

## Review

# Targeted treatment of cancer with radiofrequency electromagnetic fields amplitude-modulated at tumor-specific frequencies

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## Abstract

In the past century, there have been many attempts to treat cancer with low levels of electric and magnetic fields. We have developed noninvasive biofeedback examination devices and techniques and discovered that patients with the same tumor type exhibit biofeedback responses to the same, precise frequencies. Intrabuccal administration of 27.12 MHz radiofrequency (RF) electromagnetic fields (EMF), which are amplitude-modulated at tumor-specific frequencies, results in long-term objective responses in patients with cancer and is not associated with any significant adverse effects. Intrabuccal administration allows for therapeutic delivery of very low and safe levels of EMF throughout the body as exemplified by responses observed in the femur, liver, adrenal glands, and lungs. *In vitro* studies have demonstrated that tumor-specific frequencies identified in patients with various forms of cancer are capable of blocking the growth of tumor cells in a tissue- and tumor-specific fashion. Current experimental evidence suggests that tumor-specific modulation frequencies regulate the expression of genes involved in migration and invasion and disrupt the mitotic spindle. This novel targeted treatment approach is emerging as an appealing therapeutic option for patients with advanced cancer given its excellent tolerability. Dissection of the molecular mechanisms accounting for the anti-cancer effects of tumor-specific modulation frequencies is likely to lead to the discovery of novel pathways in cancer.

**Key words** Cancer, radiofrequency electromagnetic fields, amplitude modulation, tumor-specific frequencies

While their existence had been already hypothesized in antiquity, magnetism and electricity were first clearly described in the 16th and 18th centuries, respectively. In 1820, Oersted was the first to identify and report an interaction between electricity and magnetism by showing that a magnetic needle is deflected by electric current<sup>[1,2]</sup>. Subsequently, Faraday showed that a changing magnetic field induces an electric field, and Maxwell unified mathematically the theories of electricity and magnetism<sup>[3]</sup>. In 1895, Lorentz<sup>[4]</sup> refined the theory of electromagnetism following the discovery that the electron was the elementary particle carrying the electric charge. Additional

electromagnetic waves were found such as visible light, ultraviolet light,  $\gamma$  and X rays, leading to a description of the electromagnetic spectrum with the classification of all electromagnetic waves according to their frequencies.

The beginning of the 20th century saw the first medical applications of electromagnetic fields (EMF), notably in the diagnosis and therapy of various diseases such as cancer. The assumption was that external application of electromagnetic energy could correct disease-causing altered electromagnetic frequencies or energy fields within the body<sup>[5]</sup>.

Abrams invented various machines with the goal to cure diseases, notably cancer<sup>[6,7]</sup>. He claimed that diseases could be cured by transmitting back to the disease the same electronic "vibratory rate" it was transmitting. Between 1923 and 1924, *Scientific American* magazine set up a committee to investigate Abrams's results<sup>[8]</sup> and concluded "the claims advanced on behalf of the electronic reactions of Abrams, and electronic practice in general, are not substantiated"<sup>[6]</sup>. Lakhovsky developed the Radio-Cellulo-Oscillator in the 1920s<sup>[9]</sup>. This device produced high frequency (RF) EMF around 150 MHz. He postulated that EMF reinforced "the oscillations of the cell." Although a controversial figure in his time, he seems to have had some

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success with his treatments<sup>[10]</sup>.

Rife hypothesized that a number of bacilli were causal factors in many diseases, especially cancer. In the mid 1930s, he developed a microscope<sup>[11]</sup> able to see these bacilli and invented the Rife Frequency Generator, commonly called Rife Ray Machine, which he claimed could diagnose and eliminate diseases like cancer by tuning into electrical impulses given off by diseased tissue. The American Medical Association condemned Rife's experiments.

Until recently, virtually all medical devices aimed at treating cancer using low levels of electric and/or magnetic fields were considered quackery because of lack of scientific proof<sup>[12]</sup>.

### Diagnostic and Therapeutic Uses of Electromagnetic Fields

High-energy ionizing radiation is frequently used in medicine for both the diagnosis [computed tomography (CT) and X-ray radiography] and treatment (radiotherapy) of disease. The use of low-intensity RF EMF in medicine is much less common (Figure 1). While uncertainties regarding efficacy remain, there is increasing evidence that some forms of RF EMF exposure may be beneficial for the diagnosis and treatment of disease (Table 1).

Magnetoencephalography (MEG) is a noninvasive modality used to differentiate among neoplastic tissue types in the brain, with the potential to be used in combination with CT or magnetic resonance imaging (MRI)<sup>[13]</sup>. A modality called TRIMprob has shown promising sensitivity and specificity in the diagnosis of prostate and rectal cancers by exploiting differences in tissue resonance between neoplastic and normal tissues<sup>[14]</sup>. Thus, optimization of RF EMF diagnostic modalities to complement current screening methods may lead to improved diagnostic accuracy.

EMF has also been used as a therapeutic modality (Table 1). Pulsed EMF have shown efficacy on osteoarthritis<sup>[15]</sup>. Alternating electric fields have been used to induce fracture healing, with suggested efficacy similar to that of bone graft<sup>[16]</sup>. The proposed action of pulsed EMF is through the induction of directed migration and differentiation of bone marrow-derived mesenchymal stem cells<sup>[17]</sup>. Currently, RF EMF is used as a therapeutic option in cases ranging from tibial stress fractures to spinal cord injury.

Radiofrequency ablation (RFA) is a therapeutic option commonly used to treat malignancies including breast cancer, colorectal cancer, and hepatocellular carcinoma (HCC), and especially surgically unresectable metastases<sup>[18]</sup>. RFA is administered with medical devices operating between 460 and 550 kHz and delivering therapeutic energy to soft tissues<sup>[19]</sup>. This modality destroys tumor tissue through heat-induced necrosis by raising their temperature to approximately 100°C for approximately 15 min<sup>[18]</sup>.

Laboratory and clinical evidence suggests that certain frequencies within the RF EMF range of the spectrum may have antitumor effects without causing hyperthermia in patients with breast cancer, HCC, ovarian cancer, thyroid cancer, or glioblastoma multiforme<sup>[20-22]</sup>. The NovoTTF-100A technology applies alternating electric fields by means of electrodes placed on the skin overlying tumor-harboring body parts. This was the first EMF device of its kind approved by the Food and Drug Administration (FDA) of the United States based on the results of a phase III trial for treating recurrent glioblastoma showing efficacy similar to the standard-of-care chemotherapy regimen but with fewer adverse effects<sup>[23]</sup>.

Pulsed electric fields have been developed for localized treatment of tumors. This approach is based on the use of short electric pulses inducing either irreversible or reversible changes in cell membrane permeabilization. Irreversible changes lead to cell death, and

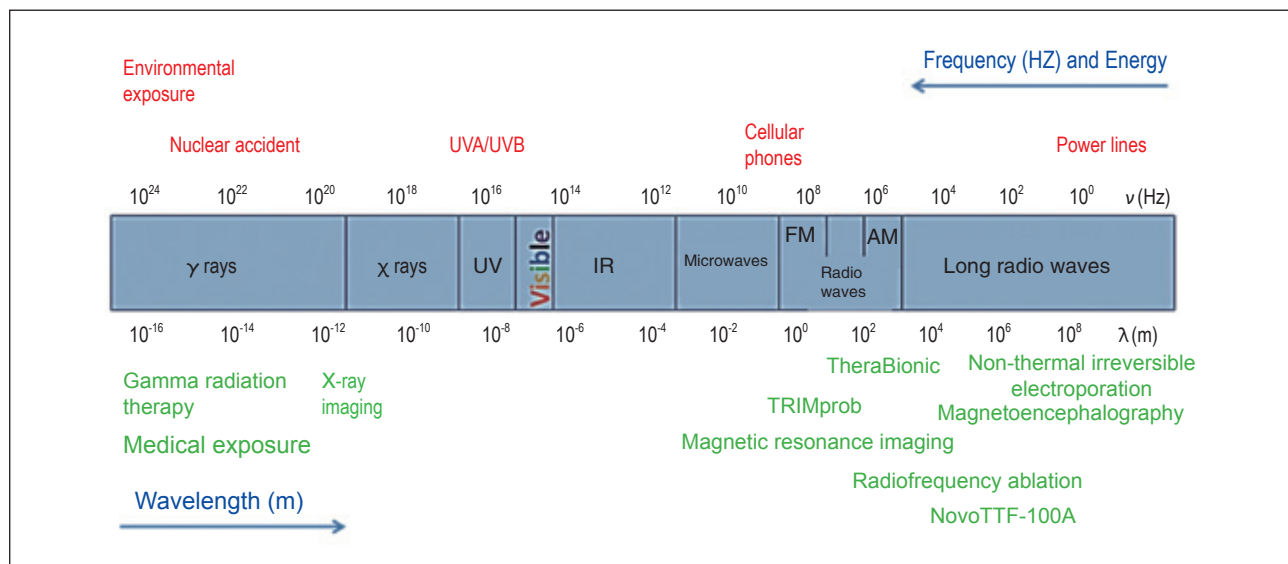


Figure 1. The electromagnetic spectrum and common exposures. The electromagnetic spectrum is depicted in blue. Environmental exposures with known or possible negative consequences are shown in red. Exposures received as part of medical diagnosis or treatment are shown in green.

**Table 1. Summary of clinical studies evaluating the efficacy of electromagnetic fields (EMF) as diagnostic or therapeutic modalities**

Application	Study	Modality	Results
Diagnostics	Pearlman <i>et al.</i> <sup>[13]</sup>	Magnetoencephalography	Non-invasive modality for differentiating among neoplastic brain tissues, designed to be used in combination with CT and/or MRI
	Vannelli <i>et al.</i> <sup>[14]</sup>	TRIMprob	Exploited tissue resonance differences between neoplastic and normal tissue, used in the diagnosis of prostate and rectal cancers
	Barbault <i>et al.</i> <sup>[20]</sup>	TheraBionic	Identified a tumor-specific frequency signature in patients diagnosed with cancer
Therapeutics	Aaron <i>et al.</i> <sup>[16]</sup>	Electric fields and EMF to facilitate fracture healing	Suggested that EMF has efficacy similar to a graft in the treatment of nonunion fractures and spinal fusions
	Pasche <i>et al.</i> <sup>[28]</sup>	LEET to treat physiologic insomnia	Demonstrated decreased sleep latency and increased sleep duration in patients treated with LEET in a double-blind cross-over study
	Stupp <i>et al.</i> <sup>[23]</sup>	NovoTTF-100A	Delivery of electric fields rather than chemotherapy in patients with recurrent glioblastoma multiforme demonstrated efficacy comparable to chemotherapy regimens
	Costa <i>et al.</i> <sup>[21]</sup>	TheraBionic	Amplitude-modulated RF EMF demonstrated safety and antitumor effects in patients with unresectable hepatocellular carcinoma

CT, computed tomography; MRI, magnetic resonance imaging; LEET, low-energy emission therapy; RF, radiofrequency.

reversible changes allow for markedly increased chemotherapy penetration<sup>[24]</sup>. The addition of therapeutic modalities based on RF EMF with minimal adverse effects is an exciting prospect for patients with cancer.

One final consideration with RF EMF-based therapies is possible synergy with frequently used chemotherapies. RF EMF in combination with bevacizumab and cyclophosphamide demonstrated no increase in adverse effects clinically, and similar findings were reported *in vitro* when EMF was used in combination with paclitaxel or cisplatin<sup>[20,22]</sup>. These findings suggest that patients may not experience additional adverse effects from undergoing both chemotherapy and RF EMF therapy; moreover, simultaneous treatment with both modalities may have a synergistic effect.

## Rationale for the Therapeutic Use of Amplitude-modulated Radiofrequency Electromagnetic Fields for the Systemic Treatment of Cancer

We have previously identified several frequencies in patients with chronic insomnia using biofeedback methods. We demonstrated that the intrabuccal administration of very low and safe levels of 27.12 MHz RF EMF, amplitude-modulated at 42.7 Hz, has a sleep-inducing effect in healthy subjects<sup>[25,26]</sup>. However, administration of the same signal to patients with insomnia did not yield any therapeutic benefits. In contrast, administration of a combination of the four frequencies most commonly identified in patients with chronic insomnia (2.7 Hz, 21.9 Hz, 42.7 Hz, and 48.9 Hz) resulted in significant improvements of total sleep time and sleep latency as assessed by polysomnographic evaluation<sup>[27,28]</sup>. These early findings suggested that a combination of several frequencies is needed to achieve a therapeutic effect on chronic insomnia.

In 2001, Barbault *et al.*<sup>[20]</sup> hypothesized that the growth of human tumors may be sensitive to specific modulation frequencies. To test this hypothesis, they initiated a patient-based research using novel biofeedback devices and techniques, which led to the discovery that patients with the same tumor type had biofeedback responses to the same frequencies, irrespective of their sex, age, or ethnic status<sup>[20]</sup>. Frequencies identified in patients with cancer were predominantly found above 1,000 Hz. This range is significantly higher than the range within which insomnia frequencies had been identified (<300 Hz). They also discovered that, in contrast to what had been observed in patients with insomnia, biofeedback responses were only observed at very precise frequencies. This prompted the development of highly precise frequency synthesizers for the detection of tumor-specific frequencies. Interestingly, the majority of frequencies identified in a given tumor type was only found in patients with the same tumor type<sup>[20]</sup>. For example, 85% of the frequencies identified in patients with HCC were only found in other patients with HCC. Similarly, 75% of the frequencies identified in patients with breast cancer were only found in patients with the same tumor type<sup>[20]</sup>. They also discovered that a small number of frequencies, e.g., 1,873.477 Hz, 2,221.323 Hz, 6,350.333 Hz, and 10,456.383 Hz, were found in the majority of patients with breast cancer, HCC, prostate cancer, and pancreatic cancer<sup>[20]</sup>. Examination of healthy individuals without a diagnosis of cancer did not reveal any biofeedback responses to the frequencies identified in patients with a diagnosis of cancer.

## Clinical Experience with Intrabuccal Administration of Amplitude-modulated Radiofrequency Electromagnetic Fields

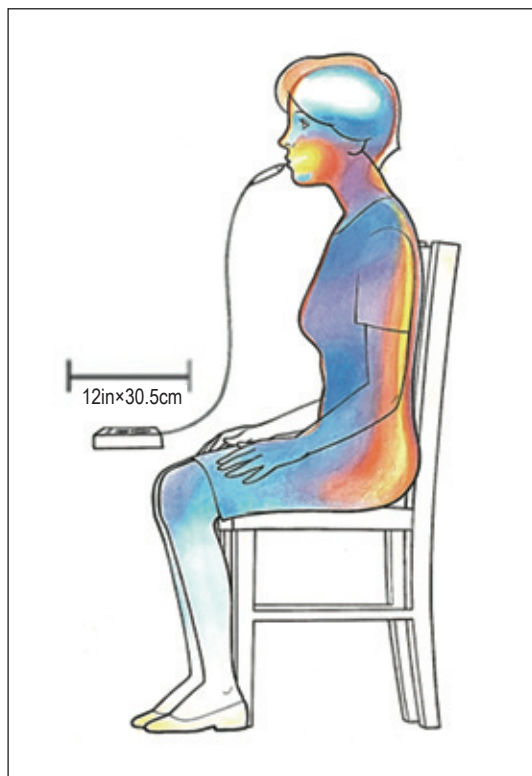
After having successfully identified tumor-specific frequencies

in 163 patients with cancer, Barbault *et al.*<sup>[20]</sup> offered compassionate treatment to 28 of these patients with advanced cancer and limited therapeutic options. Treatment was administered with a portable device built with the same high precision frequency synthesizer as that used for the identification of tumor-specific frequencies. They chose the 27.12 MHz carrier frequency, as it is universally approved for medical use. The 27.12 MHz signal was amplitude-modulated at the specific frequencies identified in patients diagnosed with cancer. The frequencies were emitted sequentially, each for 3 s from the lowest to the highest frequency, and the cycle was continuously repeated for 1 h. Treatment was administered 3 times a day, i.e., for a total of 3 h until disease progression or death. Intrabuccal administration of 27.12 MHz RF EMF with the TheraBionic device results in a whole body absorption of very low levels of EMF (**Figure 2**). The maximum specific absorption rate of the applied RF averaged over any 10 g of tissue is estimated to be less than 2 W per kg<sup>[21]</sup>.

The results of this study, with a cutoff date of April 1, 2009, are published<sup>[20]</sup>. Briefly, 1 patient with biopsy-proven stage IV breast cancer metastasized to the left femur and right adrenal gland had a complete response lasting 11 months. One patient with biopsy-proven stage IV breast cancer metastasized to the liver and skeleton had a partial response lasting 13.5 months. Additionally, 5 patients had stable disease for +34.1 months (thyroid cancer with biopsy-proven lung metastases), 6.0 months (mesothelioma metastasized to the abdomen), 5.1 months (non-small cell lung cancer), and 4.1 months (pancreatic cancer with biopsy-proven liver metastases). Two patients were still undergoing treatment as of April 1, 2009. One patient with ovarian cancer refractory to cisplatin and taxanes

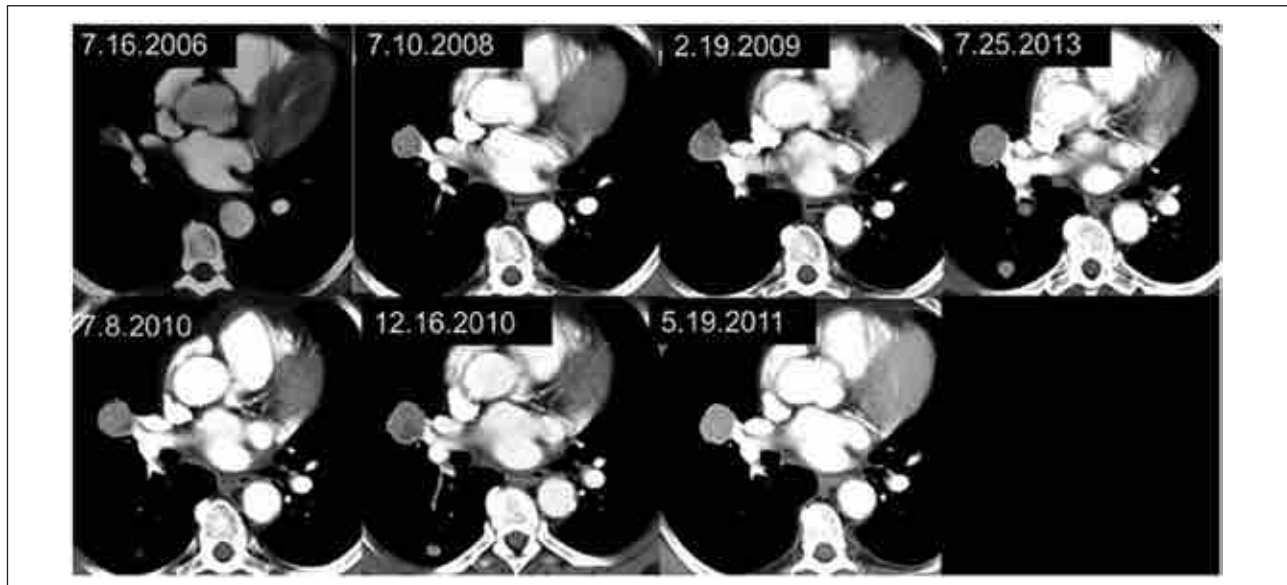
underwent treatment for 2 additional years for a total of 73 months. She had disease progression and died 78 months after enrolling in the study. The patient with stage IV thyroid cancer metastasized to the lungs remains on treatment as October 1, 2013 (**Figure 3**), and has received continuous treatment for a total of 86 months.

The excellent tolerability and the promising clinical results from the feasibility study prompted the design of an investigator-initiated phase I/II study in 41 patients with advanced HCC and Child Pugh A or B disease. The results of the study, with a cutoff date of June 9, 2011, are published<sup>[21]</sup>. Briefly, an antitumor effect was documented in 20 (48.8%) patients. Objective responses and/or long-term survival were observed in 8 (19.5%) patients. Alpha-fetoprotein levels decreased by 20% or more in 4 (9.8%) patients following initiation of therapy. Durable responses were observed in several patients with evidence of disease progression at the time of enrollment. In particular, one 76-year-old patient with HCC metastasized to the lungs and evidence of disease progression at the time of enrollment in July 2006 had a near complete response, which lasted more than 5 years (**Figure 4**). The results from this study suggest a degree of efficacy either superior or comparable to that of sorafenib (Nexavar<sup>®</sup>). Indeed, in a similar study of 137 patients with either Child Pugh A or B disease, the response rate achieved with sorafenib was 2.2% vs. 9.8% with TheraBionic<sup>[21,29]</sup>. Additionally, all patients treated with sorafenib had evidence of disease progression at 15 months, whereas 4 (9.8%) patients treated with TheraBionic had no evidence of disease progression at the same time point<sup>[21,29]</sup>. These promising results provide a strong rationale to initiate a randomized study determining the impact of TheraBionic on overall survival and time to symptomatic

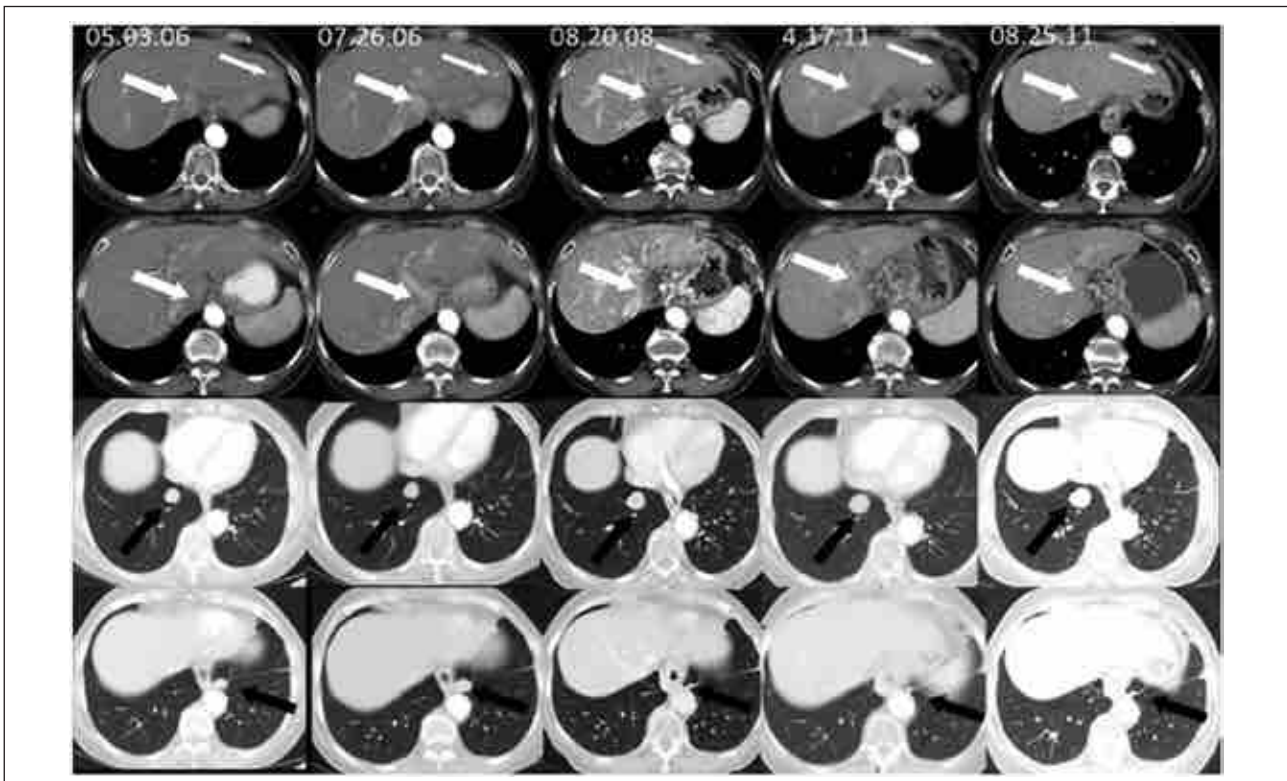


**Figure 2. Delivery of intrabuccal radiofrequency (RF) electromagnetic fields (EMF). Patient receives low levels of EMF that are minimally absorbed and distributed systemically with the body acting as an antenna. Yellow indicates the regions of the body receiving the greatest exposure.**





**Figure 3.** Long-term follow-up in a patient with recurrent, biopsy-proven thyroid carcinoma. This 67-year-old man had metastasis to the lungs. As of October 1, 2013, the patient continues to undergo treatment, 86 months after treatment initiation. CT scan images through the metastatic lesion in the right hilum demonstrate minimal changes over time.



**Figure 4.** Long-term partial response in a patient with biopsy-confirmed hepatocellular carcinoma. Shown here are images from a 76-year-old woman with hepatitis C and Child Pugh A5, Barcelona Clinic Liver Center Stage 3 (BCLC) disease, with bilateral lung metastases and evidence of progressive disease between May 3, 2006 and July 26, 2006 while enrolled in the Sorafenib Hepatocellular Carcinoma Assessment Randomized Protocol (SHARP) sorafenib registration study<sup>[60]</sup>. Treatment with amplitude-modulated EMF began on August 9, 2006. The hypervascularity of the focal hepatic lesions (arrows in first two rows) became hypo-enhanced on arterial phase (August 20, 2008); thus, portal venous phase images are shown on subsequent scans. The intrahepatic lesion size is stable regardless of enhancement pattern. The lesion in the left lung base resolved (4th row), while the right lung base lesion remained stable (3rd row) over the duration of treatment. The patient was treated for 5 years and 2 months prior to expiring of causes unrelated to her malignancy.

progression in patients with advanced HCC who have failed or do not tolerate sorafenib. Additional studies are planned in breast cancer, pancreatic cancer, ovarian cancer, and prostate cancer.

Only minor adverse effects were observed in these two studies. Four of the 69 (5.8%) patients enrolled in these two studies had grade 1 somnolence after treatment and 1 had grade 1 mucositis (1.4%). There were no grade 2, 3, or 4 toxicities in any patient, even among very long-term users. No changes in complete blood count, kidney function, or hepatic function were observed in any patient.

Clinical improvement has been observed within a few weeks of treatment initiation. Two patients with painful breast cancer bone metastases experienced significant symptomatic improvement within 2 weeks<sup>[20]</sup>. Similarly, among 11 patients with advanced HCC and pain prior to treatment initiation, 5 reported complete disappearance and 2 reported significant alleviation of pain within a few weeks of treatment initiation<sup>[21]</sup>.

As reported previously, the discovery of tumor-specific frequencies consists in the measurement of several parameters including variations in pulse amplitude<sup>[20]</sup>. This consistent but unexplained phenomenon was coined biofeedback response. To further investigate the relationship between biofeedback response and a specific diagnosis of cancer, Costa has designed a clinical study in which healthy individuals as well as patients with chronic hepatitis B, HCC, or breast cancer are exposed to randomly chosen frequencies, HCC-specific frequencies, and breast cancer-specific frequencies (clinicaltrials.org # NCT01686412). The goal of this study is to determine the sensitivity, specificity, and the pattern of biofeedback response in those four distinct groups of patients.

## Mechanism of Action

A large body of research reports a wide range of biological effects following exposure to RF EMF. The findings from these studies can be broadly grouped into categories: cellular function and metabolism; dysregulation and risk for malignancy; intercellular and systemic effects; cell morphology and differentiation; enzyme effects; pharmacologic effects (**Figure 5**). Overall, studies have focused on possible negative impacts of EMF exposure, ranging from DNA damage to a possible role as a cancer promoter. Previously, little emphasis has been placed on the possible positive impacts of controlled exposure to EMF; however, this paradigm has begun to shift.

Calcium ( $\text{Ca}^{2+}$ ) is crucial for a number of cellular processes ranging from embryonic patterning to transcription factor activation and apoptosis. EMF modulation of  $\text{Ca}^{2+}$  is especially appealing because investigators generally agree that the majority of the fields evaluated are not capable of causing direct effects on cellular structure or chromatin. Early studies illustrated enhanced  $\text{Ca}^{2+}$  flux in the brains of cats under local anesthesia following exposure to sinusoidally amplitude-modulated RF EMF, providing rationale for numerous subsequent studies<sup>[30]</sup>. Study outcomes have been dependent on cell lines used, duration of EMF exposure, choice of EMF exposure levels, and whether exposure has been continuous or pulsed<sup>[31,32]</sup>. One model of  $\text{Ca}^{2+}$  efflux has described a “windowing” phenomenon, similar to that previously described in chick brains in which efflux reaches a maximum at discrete EMF windows<sup>[33,34]</sup>. Although the specific findings and subsequent hypotheses differ, the

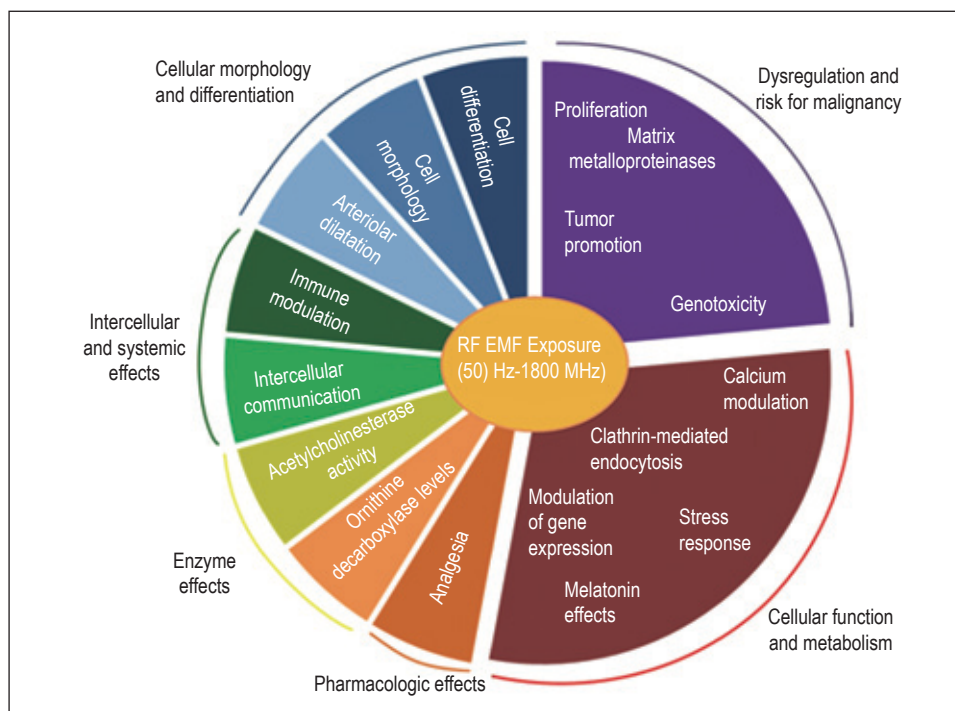


Figure 5. Reported biological effects of RF EMF exposure.

literature strongly suggests that exposure levels to EMF can impact  $\text{Ca}^{2+}$  flux; however, the biological significance of these fluctuations remains unclear and the demodulation mechanism leading to changes in  $\text{Ca}^{2+}$  flux has not yet been elucidated<sup>[32]</sup>.

Several studies have reported EMF-mediated induction of cellular stress responses through the activation of heat shock proteins (HSPs)<sup>[35]</sup>. These findings support the theory that EMF activates a common pathway mediated by HSP70, and more specifically, that EMF may interact with specific DNA sequences in genes' promoter regions<sup>[36]</sup>.

Cells use a dynamic set of redox reactions to maintain cellular homeostasis and minimize damage from oxidative processes. EMF-induced effects on cellular response to reactive oxygen species (ROS) may depend on the antioxidant state of the cells at the time of exposure<sup>[37]</sup>. Further, *in vivo* evidence suggests that intensity and duration of exposure may affect cellular oxidative response in a dose-dependent manner<sup>[38]</sup>.

These data suggest an oxidative stress response following some RF EMF exposure programs and led to the hypothesis that long-term exposure to EMF would cause chronic elevation of ROS and subsequent decrease in melatonin, leading to an increased risk for DNA damage and malignancy<sup>[39]</sup>. However, there have not been indications of increased transformation following EMF exposure alone or in combination with other stress factors, suggesting that EMF did not work in synergy with other stress factors to transform the cells<sup>[40]</sup>.

Studies evaluating the impact of RF EMF on gene expression have been inconclusive. Although some studies have reported no changes in gene expression, others have identified decreased levels of pro-inflammatory chemokines<sup>[41,42]</sup>. Modulation of gene expression was also reported in a tissue- and tumor-specific manner in cells exposed to RF EMF amplitude-modulated at specific frequencies<sup>[43]</sup>. Of note, negative studies used microarray technology or evaluated specific genes.

Melatonin maintains the natural circadian rhythms of the body, participates in the oxidative stress response, and has reported antitumor effects by mechanisms such as cell cycle inhibition, apoptosis induction, and metastasis prevention, especially in hormone-dependent malignancies<sup>[44]</sup>. Melatonin modulation following EMF exposure was also reported *in vivo*<sup>[45]</sup>. Because melatonin is thought to play a role in the prevention of metastasis, the effect of EMF on cell invasion was evaluated in breast cancer cell lines, demonstrating no change in invasion potential<sup>[46]</sup>. Although studies suggest an association between melatonin levels and RF EMF exposure, there is discordance regarding the impact on melatonin levels as well as the mechanism of melatonin and RF EMF interaction.

Studies examining DNA damage following RF EMF exposure have been inconclusive. While some studies cited double-strand breaks, evidence of chromosomal damage, and micronuclei formation, others reported no evidence of chromosomal damage or genotoxicity<sup>[47,48]</sup>. Although disparate, the literature has demonstrated that low-energy EMF does not cause predictable effects on DNA.

To begin to understand the molecular mechanism that accounts for the clinical responses observed in patients treated with the TheraBionic device, we designed and built systems to expose cell lines to the same RF signal as the one used for patient treatment<sup>[43]</sup>.

We conducted cell proliferation assays of HCC (HepG2 and Huh7) and breast cancer (MCF-7) cell lines, which were exposed to HCC-specific, breast cancer-specific, or randomly selected modulation frequencies. Exposure conditions replicated the regimen administered to patients, i.e., 3 h per day for 7 days in a row. While randomly selected modulation frequencies did not affect proliferation of any cancer cell lines, HCC-specific modulation frequencies effectively inhibited the growth of both HCC cell lines. Similarly, breast cancer-specific modulation frequencies effectively inhibited the growth of the breast cancer cell line. However, the growth of the HCC cell lines was not inhibited by breast cancer-specific modulation frequencies, nor was the growth of the breast cancer cell line inhibited by HCC-specific modulation frequencies. Additionally, the growth of immortalized normal hepatocytes (THLE-2) and breast cells (MCF-10A) was unaffected by tumor-specific modulation frequencies. These experiments provided the first *in vitro* evidence that the anti-proliferative effect of the TheraBionic device is mediated by a combination of precisely defined, tumor-specific modulation frequencies. Indeed, more than 50% of the HCC-specific, breast cancer-specific, and randomly selected modulation frequencies differed by less than 1%. Furthermore, 7 of the HCC-specific and breast cancer-specific frequencies were identical<sup>[43]</sup>. Next, we sought to determine the dose response effect of exposure to tumor-specific modulation frequencies. While 3 h or 6 h of daily exposure for 1 week resulted in significant cancer cell growth inhibition, 1 h of daily exposure for 1 week or 3 h of daily exposure for 3 days did not inhibit cancer cell growth.

Having identified a reproducible growth inhibitory effect of tumor-specific frequencies in several cancer cell lines, we used RNA-seq technology to comprehensively examine the gene expression profile of HepG2 cells exposed to HCC-specific vs. randomly selected modulation frequencies. We observed changes in expression in a small number of genes. Two of them, proteolipid protein 2 (*PLP2*) and chemokine (c motif) ligand 2 (*XCL2*), appeared to be down-regulated with an absolute fold change greater than 1.8 in HepG2 cells exposed to HCC-specific modulation frequencies. Using quantitative polymerase chain reaction (qPCR), we validated the down-regulation of these 2 genes not only in HepG2 cells but also in Huh7 cells.

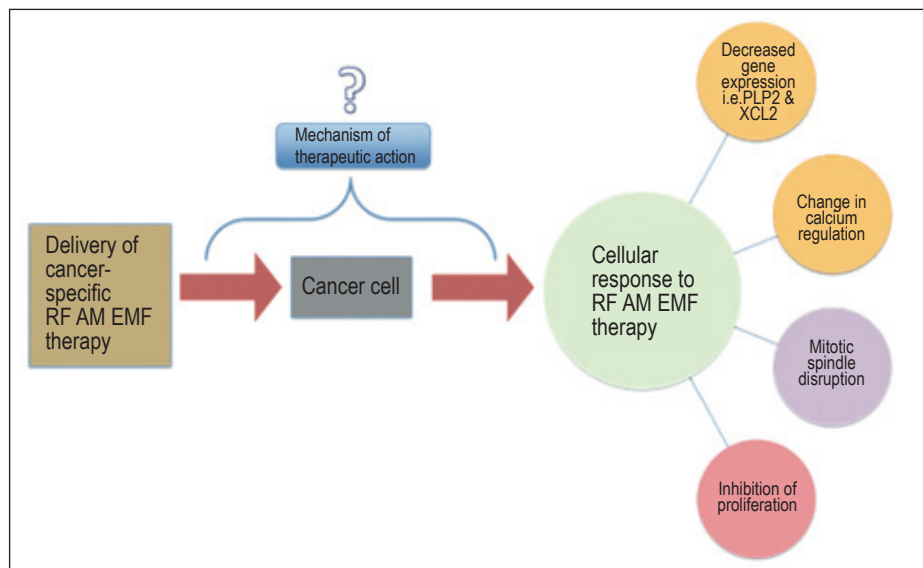
Some studies in glioblastoma and melanoma cell lines have demonstrated proliferative inhibition and mitotic spindle disruption following exposure to alternating electric fields<sup>[43,49]</sup>. We therefore investigated whether tumor-specific modulation frequencies affected the mitotic spindle. We found that more than 60% of HepG2 cells exposed to HCC-specific modulation frequencies had pronounced disruption of the mitotic spindle while none of the unexposed cells displayed the same phenotype.

## Conclusions

In summary, our clinical results provide strong evidence that the intrabuccal administration of RF EMF amplitude-modulated at tumor-specific frequencies is safe and well-tolerated and may lead to long lasting therapeutic responses in patients with advanced cancer. Our *in vitro* experiments demonstrate that cancer cell proliferation can be targeted using tumor-specific modulation frequencies, which were identified in patients diagnosed with cancer. Tumor-specific

modulation frequencies block the growth of cancer cells, modify gene expression, and disrupt the mitotic spindle (**Figure 6**). Studies are underway to dissect the biophysical mechanism leading cancer cells to respond to specific modulation frequencies identified in

patients with a corresponding diagnosis of cancer but not to randomly selected or tumor-specific frequencies identified in other tumor types. Elucidation of this mechanism of action is likely to unveil novel pathways and targets.



**Figure 6. Cancer-specific treatment and responses.** Theoretical flowchart representing the published biological responses to amplitude-modulated RF EMF therapy that may in part explain the antitumor effect.

## Conflict of interest

Boris Pasche and Alexandre Barbault have filed applications for patent protection and hold patents related to electromagnetic fields amplitude-modulated at tumor-specific frequencies as they

relate to the diagnosis and treatment of cancer. They hold stocks in TheraBionic.

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