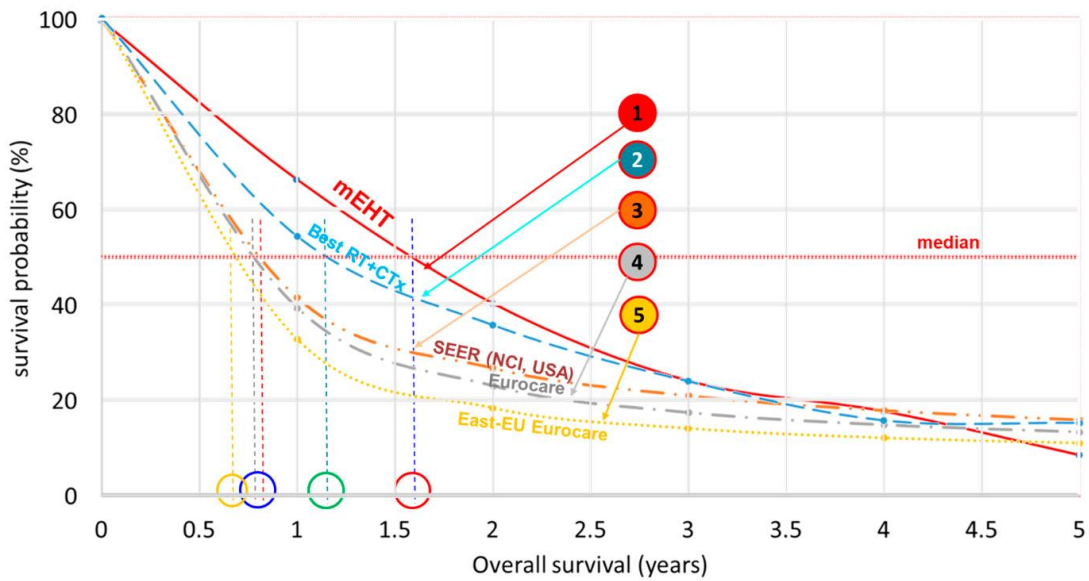
Figure 8. Comparison of the NSCLC survivals from large databases [92,130]. 1 mEHT pool, 2 Best RT+ CTx, 3 SEER (NCI, USA), 4 Eurocare–5, 5 East–EU, Eurocare–5.
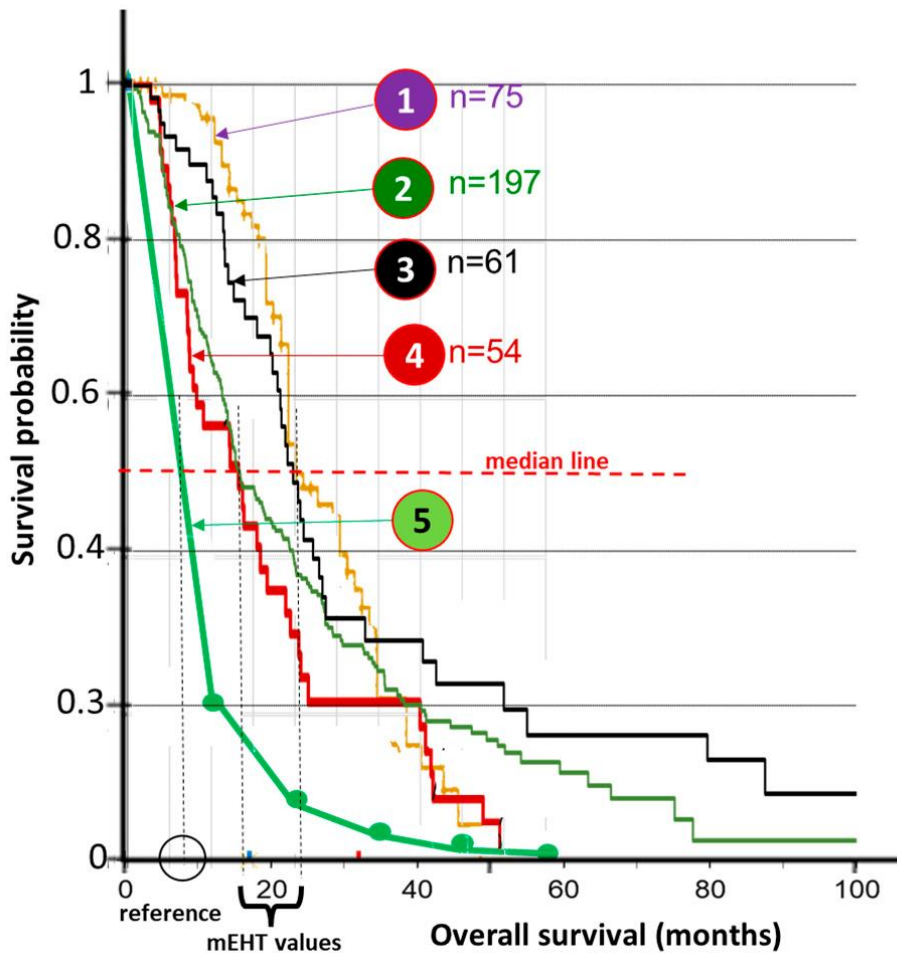
Figure 9. The measured NSCLC survival probabilities. ① = [127], ② = [129], ③ = [131], ④ = [61,99], ⑤ = SEER (NCI, USA) data from [99].

No.	Number of Patients	Treatments	OS Median (Months)	Reference
1	75	RT + ChT + OP with mEHT	16.4	Szasz, 2014 [127]
2	197	RT + ChT + OP with mEHT	15.6	Dani, et al. 2012 [129]
3	61	RT + ChT + OP with mEHT	16.4	Dani, et al. 2009 [131]
4	54	RT + ChT + BSC with mEHT	18	Parmar, et al. 2020 [99]

Table 8. The survival data of NSCLC studies.

The liver metastases of colorectal carcinoma appear to be a frequent complication in this malignancy. Multiple trials have demonstrated the efficacy of mEHT in this case, too [99,132,133]. The colorectal carcinoma survival studies also may be compared Table 9.

No.	Number of Patients	Treatments	OS Median (Months)	Reference
1	79	ChT with mEHT	48	Parmar, et al. 2020 [99]
2	218	OP + ChT with mEHT	28.5	Szasz, et al. 2010 [133]
3	50	BSC with mEHT	25	Hager, et al. 2020 [132]
4	30	ChT + BSC with mEHT	23	Hager, et al. 2020 [132]
5	40	ChT with mEHT	21.4	Ranieri, et al. 2020 [82]

Table 9. Survival data of colorectal studies.

The comparison of the measured Kaplan–Meier non-parametric distributions from different studies, Figure 10, had no significant differences but differed considerably due to the high variability of these metastatic conditions. The pooling here had no statistical basis.
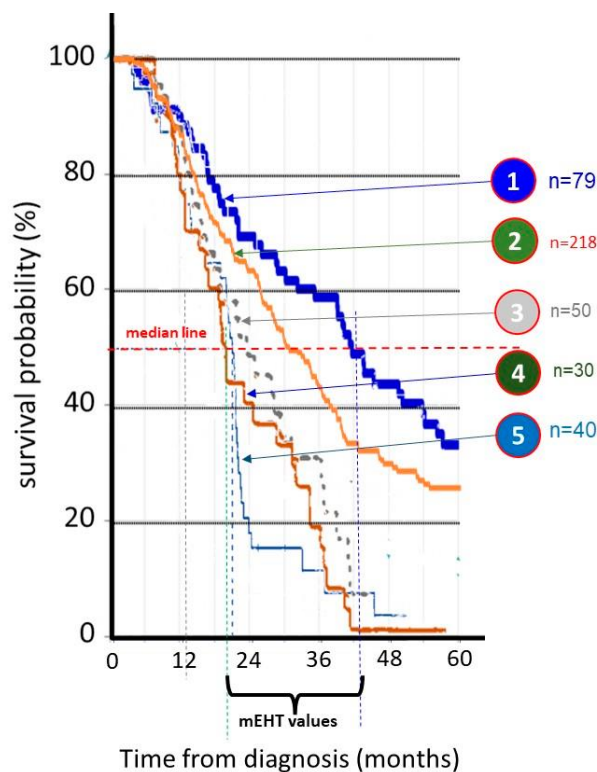
Figure 10. The colorectal cancer survival plots. ① = [99], ② = [133], ③ = [132], ④ = [132], ⑤ = [82]

2.2.4. QUALITY OF LIFE

The synergy of OS with QoL is especially important in the less–successful conventional therapies, such as the brain, pancreas, lung, and liver, which are otherwise frequent metastatic locations from various malignancies when their mortality is exceptionally high. The Phase I safety study (n = 35, involving Stage IV n = 17) for NSCLC observed adverse effects (fatigue, nausea, vomiting, diarrhea, headache) only rarely and also temporarily [60]. The function subscale of QLQ-C30 scores showed significant improvement in physical status after four weeks of treatments compared to before the therapy and getting gains in all other categories (emotional, cognitive, social, global) without

reaching the p < 0.05 significance level. However, the advantage results of mEHT on the symptoms subscale were significant after four weeks in most categories (such as fatigue, dyspnea, insomnia, appetite, and diarrhea). The decrease in nausea/vomiting, pain, and constipation were also observed without significance.

The Phase II continuation of the same NSCLC study [79] compared the QoL data in time, not to the baseline. The control arm for comparison was received by randomization of the included cohort of patients. In this randomized study, the QoL significantly improved in all conditional (functional) categories and physiometric (symptoms) categories. The data in the comparison of IPCI with mEHT favored the latter by improved overall QoL with 32.3% and 49.2%, respectively [78]. Primarily, pain relief in lung cancer was studied in a propensity case-controlled study [124], showing increasing pain after mEHT, which gradually lowered, and after a long time, the effective analgesic score decreased from the original 8.5 to –83.7%, which was more than a 90% improvement compared to baseline before therapy. Similar results were obtained with less radiation dose combined with mEHT than alone [77,83], which may result in fewer adverse effects and higher QoL of the treated patients. Improved QoL functions were measured with a Phase III randomized prospective, controlled clinical study of the same cohort of patients showing decreasing toxicity and increased quality of life [134]. Six weeks after the mEHT therapy, the cognitive function significantly improved compared to the control arm. A significant increase in social and emotional function was measured three months post-treatment, while fatigue and pain were significantly decreased simultaneously. Notable the QoL function was also improved and the ratio of mEHT-arm to the control grew by 1.6, 1.3, 2, 2, 10.8, 1.7, and 5.7 for visual analog, global health, pain reduction, nausea/vomiting reduction, fatigue reduction, cognitive functions, emotional functions, role function, and physical status, respectively.

2.2.5. IMMUNOGENIC EFFECTS

The immunogenic effects of hyperthermia are currently at the forefront of research and fit well into the general trend that immunology would require. However, hyperthermia applied by itself can also produce immunogenic effects. Cancer patients usually have a weakened immune system that requires stimulation. One of the first series of hyperthermia boosted by immunostimulants was published in 1986 [135]. The double-arm study (n = 77, 75%, surgery, 25% inoperable) of pancreas adenocarcinoma applied capacitive coupling hyperthermia 13.56 MHz plus chemotherapy (doxorubicin 30 mg/m2, 5-FU 500 mg/m2 , mitomycin C 5 mg/m2). In total, 42% of patients had chemotherapy in the inoperable group, 5% radiotherapy, 21% radio-chemotherapy, and 32% had no prior therapy due to their refractive status. The immune stimulation used granulocyte–macrophage colonystimulating factor (GM-CSF) and the results were very encouraging. The ratios of survival percentages between immune-treated and untreated groups showed 2.0-, 5.8-, and 3.0-times increase of 6, 12, and 18 months survival by immune boosting, respectively. The results were highly significant. The primary task of immunogenic stimulation is to restore homeostatic control and ensure the systemic surveillance of healthy processes. The local treatment becomes systematic in this way and allows attacking the distant micro- and macro-metastases, forming abscopal outcomes [35].

The abscopal principle could be used as a new anti-cancer vaccination strategy with immune stimuli [136], emerging "hyperthermic immunotherapy" [137], and developing tumor-directed

immunotherapy [138]. Studies have shown that combining mEHT with traditional Chinese medicine as an immune booster also has abscopal effects [139]. When the patients' immune system is strong enough to develop tumor-specific immune reactions, then the mEHT works without extra immune stimulation. A Phase III clinical trial for uterus cervix cancer proved the abscopal phenomenon [68,140], forming the complete metabolic response almost five-times more than the otherwise systemic chemotherapy in the control arm. The extra-pelvic response abscopal effect does not depend on the HIV status of the patient. The mEHT treatment of colon cancer clearly shows an abscopal effect in liver metastases [82]. A new therapeutic field is the complementarity application of checkpoint inhibitors (CPIs) combined with mEHT [141,142]. The immune action by CPI leads to a definite abscopal effect in clinical practice [143,144]. Viral immunostimulant with Newcastle viruses is a new, emerging complementary treatment with mEHT, allowing a new strategy for whole-body action [145]. The mEHT has a complex synergy with viral immune stimulation [146,147], also using the ICD process for developing tumor-specific immune activity [148].

The mEHT is a part of multimodal immunotherapy for patients with GBM [149], allowing personalized medicine in glioblastoma multiforme [150]. The complex therapy by dendritic cell vaccines and other immune stimulation to develop ICD within chemotherapy administration might improve the overall survival rate of GBM patients with long-term tumor control [112,151], as well as the induction of ICD during maintenance chemotherapy combined with subsequent multimodal immunotherapy for GBM. A study (n = 41) showed the remarkable benefits of mEHT as part of multimodal immunotherapy for brain tumors in children with DIPG [152], without significant toxicity. The median PFS and OS were 8.4 m and 14.4 m from the time of diagnosis, respectively. The two-year OS was 10.7%. Immunotherapy was applied at the time of progression, when the measured PFS and OS medians were 6.5 m and 9.1 m, respectively.

3. DISCUSSION

The evidence level is improved by comparing the survival data of various studies from different institutes. The relationship between relevant higher evidence-level studies and large databases gives efficacy information. The data of the variant of single-arm observational studies were pooled and used to compare to another type of treatment results. The pooling of data is correct because these survival plots do not vary significantly. The pool of data mimicked the later introduced market surveillance when the various results from very different medical groups were compared. The well-correlating single-arm survival showed that the application of the same protocol at different hospitals, countries, and time provided statistically equivalent data, so it is a usual requirement for worldwide approved drug applications. The technical solution allows easy and safe application [153]. The comparison of Kaplan–Meier survival times of glioblastoma multiforme in six studies made in different institutes gave an excellent agreement between the curves. The match was probably so accurate because the GBM rarely gives non-neural metastases [57], so the distant metastases do not modify the survival. However, the micro- and macrometastases could critically worsen the survival time. The frequent distant metastases of these tumors are the reason why we do not have perfect equivalence to the KM survival plot in pancreas carcinoma, NSCLC, and colorectal carcinoma, although the differences remain insignificant. The practically identical survival results increase the evidence level of the studies. Due to the insignificant differences, pooled data may be used for comparison with other kinds of cancer treatments for the same tumors. The pooled data showed the superiority of mEHT over temozolomide + radiotherapy

treatment for GBM. The mEHT pool agreed well with invasive hyperthermia and the results of other nonthermal bioelectromagnetic therapy (TTF).

The immune stimuli and the immunogenic cancer cell-death [154] are probably a considerable addition to the elongation of the patient's survival. The quality–of–life measurements had not revealed extra adverse effects of mEHT and even showed improvements in all functional and physiometric symptomatic scores. The preclinically approved immunogenic effects of mEHT [136,155–157], the human response studies [137,141,142], and the observed abscopal effects [138,143,144] probably promote the presented improvement of survival and QoL. The mEHT, as the synergic therapy of thermal and nonthermal effects, such as other therapies, has limitations. Various challenges appear in general hyperthermia treatments [158,159], which exist and are combined with some specialties in mEHT. The average power of mEHT has to be limited for optimal treatment and the energy absorption has to be balanced, due to:

1. As natural nanoparticles in the membrane, the rafts are heat-sensitive molecular clusters. The too-large absorbed energy destroys the rafts by overheating. The massive distortion of the rafts may degrade the membrane integrity and cause necrosis, losing the apoptotic "harmony" with the homeostasis, which is suboptimal.

2. The selected energy absorption of rafts heats the TME and tissues to a lesser extent. The standard applied SAR in nanoparticles, considering their weight heating, is 0.1–1.5 MW/kg. The approximation of the absorbed power of rafts in selection is SAR > 1 MW/kg [19], which is similar to the standard MNP energies [160]. The increased diffusion redistributes the initial spacing with nanoparticles [161]. The electric field impacts the diffusion of the charged and dipole particles, modifying the electrokinetics of the effusion [162] and the angiogenesis [163]. In case of electric field heating at mEHT, the electrodiffusion modifies the allocation of the gold NP–s too, positions them to the volumes of high electric field, thus promoting the heat on the TME [162]. The heating of the NPs shares the energy, reducing the effect on the membrane rafts, and, despite the increase of temperature, the apoptosis decreases [164]. The distribution of magnetic NP–s, which are modified by the increased diffusion with the temperature [165], has to be impacted too by electrophoresis and electroosmosis, and so the electric field in low frequencies (modulation frequencies of mEHT) regroups them on the same way such as in the case of the non–magnetic metallic NPs [164].

3. The thermal effect happens in nanoscopic local "points", the rafts. These molecular clusters are sensitive to overheating. When the absorbed energy is too large, it destroys the rafts and the mEHT loses its largest advantage, producing immunogenic cell death (ICD).

4. The large energy absorption extensively forces the spread of heat, and the selection of microscopic differences vanishes. A macroscopic average will characterize the target, and the cellular selection with intended molecular excitations will vanish. The thermal component will become dominant, and the

selection mechanisms cannot prevail. A limited thermal component ensures the selection of rafts.

The thermal conditions induce numerous physiological processes interacting with the body's thermal regulation, which limits the temperature gain (ΔT) with the following processes:

1. For human adults the surface heat-loss is floss~=0.15 at rest in the $0 \leq$ floss ≤ 1 scale [166], so the heat exchange is intensive enough even by intense local heating. Consequently, the bloodstream in the tumor maintains massive cooling efficacy. The cooling is inhomogeneous; it depends on the vascularization. As such, the thermal factor of mEHT is less homogenous than the nonthermal one.

2. The thermally induced vasoconstriction regulates the blood perfusion and heat conduction in tumors [167–169], while the heated healthy tissues in the surroundings have vasodilation. The relative blood flow could promote vascular invasion of the tumor border, reducing the prognostic expectations [170]. The special nano-selectivity and the applied low incident power (about 1/8th of the other conventional local hyperthermia therapies) produce a moderate (fever level) temperature in the tumor maand its surrounding tissues, which causes much less increase of the blood perfusion than the conventional hyperthermia methods, Figure 11. The thermal damage, which is usually calculated [171], is not considered in mEHT, the nonthermal factor makes the apoptosis, ICD, and immune effects also have a gradient on the tumor border, but it helps focus on the denser tumor by refraction angle.

3. The thermally promoted intensive metabolic activity deprives the ATP sources [172]. However, the massive energy demand of proliferation requests enormous ATP production, which induces anaerobic metabolism, improving the intensive proliferation [173], promoting the malignant processes [174], and leading the growth direction by an acidic invasion front [175].

4. A positive process of ATP deprivation may cause protein aggregation in the cytosol [129,176], destroying the cytoskeleton order. The collapsing cytoskeleton destabilizes the plasma membrane, and the cell necrotizes [176]. Increased temperatures can slow down or even block DNA replication [177], and the DNA strand breaks [178], which completes radiotherapy [179].

5. The intensive thermal effect acts mainly in the S-phase of the cell division cycle [180], while moderate heat shock arrests G1/S and G2/M cell-cycle checkpoints [181]. The nonthermal factor has an intensive block of the last phase of the cell cycle.
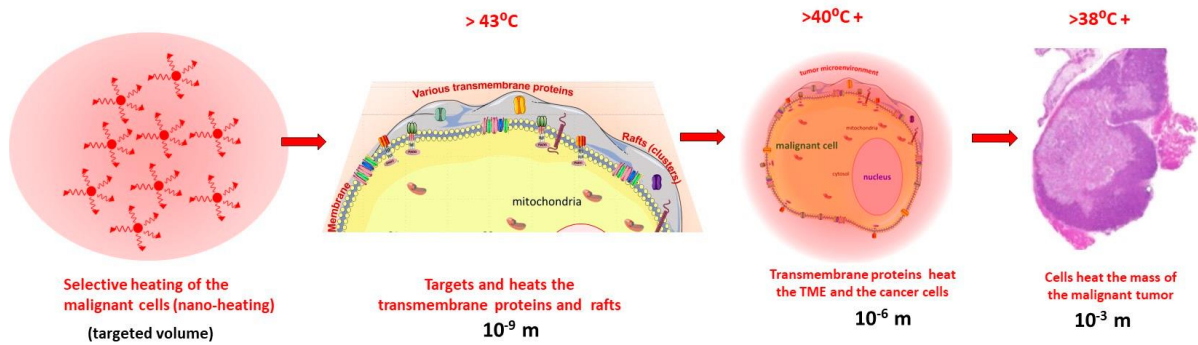
Figure 11. Heating heterogeneity. The characteristic sizes and the expected temperatures are shown in the various steps.

Furthermore, the mEHT application must consider the following conditions to avoid the suboptimal treatment:

1. The appropriate frequency is accurately selected around 10 MHz [182,183]. When the frequency is larger (>15 MHz), the membrane impedance becomes too small to select the disordered TME. The current will flow through the entire cell almost equally, neglecting the selection factor of dielectric permittivity.

2. The electromagnetic nano-targeting of rafts has similarities to the molecular targeting of drugs at cancer cells. The chemo dose is limited by poisoning. When the rafts are overheated, the raft protein may coagulate, and no selective heating is possible thereafter.

3. Hyperthermia may reversibly destabilize the raft structures [184], which could mix the time sequences of DAMP production and arrest the immune cells' activity [185].

4. The approaching of the contact current by mEHT has further limitations. RF safety standards specify the exposure limits [186]. The SAR could be extremely high in the small cross-section when the applicator does not smoothly cover the treatment area and the current flows through a small area which may burn in this touching. The challenge grows when the interface between the skin and electrode has a conductive layer such as sweat, saline, or other aqueous solution. The thin layer may be heated dangerously quickly, so the skin surface must be kept dry.

4. Conclusions

The success of the mEHT therapy of advanced, relapsed, and metastatic tumors has had multiple clinical studies. Some studies have double-arm comparisons, including a Phase III randomized, prospective controlled one for the uterus and cervix. The comparisons show a significant increase in survival time. The Kaplan−Meier survival plots were compared for the single-arm studies. The different studies at various institutes' and times showed significant correspondence, and the data could be pooled, which increases the evidence level of these observational studies. These clearly show the survival advantage of mEHT. We may conclude that mEHT significantly increases overall

survival and the quality of life; consequently, it is a feasible treatment for the presented malignant tumors.

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A Review of the Current Clinical Evidence for Loco-Regional Moderate Hyperthermia in the Adjunct Management of Cancers

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Simple Summary

There is a large gap in knowledge amongst the oncology community of moderate hyperthermia use in cancer management. This review provides an overview of clinical data on the use of loco-regional and superficial hyperthermia in the adjunct management of cancers. It is updated using higher-level evidence from prospective, comparative studies and meta-analyses. The methodology and results are summarised and tabulated according to tumour type for easy reference.

Abstract

Regional hyperthermia therapy (RHT) is a treatment that applies moderate heat to tumours in an attempt to potentiate the effects of oncological treatments and improve responses. Although it has been used for many years, the mechanisms of action are not fully understood. Heterogenous practices, poor quality assurance, conflicting clinical evidence and lack of familiarity have hindered its use. Despite this, several centres recognise its potential and have adopted it in their standard treatment protocols. In recent times, significant technical improvements have been made and there is an increasing pool of evidence that could revolutionise its use. Our narrative review aims to summarise the recently published prospective trial evidence and present the clinical effects of RHT when added to standard cancer treatments. In total, 31 studies with higher-quality evidence across various subsites are discussed herein. Although not all of these studies are level 1 evidence, benefits of moderate RHT in improving local tumour control, survival outcomes and quality of life scores were observed across the different cancer subsites with minimal increase in toxicities. This paper may serve as a reference when considering this technique for specific indications.

1. Introduction

Therapeutic hyperthermia (HT) encompasses the application of heat to targeted locations to increase the therapeutic response of oncological treatments. Various heating methods include direct (e.g., intracavitary and whole-body waterbed), infrared, perfusional (e.g., isolated limb perfusion, intravesical and intraperitoneal), nanoparticles, ultrasound and regional radiofrequency (RF) radiation [1]. Moderate HT is usually described at a range of 39–44 ∘C and its biological effects have been summarised previously and described in Figure 1 [2–4]. With the proposed mechanisms, synergisms with conventional treatments, such as radiotherapy (RT), chemotherapy (CT) and immunotherapy, should exist. Unfortunately, although positive results have been reported [5,6], robust clinical data remain elusive and marred by early negative trials [7–9]. Avid HT practitioners argue that the reasons for the hindered progress in this field are not the lack of efficacy, but the lack of funding, limited access, poorer tolerance of older technology, lack of quality assurance

processes, poor temperature monitoring and heterogenous practices [1,6,10,11].To advance the field, international groups such as the European Society of Hyperthermic Oncology (ESHO) and Society of Thermal Medicine (STM) have been formed, with the aim of promoting scientific knowledge and facilitating cooperative research. Quality assurance guidance [12–15] has also been published this past decade, to provide technical standardisations for the clinical applications of RHT. These serve to ensure the appropriate use of Regional Hyperthermia (RHT) and establish treatment standards to improve clinical outcomes.

Figure 1. Biological mechanisms of hyperthermia.

With the proposed mechanisms, synergisms with conventional treatments, such as radiotherapy (RT), chemotherapy (CT) and immunotherapy, should exist. Unfortunately, although positive results have been reported [5,6], robust clinical data remain elusive and marred by early negative trials [7–9]. Avid HT practitioners argue that the reasons for the hindered progress in this field are not the lack of efficacy, but the lack of funding, limited access, poorer tolerance of older technology, lack of quality assurance processes, poor temperature monitoring and heterogenous practices [1,6,10,11].To advance the field, international groups such as the European Society of Hyperthermic Oncology (ESHO) and Society of Thermal Medicine (STM) have been formed, with the aim of promoting scientific knowledge and facilitating cooperative research. Quality assurance guidance [12–15] has also been published this past decade, to provide technical standardisations for the clinical applications of RHT. These serve to ensure the appropriate use of Regional Hyperthermia (RHT) and establish treatment 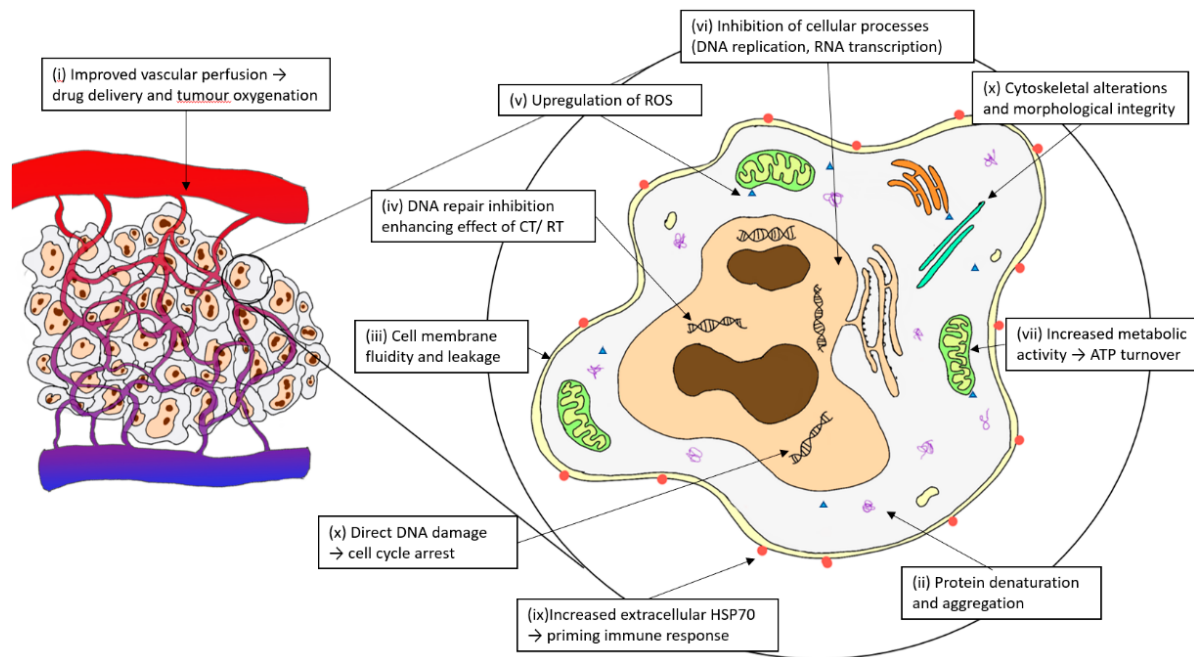standards to improve clinical outcomes. RHT technology uses a capacitive or radiative system [16], whereby antennas are externally applied over a target region. Non-ionising electromagnetic radiowaves or microwaves, using different frequencies and energy, are directed towards the tumour, where energy is deposited and converted into heat. Heat distribution is calculated and the target temperatures are monitored in real-time by minimally invasive thermoments.

2. Method

A literature review was performed using PUBMED on articles that Includeed externally applied, focused and moderate RHT. Only full-text English articles from prospective, comparative studies, meta-analyses and systematic reviews with a publication date from January 2000 to November 2022 were used. Reference and linked articles were Includeed If relevant. Trials Includeed In the meta-analysis were not re-presented to avoid duplication.

3. Results

3.1. Cervical Cancer

A Cochrane Systematic Review that compared RT alone vs. HT + RT was performed by Lutgens [22]. A total of 6 randomised controlled trials (RCTs) [7,23–28] that comprised 487 patients with locally advanced cervical cancers (LACC) were analysed. In total, 74% of patients were FIGO stage IIIB. The complete response (CR) rate (relative risk (RR) 0.56; p < 0.001), local recurrence rate (hazards ratio (HR) 0.48; p < 0.001) and overall survival (OS) (HR 0.67; p = 0.05) were significantly better with combined HT + RT. No significant difference was observed in the treatment-related acute (RR 0.99; p = 0.99) or late grade 3–4 toxicity (RR 1.01; p = 0.96). In 2016, another meta-analysis [29] using updated trial data [7,23–27] showed continued improvements in CR (+22.1%) and loco-regional control (LRC) (+23.1%) with HT; however, the survival advantage (+8%) was no longer significant. A 2019 network meta-analysis (NMA) by Datta compared the effectiveness and safety of 13 various interventional techniques for LACC [30]. 9894 patients were analysed across 59 trials, including 1 trial that compared HT + CTRT vs. CTRT [31], 1 trial that compared HT + RT vs. CTRT [32] and 4 trials that compared HT + RT vs. RT [23–26,33]. A corresponding surface under the cumulative ranking curve (SUCRA) analysis was performed to objectively rank the treatment options. The top three interventions for long-term LRC were as follows: HT + RT, CTRT + adjuvant CT and HT + CTRT. The top three interventions for OS were as follows: CTRT (3-weekly cisplatin), HT + CTRT and CTRT (not cisplatin). The three best treatment options for all endpoints (OS, LRC, grade ≥3 acute and late morbidity) were HT + RT, HT + CTRT and CTRT (3-weekly cisplatin). More recently, Yea conducted a meta-analysis comparing radical HT + CTRT vs. CTRT alone [34]. In addition, 2 RCTs [31,35] included 536 patients with LACC. Both trials used a RF capacitive heating device (Thermotron RF-8 and NRL-004 device).

Harima reported a better CR with HT in his trial (odds ratio (OR) 3.993; p = 0.047), although OS, disease-free survival (DFS) and local relapse-free survival (LRFS) improvements were not significant [31]. Wang reported a 5-year OS improvement (81.9% vs. 72.3%, p = 0.04), although LRFS was not significantly improved [35]. In the combined trial data, 5-year OS (HR 0.67; p = 0.03) was better in the group that received HT, although the LRFS improvement remained not statistically significant (HR 0.74; p = 0.16) [34]. The toxicity rates were not different between the arms. Amongst the patients who received HT, a higher CEM43T90 (≥1 min) was associated with better LRFS [17]. An ongoing phase III RCT that compared mEHT + CTRT vs. CTRT for 210 LACC patients was reported [36]. mEHT was given by an EHY2000 Oncothermia device. At 6 months, the odds ratios (OR) for achieving local disease control (LDC) and LRFS were 0.39 (p = 0.006) and 0.36 (p = 0.002), respectively, favouring mEHT + CTRT [36]. In addition, 2- and 3-year disease-free survival (DFS) was significantly improved by mEHT (HR 0.67; p = 0.017 and HR 0.70; p = 0.035, respectively).

However, 3-year OS was not significantly improved (HR 0.72; p = 0.74), except for those with stage III disease (HR 0.62; p = 0.040) [37]. Furthermore, 16.2% of participants who received mEHT reported early grade 1–2 adverse events (AEs) (adipose tissue burns, surface burns and pain), which were resolved after 3 months. There were no grade ≥3 AEs reported. Late AEs between the arms were similar. At 6 weeks, the mEHT group reported better quality of life (QoL) outcomes and better 3-month pain and fatigue scores [38]. QoL (specifically cognitive function and pain) at 2 years was significantly improved in the mEHT group. Cost-effective analysis reported mEHT+ CTRT as superior to CTRT alone, reducing the high cost of recurrent or progressive disease (PD) [37]. Interestingly, in 108 participants who underwent 18F-FDG PET/CT scans before and at 6 months post-treatment, a significantly more complete metabolic resolution (CMR) was observed in the initial PET avid lymph nodes (LN) outside the RT field (24.1% vs. 5.6%; p = 0.013), suggesting a potentiation of the abscopal effect with mEHT [39]. For recurrent cervical cancers in the pelvis following previous irradiation, Lee [40] compared CT vs. CT + mEHT (EHY2000) alone in a non-randomised cohort of 38 patients.

The overall response rate (ORR) improved with mEHT (72.2% vs. 40%; p = 0.0461). No difference in OS or toxicity was noted. The cervical cancer articles reviewed above are summarized in Table 1. We also highlight a recent review by Ijff et al. that provides further explanation and guidance on the use of RHT in LACC [41].

Author	Article Type	Investigation	Total Participants	Survival Outcome
Lutgens et al., 2010 [22]	Cochrane Systemic Review	HT + RT vs. RT alone in LACC	N = 487 (6 RCTs)	Improved CR, local recurrence rate, and better OS (HR 0.67; p = 0.05).
Datta et al., 2016 [29]	NMA	HT + RT+/−CT vs. RT+/−CT in LACC	N = 1160 (16 RCTs)	HT + RT was superior to RT alone in CR and LRC. Non-significant OS benefit. HT + CTRT resulted in best SUCRA score.
Datta et al., 2019 [30]	NMA	Compared across 13 interventional options in LACC	N = 9894 (59 RCTs)	Top 3 interventions by SUCRA: LRC: HT + RT, CTRT + adjCT and HT + CTRT. OS: CTRT (3-weekly CDDP), HT + CTRT and CTRT (non-CDDP). Cumulative: HT + RT, HT + CTRT and CTRT.
Minnaar et al., 2019 [36,37]	Phase III RCT	mEHT + CTRT vs. CTRT in LACC	N = 210	Better 6-month LDC; 2- and 3-year DFS. No OS benefit (except for FIGO III). Better QoL data with mEHT.
Yea et al., 2021 [34]	Meta-analysis	HT + CTRT vs. CTRT in LACC	N = 536 (2 RCTs)	Improved OS (HR 0.67; p = 0.03). No LRFS benefit.
Lee et al., 2017 [40]	Prospective comparative trial	mEHT + CT vs. CT in recurrent cervical cancer	N = 38	ORR improved. No OS benefit.

Table 1. HT in patients with cervical cancers. Summary of articles reviewed.

3.2. BREAST CANCER

Datta [41] performed a meta-analysis of eight trials, comparing RT vs. HT + RT (five were RCTs [8,42]) in 627 locoregional recurrent breast cancer patients. Improvement in CR was noted with HT (60.2% vs. 38.1%, RR 1.57; p < 0.0001). Survival data were not reported. The mean acute and late grade 3/4 toxicity with RT + HT was 14.4% and 5.2%, respectively. Loboda reported on 200 stage IIB–IIIA breast cancer patients randomized to neoadjuvant (NA) CT vs. NACT + HT [43]. Electromagnetic HT was given using the inductive MagTherm device. The patients that had HT experienced a greater average reduction in primary tumour size (31.24% vs. 22.95%; p = 0.034), while the ORR increased

by 15.9% (p = 0.034) and axillary LN regression improved by 14.17% (p = 0.011). The post-treatment viable tumour volume was lower if patients received HT and the proportion of women eligible for breast-conserving and reconstructive surgery increased by 13.63%. The 10-year OS was higher (p = 0.009) in patients who underwent NACT + HT.

3.3. Lung Cancer

A multi-institutional IAEA conducted RCTs in 80 LA non-small-cell lung cancer (NSCLC) patients, comparing RT + HT vs. RT alone [44]. HT was given using the capacitive RF-8 Thermotron device. There were no significant differences between the arms for the local response rate or OS. However, local progression-free survival (PFS) was significantly better with HT (p = 0.036; 1-year PFS 29.0% vs. 67.5%) and toxicity was generally mild, with no grade 3 late toxicities. Two RCTs reported outcomes in patients with refractory advanced NSCLC. Shen [45] randomised 80 patients to HT + CT vs. CT alone. An HY7000 RF HT device was used. No difference in the response rates was observed. However, QoL improvements were significantly better in the HT + CT group (82.5% vs. 47.5%; p < 0.05), especially for pain improvement. Ou [46] explored the efficacy of intravenous vitamin C with mEHT against best supportive care (BSC) in 97 patients. The 3-month disease control rate was better in the experimental arm (42.9% vs. 16.7%; p < 0.05). A prolonged median PFS (3 vs. 1.85 months; p < 0.05) and OS (9.4 vs. 5.6 months; p < 0.05) were noted and improved QoL scores were also observed with mEHT. The exploration of inflammatory markers showed differences in IL-6 and CRP levels after mEHT, although TNFa remained unchanged, suggesting some immune effect. Regarding small-cell lung cancer (SCLC), Lee [47] reported the results of a prospective case–control study with 31 patients (23 CT + mEHT; 8 CT alone). mEHT was given by an EHY2000 device. A significantly enhanced survival rate was noted with mEHT (p < 0.02). The breast and lung cancer articles reviewed above are summarized in Table 2.

Author	Article Type	Investigation	Total Participants	Survival Outcome
Breast Cancer				
Datta et al., 2016 [41]	Meta-analysis	RT vs. HT + RT in local recurrent breast cancer	N = 627 (5 RCTs, 3 cohort trials)	CR improved with HT. No survival data reported.
Loboda et al., 2020 [43]	Phase II RCT	NACT + HT vs. NACT in stage IIB–IIIA breast cancer	N = 200	Better tumour and axillary LN size reduction. Increased objective response. Higher 10-year OS rates ($p = 0.009$).
Lung Cancer				
Mitsumori et al., 2007 [44]	Phase II RCT	HT + RT vs. RT alone in LA NSCLC	N = 80	No difference in response rates or OS. Improved PFS (1-year 29.0% vs. 67.5%).
Shen et al., 2011 [45]	Phase II RCT	CT + HT vs. CT alone in advanced NSCLC	N = 80	No change in response rates. Better QoL improvements (especially pain response) with HT.
Ou et al., 2020 [46]	Phase II RCT	IV VitC + meHT vs. BSC in advanced NSCLC	N = 97	Improved disease control rate. Prolonged PFS. Better OS (9.4 m vs. 5.6 m; $p < 0.05$). Better QoL outcomes.
Lee et al., 2013 [47]	Prospective comparative trial	CT + mEHT vs. CT alone in SCLC	N = 31	Improved survival ($p < 0.02$).

Table 2. HT in patients with breast and lung cancers. Summary of articles reviewed.

3.4. Oesophageal Cancers

Hu et al. [48] performed a meta-analysis of 19 RCTs (three RCTs [49–51] had full texts available), comprising 1519 patients with locally advanced oesophageal cancers. Patients were randomly assigned into HT + CTRT, CTRT and/or RT groups. Comparison between HT + CTRT and CTRT showed improved 1-, 3-, 5- and 7-year survival rates (OR 1.79, 1.91, 9.99 and 9.49, respectively; $p < 0.05$) with HT. No differences in the recurrence or distant metastasis rate were noted. HT + CTRT was significantly superior in terms of CR (OR 2.00; $p < 0.00001$) and total effective rates (TER) (OR 3·47; $p < 0.00001$). Surprisingly, the observed gastrointestinal toxicities were less with HT + CTRT, although the radiation pneumonitis incidences were similar. Comparing HT + CTRT vs. RT alone, a significant survival advantage was also observed with HT at after 1, 2, 3 and 5 years (OR 3.20, 2.09, 2.43 and 3.47, respectively; $p < 0.05$). Lower recurrence (OR 0.39; $p = 0.0001$) and distant metastasis rates (OR 0.46; $p = 0.003$) were recorded, in addition to a higher CR (OR 2.12; $p = 0.003$) and TER (OR 4.8; $p = 0.002$). There was, however, a trend of higher toxicities with HT + CTRT.

3.5. Hepatocellular Carcinoma (HCC)

A phase II RCT of 80 patients with primary advanced unresectable HCC was performed [52]. Patients were randomised between the groups of radiofrequency HT + RT vs. RT alone. A capacitive RF system was used. The normalisation of liver enzymes and albumin levels improved more with HT ($p < 0.05$). The therapeutic efficiency (CR, PR or SD) at 3 months was better following HT (60.0% vs. 47.5%; $p < 0.001$). The 1-year recurrence (27.5% vs. 40.0%; $p < 0.001$) and mortality rates (12.5% vs. 20.0%; $p < 0.001$) were also significantly reduced in the HT group.

3.6. Pancreatic Cancer

A systemic review compared the addition of HT to RT and/or CT. A total of 14 studies (none were RCTs), consisting of 395 patients with LA or metastatic pancreatic cancer, were analysed [53]. A longer median OS (11.7 vs. 5.6 months) and better ORR (43.9% vs. 35.3%) was reported with HT. Most of the reported toxicities were mild, but there was one case of severe subcutaneous fatty burns. In the review, a prospective open-label comparative cohort was included. In total, 68 patients with LA pancreatic cancers were treated with CTRT+/–HT [54]. The median OS was better with HT (15 vs. 11 months, $p = 0.025$) without increasing toxicities.

3.7. Rectal Cancer

A total of 137 rectal cancer patients undergoing NA CTRT were randomised to RF HT (BSD 2000s) [55]. No statistical difference in the global 'Gastrointestinal Quality of Life Index' questionnaire at four time points was detected. Response or survival data were not reported, and a trend of increasing toxicity and post-op complications occurred in the HT group. A Cochrane Review [56] of pre-operative RT+/–HT in patients with LA rectal cancer used 6 RCTs that comprised 520

patients [28,57–60]. The 2-year OS was better with HT (HR 2.06; p = 0.001), but this difference disappeared after a longer period (3-, 4- and 5-year OS). The CR rates were higher with HT (RR 2.81; p = 0.01). Acute toxicity was not different between the treatment arms. Late toxicity data were not reported. More recently, a matched cohort of 120 LA rectal cancer patients receiving NA CTRT+/-mEHT was reported [61]. In the mEHT (EHY2000) arm, the median RT dose was lower. Larger tumours (>65 cm3) showed improved regression (31.6% vs. 0%; p = 0.024) and gastrointestinal toxicities were less (64.5% vs. 87.9%; p = 0 .01). No difference in the 2-year DFS, OS, LRRFS or DMFS was noted.

3.8. ANAL CANCER

Ott [62] reported the outcomes of 112 consecutive patients with UICC stage I–IV anal cancer who received CTRT. A total of 50 patients received additional radiative HT (BSD 2000-3D). At the 5-year follow-up point, the OS (95.8% vs. 74.5%; p = 0.045), DFS (89.1% vs. 70.4%; p = 0.027), LRFS (97.7% vs. 78.7%; p = 0.006), and colostomy-free survival rates (87.7% vs. 69.0%; p = 0.016) were better with HT. Disease-specific, regional failure-free, and distant metastasis-free survival rates were not different. The adjusted HRs for death (0.25; p = 0.036) and local recurrence (0.14; p = 0.06) improved with HT. With the exception of haematotoxicity, which was higher with HT (66% vs. 43%; p = 0.032), the reported early grade 3–4 toxicities were comparable between treatment arms. The incidences of late side effects were similar, except for a higher telangiectasia rate in HT (38% vs. 16.1%; p = 0.009). The esophageal, HCC, pancreatic, and anorectal cancer articles reviewed above are summarized in Table 3.

Author	Article Type	Investigation	Total Participants	Survival Outcome
		Oesophageal Cancer		
Hu et al., 2017 [48]	Meta-analysis	HT + CTRT vs. CTRT or RT alone	N = 1519 (19 RCTs)	HT + CTRT vs. CTRT: better CR and TER. No difference in recurrence and distal metastases rates. Improved 1-, 3-, 5- and 7-year OS. HT + CTRT vs. RT alone: better CR and TER. Lower recurrence and distal metastases rates. Improved 1-, 2-, 3- and 5-year OS.
		HCC		
Dong et al, 2016 [52]	Phase II RCT	HT + RT vs. RT alone in advanced HCC	N = 80	Improved liver enzyme and TER. Reduced recurrence rates. Reduced 1-year mortality (12.5% vs. 20.0%; $p < 0.001$).
		Pancreatic Cancer		
Van de Horst et al., 2017 [53]	Systematic review	Addition of HT to RT and/or CT	N = 395 (14 cohort trials)	Improved median OS and ORR, but not statistically analysed.
Maluta et al., 2011 [54]	Prospective comparative trial	HT + CTRT vs. CTRT in LA pancreas cancer	N = 68	Improved median OS ($p = 0.025$).
		Rectal Cancer		
Schulze et al., 2006 [55]	Phase II RCT	HT + CTRT vs. CTRT in NA rectal cancer	N = 137	No difference in QoL. No survival/response data.
Haas-Kock et al., 2009 [56]	Cochrane Systematic Review	HT + RT vs. RT alone in NA rectal cancer	N = 520 (6 RCTs)	CR higher (RR 2.81; $p = 0.01$). 2-year OS improved (HR 2.06; $p = 0.001$), but not for 3-,4- or 5-year OS.
Kim et al., 2021 [61]	Prospective comparative trial	mEHT + CTRT vs. CTRT in NA rectal cancer	N = 120	More regression in large tumours. No difference in DFS, OS, recurrent or distal metastases rates.
		Anal Cancer		
Ott et al., 2018 [62]	Prospective comparative trial	HT + CTRT vs. CTRT alone	N = 112	No difference in regional failure and distal metastases. Improved 5-year DFS, LRFS and OS (95.8% vs. 74.5%; $p = 0.045$).

Table 3. HT in patients with gastrointestinal and hepato–pancreatic cancers. Summary of articles reviewed.

3.9. HEAD AND NECK CANCERS (HNCS) AND NASOPHARYNGEAL CARCINOMAS (NPC)

Kang [63] reported the outcomes of a phase II RCT using CTRT + HT in the treatment of 154 N2/3 NPC patients. The patients were randomised to microwave HT (Pingliang 778WR–L–4) to the metastatic LN. At 3 months post–treatment, cervical LN CR was better (81.6% vs. 62.8%; p = 0.014) with HT. The 5–year LC (96.1% vs. 76.9%; p = 0.001), DFS (51.3% vs. 20.5; p = 0.001) and OS (68.4% vs. 50.0%, p = 0.001) rates were improved with HT. Dermatitis incidence was not significantly higher and no severe complications were observed in any of the patients during the 5–year follow–up. In the patients receiving HT, the 3–month and 5–year LN regressions rates were better if higher temperatures (T90 ≥ 43 ∘C) or 4–10 sessions were given. Another phase II RCT compared the outcomes of 83 NPC patients that had definitive CTRT+/–HT [64]. Capacitive RF HT was given using HG-2000/NRL-002 applicators. The median DFS was better with HT (61 vs. 38 months; p = 0.048). In addition, 3–year OS was also improved (73.0% vs. 53.5%; p = 0.041). Post–treatment NPC–

specific QoL scores were also better preserved with HT. A meta-analysis evaluated the outcomes of HT + RT vs. RT alone in HNCs [65]. A total of 451 cases from 6 studies [8,66–70] were included (five RCTs; one NPC–only trial). No concurrent CT or surgery was used, and RT dose was variable. Overall CR was higher with the addition of HT (39.6% vs. 62.5%; OR 2.92; p = 0.001). Acute and late grade 3/4 toxicities were similar in both the groups. Five trials reported long–term survival outcomes using different end points. Patients fared better with HT + RT. The longest survival figures, as reported by Valdagni [68], showed improved 5-year freedom from local relapse (68.6% vs. 24.2%; p = 0.015) and OS (53.3% vs. 0%; p = 0.02) with HT. A multicentre phase II Chinese RCT compared the induction of CT + HT vs. CT alone in 120 LA resectable oral squamous cell carcinoma (OSCC) patients [71]. An ultrasonic HT system was used. Treatment was followed by radical surgery and post-operative RT. The clinical response rate was better with HT (65.45% vs. 40.0%; p = 0.0088). DFS improved (HR 0.5671; p = 0.0335), but not OS (HR 0.6022; p = 0.0551). No unexpected toxicity or increase in perioperative morbidity was noted. A 3.33% grade 1/2 skin toxicity rate was associated with HT. OS and DFS were associated with better clinical response in the subgroup analysis. The HNCs and NPCs articles discussed are summarized in Table 4.

Author	Article Type	Investigation	Total Participants	Survival Outcome
Kang et al., 2013 [63]	Phase II RCT	HT + CTRT vs. CTRT in N2-3 NPC	N = 154	Improved 3-month CR. 5-year LCR and DFS better. 3- and 5-year OS improved.
Zhao et al., 2014 [64]	Phase II RCT	HT + CTRT vs. CTRT in NPC	N = 83	DFS improved. 3-year OS better (73.0% vs. 53.5%; p = 0.041). Better QoL preservation.
Datta et al., 2016 [65]	Meta-analysis	HT + RT vs. RT in HNCs	N = 451 (5RCTs; 1 non-RCT)	Improved overall CR (OR = 2.92; p = 0.001). Survival not analysed.
Ren et al., 2021 [71]	Phase II RCT	Induction CT + HT vs. CT alone in OSCC	N = 120	Improved clinical response rates. Improved DFS (HR 0.57; p = 0.034). No significant OS advantage.

Table 4. HT in patients with head and neck cancers, including NPC. Summary of articles reviewed.

3.10. SOFT TISSUE SARCOMA (STS)

A total of 341 patients with localised high-risk STS were randomised to NACT+/-RHT (BSD-2000 system) in the EORTC 62961-ESHO 95 multicentre phase III RCT [72,73]. Patients were stratified according to presentation, centre and site. In patients with extremity sarcomas, higher treatment responses (28.8% vs. 12.7%; p = 0.002) and R0 resection rates were observed with combined treatment. Similarly, better response rates were observed in retroperitoneal and abdominal STS groups (34.7% vs. 15.6%; p = 0.034) [74]. Patients who received HT had better LPFS (2 year: 76% vs. 61%; HR 0.58; p = 0.003) and DFS (HR 0.70; p = 0.0011). In per–protocol analysis, the HT group had better OS (HR 0.66, p = 0.038) [73]. After longer follow–ups (>11 years), further separation of the survival curves was noted. HT improved median LPFS (67.3 vs. 29.2 months; RH = 0.65, p = 0.002), median DFS (7.4 vs. 33.3 months; HR = 0.71, p = 0.01) and median (15.4 vs. 6.2 years, HR = 0.73; p = 0.04) 5-year (62.7% vs. 51.3%) and 10-year OS (52.6% vs. 42.7%). The survival benefit of RHT was noted across all subgroups. Five deaths (3.1%) were attributable to treatment in the combined group vs. two deaths (1.2%) in the NACT–alone group [72]. Toxicities, e.g., leukopenia (grade 3/4), were more frequent with HT (77.6% vs. 63.5%; p = 0.005). HTrelated grade 3–4 AEs were as follows:

4.3% pain, 4.9% bolus pressure, and 0.6% skin burns [73]. Out of 94 patients with macroscopically resected retroperitoneal or abdominal STS, early progression occurred in 10 patients (22.2%) treated with NACT only vs. none with RHT (p < 0.001). In addition, 5-year LPFS (56% vs. 45%; p = 0.044) and DFS (34% vs. 27%; p = 0.040) improved with RHT. OS, perioperative morbidity, and mortality were not different between arms [74]. Immune infiltrates in the biopsies at baseline and after induction treatment were analysed in 109 patients. Post-treatment high tumour–infiltrating lymphocytes (TILs) correlated with better LPFS. A strong association between high TILs or CD8 T cell infiltration and tumour response was noted for patients receiving RHT (p = 0.02), but not for the control. It was concluded that HT appeared to prime the tumour microenvironment, probably enabling enhanced anti-tumour immune activity in high-risk STS [75].

3.11. BLADDER

A Dutch multicentre prospective RCT was performed in 101 muscle-invasive bladder cancer (MIBC) patients, who were randomised to RT vs. HT + RT [28]. HT was given using various radiative RF systems.
 Improved CR was noted with HT (73% vs. 51%; p = 0.01). However, at 3 years, the difference in LC and OS was non-significant.

3.12. GLIOMA

In a prospective case–control study, 38 glioblastoma patients underwent CTRT or CTRT + HT [76]. HT was given via a capacitive system (Celsius 42+). Pre- (V1) and post- (V2) treatment MRI comparisons showed improvements in tumour reduction (ratio (V2/V1) 1.12 vs. 0.66 at 6 months) in favour of HT. The OS at 15 months and performance score change was not significantly different between the groups. HT was well tolerated without any significant AEs. The STS, Bladder cancer and Gliomas articles discussed above are summarized in Table 5.

Author	Article Type	Investigation	Total Participants	Survival Outcome
Soft Tissue Sarcoma				
Issels et al., 2018 [72]	Phase III RCT	HT + NACT vs. NACT alone in localised high-risk STS	N = 341	Improved response. Improved LPFS and DFS. OS improved (HR = 0.73; p = 0.04).
Bladder Cancer				
Van der Zee et al., 2000 [28]	Phase III RCT	HT + RT vs. RT alone in advanced pelvic tumours	N = 101 (MIBC)	Improved CR. No difference in OS and LC rates.
Glioma				
Mahdavi et al., 2020 [76]	Prospective comparative trial	CTRT vs. CTRT + HT in glioblastoma	N = 38	Improved response. No difference in OS or performance score change.

Table 5. HT in patients with soft tissue sarcoma, bladder cancer and glioma. Summary of articles reviewed.

3.13. Palliation

In total, 108 patients with incurable superficial lesions <3 cm from the surface were randomised to RT+/-HT [77]. HT was given using microwave spiral strip applicators. CR improved with HT (66.1% vs. 42.3%; OR 2.7, p = 0.02). Previously irradiated patients had the greatest incremental gain in CR (68.2% vs. 23.5%). HT was generally well tolerated, but a higher portion of grade 1–3 skin burn toxicities (46% vs. 5.7%), with one patient having a third-degree skin burn, was observed. No OS benefit was noted.

A Chinese trial compared local mEHT (EHY2000) in combination with traditional Chinese medicines (TCM) vs. the control of intraperitoneal chemoinfusion (IPCI) for the palliation of peritoneal carcinomatosis with malignant ascites (PCMA) [78]. A total of 260 patients were randomized between the 2 arms. In the experimental arm, superior ORR (77.69% vs. 63.85%; p < 0.05), QoL scores (48.23% vs. 32.3%; p < 0.05) and lower adverse reactions rates (2.3% vs. 12.3%; p < 0.05) were observed. All the AEs were grade 1. No survival data were reported. Furthermore, 103 patients with multiple liver metastases from breast cancer were assigned to CT+/-RHT (MagTherm) [79]. Higher therapeutic efficacy (PR + SD) (75.9% vs. 42%, p < 0.01) and QoL scores were noted with RHT. The median time to progression was prolonged with RHT (8.51 vs. 4.32 months; p < 0.05) and no serious AEs were reported. A total of 57 patients with painful bone metastases were randomised to RT (30Gy/10#)+/-HT (Thermotron RF-8) in a phase III RCT [80]. Improved complete pain responses (37.9% vs. 7.1%; p = 0.006) and pain control durability (28 days vs. not reached (NR); p < 0.001) were observed with HT. QoL improved in the first month, but not the third month. No change in skin or grade ≥3 toxicities were noted. However, 48.3% reported mild heating pain and 20.6% had elevated body temperatures that were resolved shortly after. Obese patients were more likely to experience subcutaneous fat induration. The trial was stopped following interim analysis due to a significant clinical effectiveness and slow recruitment. The palliative articles discussed above are summarized in Table 6.

Author	Article Type	Investigation	Total Participants	Survival Outcome
Jones et al., 2005 [77]	Phase II RCT	RT + HT vs. RT alone in superficial skin tumours	N = 108	Improved CR. No OS benefit.
Pang et al., 2017 [78]	Phase II RCT	mEHT + TCM vs. IPCI in PCMA	N = 260	Higher ORR. Improved QoL.
Kilmanov et al., 2018 [79]	Phase II RCT	HT + CT vs. CT alone in breast cancer and liver metastases	N = 103	Higher PR and SD. Improved QoL. Longer median time to progression.
Chi et al., 2018 [80]	Phase III RCT	RT + HT vs. RT alone in painful bone metastases	N = 108	Improved pain response. Longer time to pain progression. Improved 1-month QoL.

Table 6. HT for patients with palliation. Summary of articles reviewed.

4. Discussion

A total of 31 articles that used RHT across various cancer types are reviewed here. These include 9 systematic reviews and meta-analyses, and 22 prospective trials (16 randomised trials) published between January 2000 and November 2022. Trials before 2000, although informative, were not

reviewed, as their treatment practices may not be current and applicable. Retrospective studies and case series, although important, were also not included in our review, due to an inherent risk of confounding biases. In these studies, HT was deployed using a variety of technologies and settings. Nevertheless, a general trend of improvements in the therapeutic effects, such as the tumour response, local control rates and QoL outcomes (notably pain), can be observed when RHT is added to standard treatments. Reassuringly, the overall severe toxicity rates are not increased. However, low-grade and temporary skin or pain toxicities were higher in several studies. Importantly, several studies also report significant improvements in OS when RHT was employed. For example, a meta-analysis by Yea reports improved 5-year OS with HT in LACC patients undergoing radical CTRT [34]. In a multi-national phase III RCT with patients undergoing NA CT for STS, the median OS more than doubled with minimal toxicities [72], resulting in HT being included in both the NCCN and ESMO guidelines. Other presented studies also point to the potential of using RHT alone, as an immune stimulator and even allowing for treatment de-escalations. Whilst these findings are encouraging, one should be circumspect when interpreting the results. Several of the meta-analyses had combined studies that spanned a wide duration (including older studies before 2000), using different study types (observational and RCTs), and included trial data that are not publicly available. This could result in significant heterogeneity of the patient cohorts and interventions, which may compromise the validity and applicability of the results. There is also a risk of publication bias. Despite this, the data do present an estimate of the true effect.

The limitations of our narrative review were that other methods, such as whole-body HT and interstitial/intracavitary HT, were not reviewed. Retrospective and single-armed cohort studies were also not included. The methodology of the trials reported here was also not formally assessed for quality and the results were not synthesised, which precludes us from drawing any firm conclusions. However, the purpose of our review is to identify and present higher-level reports that would provide oncologists with a broad overview of RHT in the adjunctive management of cancer. In conclusion, the efficaciousness of RHT as an adjunct to modern cancer treatments appears promising. It is encouraging to note that there is an increasing amount of research on this subject, with most of the presented reports herein published within the last decade. Although limited, there is some high-quality clinical evidence that RHT offers benefits in certain scenarios, and more RCTs are needed.

ABBREVIATIONS

AE Adverse event
BSC Best supportive care
CEM43 Cumulative equivalent minutes at a temperature of 43 °C
CR Complete response

CRP C-reactive protein

CT Chemotherapy

DFS Disease-free survival

DMFS Distant metastasis-free survival

ESHO European Society of Hyperthermic Oncology

ESMO European Society of Medical Oncology

HCC Hepatocellular carcinoma

HNC Head and neck cancer

HR Hazards ratio

HT Hyperthermia

IAEA International Atomic Energy Agency

IL-6 Interleukin 6

IPCI Intraperitoneal chemoinfusion

LA Locally advanced

LACC Locally advanced cervical cancer

LC Local control

LDC Local disease control

LN Lymph nodes

LPFS Local progression-free survival

LRC Loco-regional control

LRFS Local relapse-free survival

LRRFS Locoregional recurrence-free survival

mEHT Modulated electro hyperthermia

NA Neoadjuvant

NCCN National Comprehensive Cancer Network

NMA Network meta-analysis

NPC Nasopharyngeal carcinoma

NSCLC Non-small-cell lung cancer

MIBC Muscle invasive bladder cancer

OR Odds ratio

ORR Overall response rate

OS Overall survival

PCMA Peritoneal carcinomatosis with malignant ascites

PET Positron emission tomography

PFS Progression-free survival

PR Partial response

RHT Regional hyperthermia

RR Relative risk

RCT Randomised controlled trial

RF Radiofrequency

RT Radiotherapy

SCLC Small-cell lung cancer

SD Stable disease

STM Society of Thermal Medicine

STS Soft tissue sarcoma

SUCRA Surface under the cumulative ranking curve

TER Total effective rate

TIL Tumour–infiltrating lymphocyte
TNFa Tumour necrosis factor alpha
QoL Quality of life

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EHY-2030

Loco-regional modulated electro-hyperthermia (mEHT) device

The EHY-2030 is Oncotherm's latest development for the loco-regional treatment of tumors. The newly designed device includes the Smart Electrode system and the integrated Patient Management System (PMS) and a user-friendly touch screen display with full system control. The new RF generator with impulse mode increased peak power has been developed with a new intelligently controlled step motor tuning system for rapid impedance matching to achieve shorter tuning-time.

The EHY-2030 device is a CE-marked medical device, and is intended for professional use.