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Use of non-ionizing electromagnetic fields for the treatment of cancer

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1. ABSTRACT

Cancer treatment and treatment options are quite limited in circumstances such as when the tumor is inoperable, in brain cancers when the drugs cannot penetrate the blood-brain-barrier, or when there is no tumor-specific target for generation of effective therapeutic antibodies. Despite the fact that electromagnetic fields (EMF) in medicine have been used for therapeutic or diagnostic purposes, the use of non-ionizing EMF for cancer treatment is a new emerging concept. Here we summarize the history of EMF from the 1890's to the novel and new innovative methods that target and treat cancer by non-ionizing radiation.

2. INTRODUCTION

In this review, we summarize current technologies that utilize non-ionizing RF EMF for cancer therapy and the existing research that may potentially elucidate the mechanisms underlying their anti-cancer effects. These include tissue heating/ablation, altered mitotic spindle formation and channel specific calcium

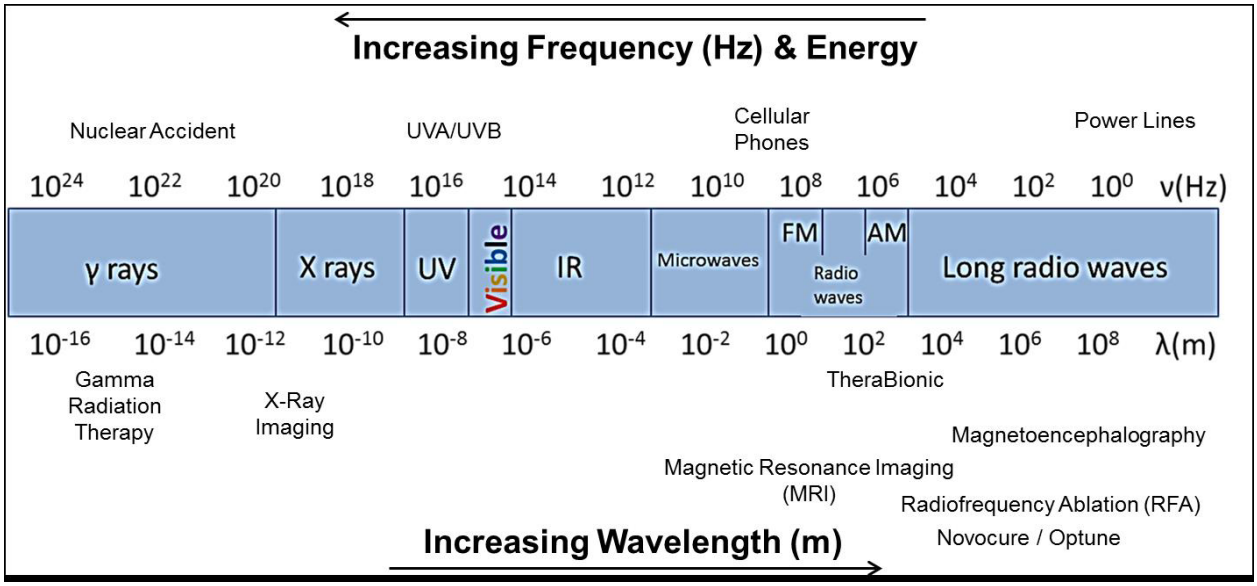

Figure 1. The EMF spectrum. As frequency and energy increase the wavelength decreases. Multiple medically related items and common environmental events are displayed in their respective range in the electromagnetic spectrum (15).

signaling. We also discuss the development of these technologies and field of research by reviewing the history of Radiofrequency Electromagnetic Fields.

3. HISTORY OF RADIOFREQUENCY ELECTROMAGNETIC FIELDS (RF EMF)

The notion that electromagnetic radiation (see Figure 1 for EMF spectrum) could have a biological impact by releasing heat in tissues emerged in the 1890's as electricity began to be produced in a controlled form. Arsène d'Arsonval was one of the first to identify increases in temperature and metabolism of the microbial cell in contact with electricity, and then, with Albert Charrin, he reported the attenuation of diphtheria and pyocyanic toxins by radiation at a frequency of 2 x 10^3 cycles per second (200 kHz) without a significant increase in temperature (1–3). In 1924, it was shown that when tumorous plants were subjected to ultra-short wavelengths, tumors would initially grow rapidly but then completely and selectively necrose (4). Some years later, it was reported that malignant tumors in mice could be destroyed by currents of very high frequency (VHF) (5). These reports also opened a large debate over thermal, which predominate, and non-thermal effects on living tissue (6–8). This scientific research was primarily conducted in a medical environment, which was interested in therapeutic applications.

At about the same time the invention of the split-anode magnetron (1920 General Electric research laboratories, New York; Albert W. Hull) and mainly the klystron (1938 Stanford University; Varian brothers, W.W. Hausen and D.L. Webster), which generated higher frequencies and power outputs, led to the development of new microwave energy generators and expanded their potential uses (9,10). While this was of interest to physicians at the Mayo Clinic in 1937, the power was far too low for therapeutic use. Over time the power levels began to increase and in 1938, the magnetron could produce 100 watts of power, then in 1939 it was found that the klystron could produce several hundred watts of power. As power began to reach a level high enough for therapeutic use, the magnetron and klystron became "mysteriously" unavailable (11). It was not until much later that it was discovered that the development of the magnetron and klystron were only designated for military application during World War II, in particular for radar, which did not seem harmful to personnel (12). Specifically, the development of a multicavity, air-cooled magnetron (1940 University of Birmingham, England; John Randall and Harry Boot) had been very important in perfecting radar (13). The same year, this multi-cavity magnetron was brought to the United States, after which the development of tubes that could produce a power output as high as one million watts was generated (14). The microwaves these new tubes could create had optical properties that could be reflected, refracted and diffracted. The cavity magnetron became largely used in radar technology by the Allies; the klystron being preferred by the Germans. The impact of war on research was without a doubt important.

In 1946 a microwave generator (cavity magnetron) became available to the Mayo Clinic for renewed studies on living animals (11). Much more careful investigation needed to be conducted with microwave energy to understand its way of functioning

and its possible safe place in medical therapy (16). During the 1950's a considerable push was under way to examine the biological effects of microwave radiation and possible harmful effects to the human body because "they have important uses in defense projects, industrial developments, and basic physical research" (17). A 1957 report described the death of a man standing in the direct beam of a radar transmitter. It was reported that the man experienced a sensation of heat, which quickly became intolerable in less than a minute. Within 30 minutes he developed acute abdominal pain and vomiting, which prompted surgical opening of the abdomen and draining of approximately 500 cc of serosanguinous fluid with the excision of the appendix that appeared gangrenous. The post-operative course was at first good but abdominal distention recurred and inflammation of the intestines with evisceration of the wound led the patient to his death ten days after the incident (18). It reinforced considerable interest and research in the biological aspects of exposure to radio frequency electromagnetic field (RF EMF) (19). U.S. government officials and business companies, such as Chief of R&D of Ordnance Missile Laboratory, Sylvania Electric Company and Bell telephone labs, began to issue statements related to the untoward effects of high powered radar for which safety limits should be determined (1,19). In March 1959, experiments to determine the effects of close-range exposure of the brain of a monkey to high intensity radio waves were conducted by the National Institutes of Health and reported before the House of Representatives Appropriations subcommittee. In examining the brains of ten monkeys, which died during the experiments, no pathological cause of death could be found. In a separate set of 10 monkeys whose exposure was cut short of death, the monkeys had convulsions resembling Parkinson's disease in humans (20). Another main aspect of research was to find out non-thermal non-ionizing biological effect of living tissue (21).

In 1968, James R. Hamer, reported that in 29 human subjects exposed to sinusoidal electric fields at field magnitudes of four volts per meter in the frequency range of 2–12 Hertz, reaction time performance was found to be approximately 1.6 milliseconds faster during "field on" compared to "field off" conditions. The experiments revealed that the effects were frequency sensitive and not merely due to the presence of the field (22). This work was a prime example showing that exposure to a low level, low frequency electric field could impact a biological system in a non-thermal, non-ionizing manner.

In 1969, Gavalas, Walter, Hamer and Adey reported that exposure to low-level, low frequency sinusoidal electric fields had an effect on the behavior and pattern of electrical activity (EEG) of monkey brains. Behaviorally, monkeys displayed a shorter inter response (time between signal and response behavior, i.e. push a lever in front of each subject) when exposed to 7 cycles per second but not to 10 cycles per second electric fields. EEG results showed an increase in percent power at the frequency of the fields for the hippocampus but less consistently in the amygdala and center median (the brain structures used for recording EEG and measuring percent power). Percent power is calculated by averaging spectral intensity and coherences for each brain structure. The "Coherence," parameter is calculated by analyzing the coherence between the imposed field and the activity in each structure, as well as between the brain structures themselves. The "Spectral intensity," is a specialized statistical test for the effect of the imposed field on recorded activity. The increased percent power, in some brain structures, was observed during two different conditions, one being 7 cycles per second and the other being 10 cycles per second, a previously reported frequency exposure used by Hamer and identified to have an effect on human reaction time (23).

In 1973 Bawin, Gavalas-Medici and Adey studied the effects of exposures to low intensity, very high frequency (VHF-147 MHz) electromagnetic fields, amplitude-modulated at biologically relevant frequencies (1–25 Hz) on cats with chronically implanted electrodes. To minimize interference with VHF fields due to behavioral responses and/or gross body movements, cats underwent pattern conditioning of specific brain locations. This was accomplished by directly conditioning specific patterns in specific brain locations which would then allow consideration of the overt behavior as a correlate of the conditioned response (24). The authors found that low level VHF, amplitude-modulated at specific frequencies, produced marked effects on conditioned specific brain rhythms (enhanced regularity of patterns, sharpening of the spectral peaks around the central frequency of the response, extremely prolonged resistance to extinction). This work brought attention to the realm of non-thermal biological response to EMF by showing changes in brain wave patterns. Up until this time, much of the work had been accomplished by Russian and Eastern European investigators, although Gavalas *et al*., 1970, may have been first to report changes in brain electrical activity (23,24). Building on their previous work, in 1975, Bawin *et al.* identified enhanced calcium efflux from chick brain tissue in a test tube following exposure to amplitude-modulated (AM) radio frequency (RF) waves. This effect appeared to occur without involvement of heating and appeared to be mediated by release of calcium. Specifically, the radiofrequency-dependent calcium efflux from chick brains was only reported when a carrier wave (147 MHZ) was sinusoidally amplitude-modulated (see Figure 2 for example of amplitude modulation) at specific frequencies of 6, 9, 11, 16, and 20 Hz. No altered efflux compared to control was found without modulation nor at 0.5., 3, 25 or 35 Hz modulations

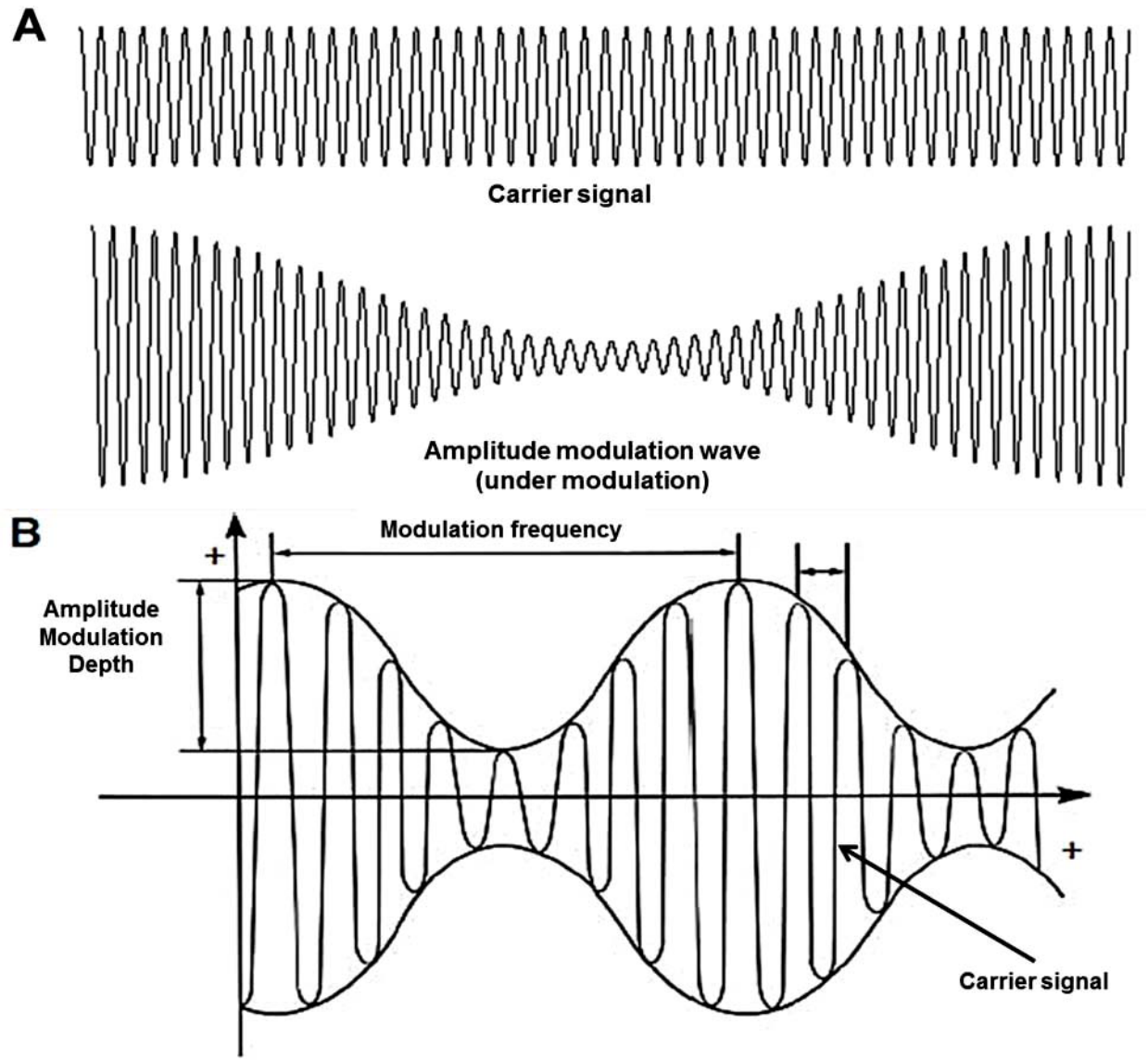

Figure 2. A & B. Radio frequency carrier signal as a function of time (horizontal axis). A. The physical appearance of an unaltered carrier signal (i.e. a radiofrequency wave) and amplitude-modulated carrier signal. B. An amplitude-modulated carrier signal showing modulation depth and frequency post modulation (29–31).

(25). Another, more limited report by the Bawin group show that 450 MHz EMF, amplitude modulated at 16 Hz, enhanced calcium release in a narrow window of intensities (26). Additionally, when chick brains were co-treated with cyanide (a compound which prevents electron transport in the cytochrome, shutting down metabolism) calcium efflux still occurred, which indicates that amplitude modulation-dependent calcium efflux does not depend on metabolic processes. These findings provided the experimental basis that suggested a molecular mechanism explaining Bawin *et al*.'s (1973) work on the inhibition and excitation of the cerebral cortex of cats, as well as the work of Hamer, Gavalas, and Subbota (22,25,27,28).

On the heels of Bawin's work, the focus began to turn to the molecular mechanism behind EMF exposure effects on biological systems and the understanding that EMF demodulation could explain such a mechanism.

In 1980 Blackman *et al*. independently reproduced the work of Bawin *et al.* by showing that calcium efflux from chick brains occur in a windowed, sinusoidally amplitude-modulated, frequency-specific fashion (32). Moreover, Blackman built on the research performed by Bawin *et al.* and showed that calcium efflux depends on specific amplitude-modulations independently of the carrier wave (50 MHz). Importantly,

Table 1. Summary of RF treatment modalities used in oncology

Modality	Indications/Mode of delivery	Mechanism of Action
Radiofrequency Ablation (37–39)	Predominantly used for the treatment of liver metastases. Treatment is administered during surgical procedure.	Tumor necrosis via thermal ablation
Novocure (55,73,74)	Treatment of glioblastoma following tumor resection and radiation therapy. Treatment is administered for 18 hr daily by means of electrodes glued to the skin.	Mitotic spindle disruption in proliferating cancer cells. Exact molecular mechanism is unknown
Therabionic (31,49,52)	Treatment of advanced HCC with effect on the primary tumor and its metastases. Treatment is administered 3 hr daily by means of a spoon-shaped antenna placed in the patient's mouth that delivers EMF to the entire body	Direct anti-proliferative effect of cancer cells. Mitotic spindle disruption. Exact molecular mechanism is unknown

Blackman *et al.* validated specific "modulation frequency windows" at which calcium efflux occurred. They also demonstrated that such effects only occurred within certain levels of power exposure and were amplitude-modulation dependent; an effect first identified by Bawin *et al* (1975) (25,33,34).

In 1984 Dutta *et al.* published work focusing on calcium ions and their relationship to microwave radiation (915 MHz) with or without sinusoidal, amplitude-modulation (80%) at 16 Hz at various specific absorption rates (SAR). They found that in human neuroblastoma cells (IMR-32) calcium efflux occurred in a power and amplitude-modulation frequency-specific fashion. Specifically, a significant efflux of calcium ions was found to occur at two SAR values 0.05 and 1 mW/g of an amplitude-modulated (16Hz AM) microwave (915 MHz-carrier wave) compared to unexposed samples further validating the work of Bawin, Adey, Blackman, and Joines (35). An additional validation of this phenomenon was provided in 1990 by Schwartz who reported enhanced calcium ion release from isolated, beating frog hearts only when they were exposed to 240 MHz EMF, sinusoidally amplitude modulated at 16 Hz, but not when exposed at 0.5 Hz nor when the EMF was unmodulated (36).

The research and development of EMF in biological systems has now spanned over 100 years, from d'Arsonval to World War II to calcium efflux, and now we are beginning to see the rise of therapeutic non-thermal, non-ionizing EMF exposure. In this review we highlight some of the most innovative and promising therapeutic research currently being performed in the field of cancer.

4. MINIMALLY-INVASIVE RF EMF FOR THERAPEUTIC USE IN CANCER

4.1. Nano-Radio-Frequency Ablation (NaRFA)

Non-ionizing radio frequency (RF) radiation is a common thermal therapy approach used in clinical oncology (37). In particular, radiofrequency ablation's (RFA) approach of hyperthermia (temperatures above 47°C) will expose target tissue to high temperatures to destroy the tissue directly or render cancer cells more susceptible to other treatment modalities (thermal sensitization; 41–45°C) (37) (Table 1). While this technique does show success, RFA is a localized and invasive method that requires a needle to penetrate directly into the tumor. Even though this technique is very effective and widely used, specifically for the treatment of hepatic (primary or metastatic), kidney, liver and a number of other neoplasms, this indication is limited by tumor size and tumor location (38, 39). Close proximity of the tumor to the biliary tree or blood vessels is considered a contraindication to its use. Moreover, targeting a localized tissue and selecting an appropriate or efficient method of heat delivery remains an issue. Multiple sources of energy for heat delivery include microwaves, radiofrequency, laser and ultrasound (37). Here we briefly summarize a few novel uses of radio frequency exposure as a method of localized RFA using nano-particles.

Nano-Radio-Frequency Ablation (NaRFA) is an experimental method of non-invasive RFA with the potential to improve the efficacy of thermal damage to tumors while minimizing damage to normal healthy tissues. In order to accomplish this, tumors are loaded with nanoparticles that enhance the conversion of external energy source (RF) into heat, creating an inside-out hyperthermia. This is possible because RF fields are able to penetrate deep into the body without the need for an invasive procedure (37). One such example of NaRFA is carbon based nanomaterials. Single-walled carbon nanotubes (SWNTs) can be modified to increase efficacy by improving specificity through surface engineering of SWNTs to have ligands, which can target receptors specific to cancer cells (40,41). In a study performed by Gannon *et al.* RF exposure of SWNT caused cytotoxicity of cancer cells *in vitro* and *in vivo* while being well tolerated by rabbits bearing tumors. A second example can be found in Carbon coated metallic nanoparticles (C-Co-NPs). C-Co-NPs are 7-nm cubic crystalline graphitic carbon decorated ferromagnetic cobalt nanoparticles (40). These nanoparticles have been shown to effectively enter HeLa cells and when exposed to RF

pulses of 350 kHz the nanoparticles generate localized heat, a process that is dependent on RF power and nanoparticle concentration. The treated HeLa cells showed DNA fragmentation, nucleus rupturing and membrane disintegration (40). An additional example is found in the work of Tamarov *et al.* and their use of crystalline silicon (Si) based nanomaterials. An aqueous suspension of Si nanoparticles is able to generate temperatures above 45–50 ^{o}C when exposed to 27 MHz RF EMF (41). Moreover, Si nanoparticles are biocompatible and biodegradable in biological tissues, decaying into orthosilicic acid, $Si(OH)_4$, which will be voided through the urinary tract. *In vivo* work displayed inhibition of tumor growth and led to a decrease in tumor volume (42).

4.1.1. Mechanism of Action for Nano-Radio-Frequency Ablation (NaRFA)

Heat generation in nanoparticles when exposed to low RF fields remains a contentious subject (37, 43). The dominant mechanism behind RF heating is joule heating (heat released due to resistivity of nanoparticle; Power (P) is dissipated in the form of heat, and given I is electric current and R is resistance, $P = I^2R$). No optimal RF conditions for effective heating have been reported and power has been reported to range from 40–800 W (37, 40, 44). A number of other factors such as electrical conductivity, size, shape, and concentration of the nanoparticles contribute to heating effects as well (37). While systems such as NaRFA appear to be promising it should be noted that there is a need for more *in vivo*, clinical data as well as technological refinement to reduce unwanted tissue damage and increased specificity of the nanomaterial to the target (37, 40). Moreover, there are currently at least two other separate RF technologies in existence that have no need for nanoparticles and have shown beneficial activity in cancer patients.

4.2. TheraBionic™: Tumor-Specific AM RF EMF

During the 1990's Pasche *et al.* demonstrated that intrabuccal administration of low and safe levels of 27.12 MHz RF EMF, amplitude-modulated at 42.7 Hz, has a sleep-inducing effect in healthy patients but does not improve sleep in patients with a diagnosis of insomnia (45,46). However, when patients with a diagnosis of insomnia were treated with the same carrier signal amplitude-modulated at four different frequencies (2.7 Hz, 21.9 Hz, 42.7 Hz and 48.9 Hz; i.e. insomnia-specific modulation) they experienced shorter sleep latency, longer total sleep time, increased sleep efficiency, and increased numbers of sleep cycles compared to controls (47,48). In early 2000, Pasche and Barbault hypothesized that tumor-specific modulation frequencies could be used to treat cancer. In 2009, Barbault *et al.* published the results of their investigations to determine if tumors may be sensitive to specific RF EMF modulated at specific frequencies. Using devices emitting a carrier frequency of either 433 MHz or 27 MHz the authors exposed 163 patients, who had a diagnosis of cancer, to RF EMF amplitude-modulated in the range of 0.1Hz to 114 kHz and the results of the study were remarkable (49). The authors reported that patients with cancer, but not healthy patients, had changes in skin electrical resistance, pulse amplitude and blood pressure (biofeedback responses) when exposed to a subset of very discrete modulation frequencies. Interestingly, patients with the same tumor type were found to exhibit biofeedback responses when exposed to the same discrete modulation frequencies creating a frequency set specific to tumor type. Moreover, the majority of frequencies found for any given tumor type were specific to that tumor type only and only 4 frequencies (1873.5 Hz, 2221.3 Hz, 6350.3 Hz and 10456.4 Hz) were found to overlap in multiple tumor types specifically, breast cancer, hepatocellular carcinoma, prostate cancer and pancreatic cancer (49). Post frequency identification, the authors then proposed to determine if treatment with the recently discovered tumor-specific frequency sets to corresponding cancer patients would have a therapeutic effect and hence the authors offered compassionate treatment to patients with limited therapeutic options. Again, the results were remarkable, of sixteen patients evaluable for response, one patient with hormone-refractory breast cancer metastatic to the adrenal gland and bones had a complete response lasting 11 months. One patient with hormone-refractory breast cancer metastatic to liver and bones had a partial response lasting 13.5 months, Four patients had stable disease lasting more than: 7 years (thyroid cancer metastatic to the lung), 5.1 months (non-small cell lung cancer), 4.1 months (pancreatic cancer metastatic to liver) and 4.0 months (leiomyosarcoma metastatic to liver) (15, 49). These results indicate that treatment not only has an impact on the primary tumor but can also treat metastatic tumors implying that this treatment is systemic.

Building upon their findings from 2009, Costa *et al.* conducted a single-group; single-center, open-label, phase I/II study in patients with advanced hepatocellular carcinoma (HCC). In this study, more than 75% of patients had radiological evidence of disease progression and half of the patients had poor liver function with limited treatment options at the time of treatment initiation. All patients were exposed to electromagnetic fields amplitude modulated at HCC-specific frequencies. A total of 41 patients with advanced HCC and Child Pugh A or B disease were accrued and self-administered treatment three times daily for 60 minute (180 min) until disease progression or death and imaging studies were obtained every eight weeks. The results supported the initial experience with the same device: four patients had objective tumor response. One patient with

prior progressive disease experienced durable near complete response lasting more than 5 years, and fourteen patients had stable disease for more than 6 months. The median progression-free survival (PFS) was 4.4 months (95% CI: 2.1–5.3) and median overall survival (OS) was 6.7 months (95% CI: 3.0–10.2). Subset analysis of the patients with similar diagnostic criteria as those applied in phase III studies such as the SHARP and Asia-Pacific sorafenib studies (Llovet *et al.*, 2008;Cheng *et al.*, 2009), i.e. biopsy-proven disease and assessment of disease with CT, shows an objective response rate (RR) by RECIST of 18.2.% (2/11), and median PFS and OS of 4.9 months (95% CI .6 to 10.8 months) and 10.8 months (95% CI 2.1 to 34.0 months) (50,51). Overall, there were six long-term survivors with an OS greater than 24 months and four long-term survivors greater than three years. Despite long-term treatment duration and poor liver function in the majority of patients, treatment was well tolerated and no NCI grade 2, 3, or 4 toxicities were reported (31).

To further evaluate the results found by the work of Barbault *et al.* (2009) and Costa *et al.* (2011), Zimmerman *et al.* (2012) performed *in vitro* studies to begin to elucidate the mechanism of this novel therapy. Using specifically designed exposure devices to replicate the clinical exposure settings, Zimmerman *et al.* investigated whether the proliferation of HCC (HepG2 and Huh-7), breast cancer (MCF-7) and corresponding non-malignant THLE-2 (represent normal liver cells), MCF-10A (represent normal breast cells) cell lines would be affected by the tumor-specific modulation RF EMF that were found in the clinical setting. Using tumor-specific RF EMF vs control exposure, comprised of randomly chosen modulation frequencies within the same range as cancer-specific frequencies, authors found that tumor-specific frequencies were able to inhibit the proliferation of cancer cells when used in a corresponding fashion i.e. HCC-cell lines exposed to HCC-specific frequencies. Yet, when HCC-specific frequencies were used on breast cancer cells, or vice versa, no proliferative inhibition was noted. Furthermore, HCC-specific and breast cancer-specific frequencies did not inhibit the proliferation of THLE-2 or MCF-10A cells, respectively. These findings led to the conclusion that exposure to tumor-specific RF EMF not only had an inhibitory effect on the proliferation of cells but that it did so in a cancer-specific fashion, apparently not affecting normal healthy cells (15,52). The authors also discovered that mitotic spindle formation was greatly disrupted in HCC-specific treated HepG2 cell and genes relating to migration (*PLP2)* and invasion (*XCL2)* were found to be significantly downregulated as shown via RNA-seq and confirmed by qPCR (52). The exact mechanism of action of this new therapeutic approach is unknown.

4.3. Novocure™

NovoTTF-100A (brand name Optune®) is a device that delivers low-magnitude (1–3 V/cm), intermediate frequency (100–300 kHz) tumor treating electric fields (TTFields) by transducer arrays that are applied directly to the scalp (53–55). The Novo TTF-100A system slows tumor growth and inhibits mitosis. More specifically, TTFields have been shown to disrupt glioblastoma cells during mitosis, resulting in apoptosis, aneuploidy, asymmetric chromosome segregation, and defects in centrioles and mitotic spindles. Additionally, TTFields causes cytoplasmic stress which targets tumor cells for immunological destruction and clearance TTFields have been demonstrated to inhibit proliferation in multiple cancer cell lines, e.g., human melanoma, lung, prostate, pancreas, breast and glioma after 24 hours of continuous exposure while not having any impact on normal non-dividing cells (53, 54). In addition, mice bearing tumors (mouse melanoma and rat glioma) also showed growth inhibition and a decrease in angiogenesis after less than one week of treatment.

Optune® is approved by the U.S. FDA for use as a treatment for adult patients with histologically-confirmed glioblastoma (55). The activity of TTFields is intensity and frequency specific and is inversely proportional to tumor cell size. Hence, the NovoTTF device can be optimized for multiple tumor types such as pancreas adenocarcinoma, ovarian cancer and non-small cell lung cancer (54). Of importance, the device and treatment is considered to be safe as normally dividing cells would require a different frequency set to have mitotic interference making the TTFields specific to dividing cancer cells (54). Due to the effects of the TTFields being directional (TTF fields function best when applied in the direction of the separation axis of the dividing cell) two sequential field directions are applied to patients by wearing two pairs of transducer arrays that generate fields that switch direction by 90°. Lastly, TTFields do not attenuate over distance(s) used in treatment and are minimally impacted by biological tissues. This gives TTFields the capability to cover large body regions that may be commonly affected by metastases deep within organs, so long as the leads are placed over the metastatic area. The clinical recommended treatment time is a minimum of 18 hours continuous treatment per day (54).

In 2014, the Data Safety Monitoring Board recommended that the Phase III clinical trial of this device in patients with newly diagnosed glioblastoma be stopped after it was reported that during the interim analysis of 315 patients who received standard chemoradiation therapy, adding TTFields to maintenance temozolomide chemotherapy resulted in significant improvements in progression-free survival (PFS) and overall survival (OS) (56). Specifically,

median PFS in the intent–to–treat population was 7.1 months (95% CI, 5.9–8.2 months) in the TTFields plus temozolomide group and 4.0 months (95% CI, 3.3–5.2 months) in the temozolomide alone group; hazard ratio (HR) 0.62; (98.7% CI, 0.43–0.89); P = 0.001). The median overall survival in the per-protocol population was 20.5 months (95% CI, 16.7–25.0 months) in the TTFields plus temozolomide group (n=196) and 15.6 months (95% CI, 13.3–19.1 months) in the temozolomide alone group (n=84); HR 0.64 (99.4% CI, 0.42–0.98); P=0.004) (56).

4.3.1. Novocure™ TTF (Tumor treating fields) Mechanism

The use of intermediate-frequency electric fields (kHz-MHz range) alternate too fast to cause nerve-muscle stimulation and involve a minimal amount of heating; until the mid-2000's fields in this range were generally accepted as having no biological effect (54, 57). The mechanism of action involves destabilization of spindle microtubules, consequently leading to mitotic catastrophe. It is unknown whether this effect occurs by direct interference of the addition of tubulin subunits to microtubules or by destruction of existing microtubules structures (57). Hence, cells entering mitosis are those most likely to respond to treatment and would exclusively impact dividing cells (54, 57). Additionally, after spindle disruption by TTFields, and the prolonged mitotic arrest that may occur, subsequent cell death is more likely the outcome than mitotic arrest and yet it is still not understood what initially triggers the caspase dependent apoptosis. Data generated by Giladi *et al.* suggests that the accumulation of significant aneuploidy, in tandem with mitotic arrest, contributes to the compromised viability of cancer cells (57).

5. NOVOCURE™ AND THERABIONIC™: TWO NOVEL MODALITIES FOR CANCER TREATMENT

The mechanism(s) of action for RF EMF on biological systems beyond heating have not been fully established. Hence, we discuss published research that will have important biological relevance to the likely mechanistic difference that may underlie two distinct therapeutic options offered by Novocure's TTF-100A (Optune®) and the Therabionic™ device.

5.1. Relevant Literature for Novocure/Optune®

Novocure™'s Optune® identified the improper formation of microtubules as key to the inhibitory action on GBM. In literature relevant to the intracellular mechanics of centrioles the presence of electromagnetic forces is evident (58). Microtubules are hollow cylinders composed of 13 longitudinal filaments. The filaments are strings of alpha/beta tubulin dimers connected end to end with the alpha/beta tubulin dimers having positive and negative charges at their ends. During filament movement, by means of their vibration, oscillation of these charged dimers produces an electromagnetic field (58). Evidence of cellular electromagnetic field activity occurs during mitosis when centriole pairs are separated to diametrically opposite sides of the nucleus and extend out microtubules toward each other to begin the separation the cell in two (58). This electromagnetically active area certainly appears to continue to be a prime target for Novocure™'s treatment and future research.

5.2. Relevant Literature for Therabionic™

The work described in the 2009 Barbault *et al.* paper reports that discovery of frequencies, used in the treatment of cancer, was based on the measurement of variations in skin electrical resistance, pulse amplitude and blood pressure (49). Calcium (Ca^{2+}), Ca^{2+} signaling and Ca^{2+} channels are an important feature of blood pressure regulation and cardiovascular health. Here we highlight the work performed by Buckner *et al.* that potentially sheds light on the work related to calcium ion channels (59, 60). The studies performed by Buckner *et al.* show that exposure to a specially designed, weak (2–10 μTesla), frequency-modulated, patterned EMF signal called the Thomas-EMF signal, can inhibit the growth of malignant cells by promoting Ca^{2+} uptake through T-type voltage-gated calcium channels (VGCC) (61). This effect does not appear to be mediated by L-type voltage-gated calcium channels (61). The Thomas-EMF pattern is a digital file composed of 849 points programmed to deliver each point for 3 milliseconds. Exposure to the Thomas-EMF pattern at various time intervals has been previously associated with an analgesic response, an outcome whose mechanism was suspected to be due to or include metal binding ions (Ca^{2+} and K^{+}) (62). Additionally, the Thomas-EMF pattern was designed to affect membrane activity associated with epileptic seizures, a disease state known to be related to alterations in various types of ion channels (Ca^{2+}, K^{+}, Na^{+}, GABA) (61–63). Buckner *et al.* exposed cultured cells of mouse and human origin (B16-BL6, MDA-MB-231, MCF-7, HSG, HBL-100, HEK293 and HeLa) or mice (bearing tumors from hind flank injected B16-BL6 cells) to Thomas-EMF signal (2–10 microT). Proliferative inhibition was found to occur in malignant cells only, e.g., MDA-MB-231, MCF-7 and HeLa cells and in mice bearing tumors whereas non-malignant cells, e.g., HBL-100, HEK293 and HSG cells were unaffected. In attempting to understand the mechanism for proliferative inhibition Buckner *et al.* reported that in malignant cells an increase in Ca^{2+} influx occurred, specifically through the T-type VGCC while in non-malignant cells no increase in Ca^{2+} influx was found. Moreover, blocking Ca^{2+} influx with T-type VGCC

blockers appeared to block the ability of Thomas-EMF signal to inhibit cell proliferation. Additionally, malignant cells, exposed to Thomas-EMF signal, also showed a slowed entry into the S-phase of the cell cycle as noted by temporal changes in cyclin expression but, did not show cell death or DNA fragmentation (61). Hence, Buckner *et al.* concluded that specific EMF patterns can affect biological systems by allowing for increased cytoplasmic Ca^{2+} which then impacts the cell cycle by changes in cyclin expression (61, 64, 65). This provides a potential anti-cancer therapy that acts through the T-type VGCC to allow inappropriate influx of Ca^{2+} resulting in proliferative inhibition (61).

The research reports published by Buckner *et al.* appear to have relevance to Therabionic's cancer treatment, particularly given the fact that their initial focus of work was on the treatment of insomnia, a disease state that can be mediated by Ca^{2+} and T-type VGCC dysregulation (48, 66, 67). Moreover, enhanced Ca^{2+} flux has been shown to be affected by RF exposure in research that dates as far back as the 1970s and in a modulation specific fashion (68–72). Hence, hypothetically, amplitude-modulated RF exposure eliciting a calcium dependent anti-cancer specific response could represent a promising, if not paradigm changing, direction in the treatment of cancer (30).

6. CONCLUSION

In closing, with the number of tumor types currently under investigation with the Novocure™ technology combined with the tumor types in which Therabionic™ has already shown some efficacy, treatment, either local or systemic, of tumors with electromagnetic fields should still be considered in its infancy. Moreover, as a field of research, we expect that these technologies will quickly expand over the next ten years to become possibly as common place as chemotherapy with the hope that at the very worst it will allow cancer to become a chronic condition instead of a life-ending disease.

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