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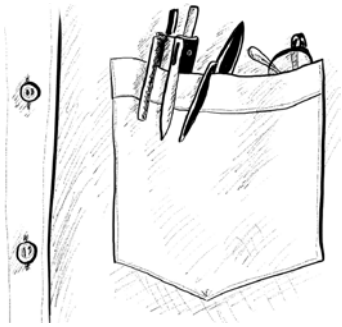

TRIALS & TRIBULATIONS

How tumor-specific modulation frequencies were discovered

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In the spring of 2001, I visited a longtime friend and collaborator, Alexandre Barbault, to share with him my vision of using low levels radiofrequency electromagnetic fields for the treatment of cancer.

We had worked together for the previous 15 years, developing a medical device emitting low levels 27 MHz radiofrequency electromagnetic fields with the goal to treat insomnia. This work had been fruitful, as we had identified specific modulation frequencies with a sleep-restoring effect in humans(*1-3*).

Despite clinical evidence of efficacy from a multicenter randomized study conducted in the US that included 106 patients with chronic insomnia(*4*), there were lingering concerns about the long-term toxicity of radiofrequency electromagnetic fields because of the emerging cell phone controversy in the 1990s, which suggested that long-term use of cell phones was associated with increased cancer risk, especially with respect to brain tumors.

Symtonic, the company built around this technology, was not able to find partners to bring this device to the market as most pharmaceutical companies were concerned about the long-term liability of a novel technology making use of radiofrequency electromagnetic fields.

Following completion of my clinical training in Hematology/Oncology at Memorial Sloan Kettering Cancer Center and a postdoctoral fellowship in the laboratory of Joan Massagué at Sloan Kettering Institute, I had garnered peer reviewed funding from the National Cancer Institute and moved to Northwestern to develop my own laboratory research focusing on TGF-ß and cancer susceptibility.

Reflecting on the work conducted with Alexandre Barbault, I postulated that specific modulation frequencies could target tumor growth. This hypothesis was based on our own previous work identifying specific modulation frequencies with a sleep-inducing effect in patients with a diagnosis chronic insomnia, but not in patients without sleep problems.(*5*)

This hypothesis was further supported by the pioneering work of Drs. Ross Adey and Carl Blackman, who had identified and validated in mammalian models the so-called "window effect," which resulted in calcium efflux in mammalian models exposed to low levels radiofrequency electromagnetic fields when amplitude modulated at specific frequencies. This effect did not occur with unmodulated radiofrequency electromagnetic fields or when the radiofrequency electromagnetic fields were amplitude modulated outside these windows.(*6-8*)

These findings were consistent with the existing scientific literature at the beginning of the 21st century indicating that mammalian cells were insensitive to athermal radiofrequency electromagnetic fields, i.e. radiofrequency electromagnetic fields that did not result in any measurable heating of a biological system. However, Adey and Blackman discoveries strongly suggested that low levels of radiofrequency electromagnetic fields could affect calcium flux in brain cells, but only when the fields were amplitude modulated at specific frequencies.

As a freshly trained oncologist, I had become aware that most chemotherapy drugs had serious toxicity, which was considered acceptable given their potential to control disease progression and extend life. I also realized that the toxicity profile of chemotherapy was far more concerning than the hypothetical long-term risk of exposure to low levels of radiofrequency electromagnetic fields. I concluded that assessing the potential antitumor effects of low levels radiofrequency electromagnetic fields would be a clinically attractive and acceptable option, especially for patients with limited treatment options.

I asked Alexandre Barbault whether he would be willing to test this hypothesis with me and embark on a new adventure assessing the potential of this approach for the treatment of cancer. He agreed, and we decided to give ourselves three years to determine whether this postulate was worth pursuing or not. We also agreed that we would fund these studies ourselves.

In December 2001, Barbault and I met in Switzerland and started examining patients with a diagnosis of cancer by exposing them to low levels radiofrequency electromagnetic fields, which were amplitude modulated from 0.1 Hz to more than 1 kHz.(*5*) A proprietary methodology was used to identify cancer specific frequencies, employing the evaluation of the patient's pulse pressure, the difference between the systolic and diastolic blood pressure, during exposure to amplitude modulated radiofrequency electromagnetic fields.(*9*)

Correlations between hemodynamic parameters and radiofrequencies defined specific frequencies. We discovered that changes in pulse pressure in patients with a diagnosis of cancer were predominantly identified at modulation frequencies above 1000 Hz. These findings prompted the design and development of novel emitting devices with a signal synthesizer of high precision as our initial emitting devices lacked precision at higher frequencies. These new devices were equipped with a Direct Digital Synthesis (DDS) based synthesizer with a frequency precision of 10^{-7} and were developed in collaboration with Niels Kuster at the Swiss Federal Institute of Technology in Zurich, Switzerland.(*9*)

Using this new equipment, we found that patients with the same tumor type, i.e. breast cancer or hepatocellular carcinoma, exhibited reproducible hemodynamic changes in pulse pressure when exposed to the same frequency modulations. Specifically, 78 percent of the 1024 frequencies discovered were tumor-specific, i.e. hemodynamic changes were only detected in patients with the same tumor type, irrespective of their age, gender, and ethnic status. The remainder of the frequencies were not tumor-specific, i.e. changes were detected in patients with different primary tumors. These findings suggested the existence of a tumor frequency profile, like the gene expression profile identified in many tumor types.

Having gathered experimental evidence that patients with a given tumor type exhibit hemodynamic changes in pulse pressure when exposed to specific modulation frequencies, we tested the hypothesis that administration of these frequencies could be used as a novel cancer treatment. We designed a feasibility study in which 28 patients with advanced cancer and limited therapeutic options were offered compassionate treatment with an experimental device emitting 27 MHz radiofrequency electromagnetic fields, which were amplitude modulated at the same specific frequencies identified in patients with the same primary tumor type, i.e. frequencies previously discovered in patients with breast cancer were used to treat patients with a diagnosis of breast cancer.(*9*)

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All patients had discontinued any other anticancer therapy for at least 4 weeks prior to treatment with radiofrequency electromagnetic fields. The output of the device was adjusted to 100 mW into a 50 Ohm load using a sinusoidal modulated test signal. Treatment consisted of 27 MHz radiofrequency electromagnetic fields, which were sinusoidally amplitude modulated for 3 seconds at each of the tumor-specific frequencies previously discovered in patients with the same tumor type.

A spoon-shaped antenna was connected to the battery-powered device and the spoon was placed on the anterior part of the patient's tongue for treatment (Fig. 1). Treatment was administered for 60 minutes 3 times a day until progression of disease. Sixteen of the 28 patients enrolled in the study could be evaluated for response according to the RECIST criteria(*10*) and all imaging studies were independently reviewed by Drs. Brad Bottger and Reggie Munden, two U.S. board certified radiologists.

The results were encouraging. One patient with hormone refractory stage IV breast cancer metastatic to bone and the adrenal gland had a complete response lasting 11 months. Another patient with hormone refractory stage IV breast cancer metastatic to the liver and bone had a partial response lasting 13.5 months. Five additional patients had stable disease for at least 4 months. One of them with thyroid cancer metastatic to the lungs had stable disease for 7 years. This patient is still alive and receiving daily treatments with the device as of October 2018, more than twelve years after enrolling into the study.(*9, 11*)

Importantly, treatment was well tolerated with grade 1 fatigue and grade 1 mucositis being the only side effects reported, even after years of treatment. These results demonstrated that low levels of amplitude modulated radiofrequency electromagnetic fields administered by means of a spoon-shaped antenna placed in the patient's mouth had a systemic effect in patients with advanced cancer, were well tolerated, and could be easily administered by the patients themselves in the comfort of their home.

Figure 1: Patient receiving treatment with the TheraBionic P1 device

These exciting results led Frederico Costa, a former Sloan-Kettering colleague of mine, to propose a trial testing the safety and effectiveness of the therapy in patients with a diagnosis of advanced hepatocellular carcinoma, a group of patients with limited therapeutic options. Using Barbault and Pasche's newly developed medical devices and hepatocellular carcinoma specific frequencies discovered by Barbault and Pasche, Pasche and Costa designed and Costa conducted a phase I/II study in patients with advanced hepatocellular carcinoma and limited therapeutic options.(*12*)

The study was conducted at the University of São Paulo, Brazil, which was a major site for the recruitment of patients in the Sorafenib Hepatocellular Carcinoma Assessment Randomized Protocol (SHARP) registration study,(*13*) which led to the approval of sorafenib for the treatment of advanced hepatocellular carcinoma. The TheraBionic phase I/II study was run in parallel with the SHARP study and

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enrolled patients with Child Pugh A or B advanced hepatocellular carcinoma and limited therapeutic options. Prior systemic treatment with chemotherapy or sorafenib was allowed.

The results of this study were also compelling. Similar to the findings of the feasibility study,(9) treatment with amplitude modulated radiofrequency electromagnetic fields was well tolerated, even after several years of continuous treatment, and there were no NCI grade 2, 3 or 4 toxicities. The study met its primary efficacy end point, which was progression free survival equal or greater than 6 months in 20 percent of patients.

Indeed, 14 (34.1%) of the 41 patients enrolled in the study had stable disease for more than 6 months. Median progression free survival was 4.4 months and median overall survival was 6.7 months. One patient previously enrolled in the SHARP study(13) and with evidence of disease progression at the time of enrollment, remained on therapy with a near complete response for 5 years and two months prior to expiring to causes unrelated to her malignancy.(11, 12)

There were four partial responses resulting in a 9.8 percent response rate, which were independently reviewed by Desiree Morgan, a U.S. board certified radiologist. Drs. Al Benson from Northwestern and Leonard Saltz from Sloan-Kettering reviewed the data and were impressed by the single agent activity of amplitude modulated radiofrequency electromagnetic fields in these patients. We compared these results with those by Abou-Alfa et al.(14) who conducted a large phase II study assessing the effects of sorafenib in patients with HCC and Child–Pugh A and B who had not received previous systemic treatment.

Abou-Alfa et al. observed partial responses using the WHO criteria in 2.2 percent of patients. This compares to 9.8 percent with the TheraBionic device, which is an over fourfold higher percentage. Investigator-assessed median time to progression in the sorafenib study was 4.2 months, and median OS was 9.2 months. Of note, all 137 patients from that study had evidence of disease progression after 14.8 months. At the same time point, four (9.8%) of the patients enrolled in the TheraBionic study did not have evidence of disease progression.

These findings suggest that treatment with the TheraBionic device may increase the time to radiological progression in advanced HCC. Importantly, the ratio of Child-Pugh A patients vs. Child-Pugh B patients was higher in the Abou-Alfa (2006) study than in the Costa et al. (2011) study. Thus, better outcome in the Costa et al. (2011) study cannot be attributed to better general physical condition.

In 2007, Barbault and I filed a patent application entitled "Electronic system for influencing cellular functions in a warm-blooded mammalian subject", which described the novel device we had developed as well as the tumor-specific frequencies we had identified. The same year, we established TheraBionic LLC to further develop our novel technology.

In 2008, I moved from Northwestern to the University of Alabama at Birmingham to become chief of the Division of Hematology/Oncology and associate director for translational research at the UAB comprehensive Cancer Center. In 2009, the results from our feasibility study were published and attracted the attention of Jackie Zimmerman, a UAB MD/PhD student who was interested in undertaking her graduate work in my laboratory.

Despite my suggestion to focus on projects related to TGF-ß, which were funded by two separate R01 awards from the National Cancer Institute, Zimmerman insisted on studying the biological effects of amplitude-modulated radiofrequency electromagnetic fields in cancer. I explained to her that we had not yet uncovered any evidence of in vitro activity on tumor cells. Based on the mode of discovery of tumor specific frequencies, my hypothesis was that systemic administration of these frequencies was a prerequisite for antitumor effect and that we might not observe any direct antitumor effects on cancer cells.

We both agreed that the only way to test this hypothesis was to create an in vitro exposure model replicating the in vivo conditions. Working closely with Ivan Brezovich, director of the Medical Physics Division in the UAB Department of Radiation Oncology, we developed a system for in vitro exposure replicating human exposure.(15)

Within a few months, Zimmerman generated experimental evidence that breast cancer modulation frequencies inhibited the proliferation of the MCF-7 breast cancer cell line. This "reverse translational work" testing the antitumor effects of modulation frequencies identified in patients with a diagnosis of cancer was expanded to other cancer cell lines using both corresponding and non-corresponding tumor-specific frequencies as well as randomly chosen frequencies.

Zimmerman and collaborators demonstrated that the proliferation of breast cancer cells was inhibited by breast cancer specific modulation frequencies. Similarly, proliferation of hepatocellular carcinoma cells was inhibited by hepatocellular carcinoma specific frequencies. Breast cancer specific modulation frequencies, however, did not affect the proliferation of hepatocellular carcinoma cells and vice versa. Additionally, randomly chosen modulation frequencies did not affect the proliferation of either breast cancer cells or hepatocellular carcinoma cells. Furthermore, tumor-specific modulation frequencies did not affect the growth of noncancerous cells.

Michael Pennison, another graduate student in my laboratory, asked the question whether amplitude modulated radiofrequency electromagnetic fields would disrupt the mitotic spindle of tumor cells, similarly to the mechanism of action of the tumor treating fields technology developed by Yoram Palti and collaborators.(*16, 17*) He found that there was pronounced disruption of the mitotic spindle of hepatocellular carcinoma cells after exposure to amplitude modulated radiofrequency electromagnetic fields.(*15*)

The work of Zimmerman and Pennison has been significantly expanded by Hugo Jimenez, whose work has dissected the mechanism of action of amplitude modulated radiofrequency electromagnetic fields both in vitro and in vivo using a custom-designed small animal model exposure system, which replicates in mice the same levels of exposure as when patients use the TheraBionic device.(*18*)

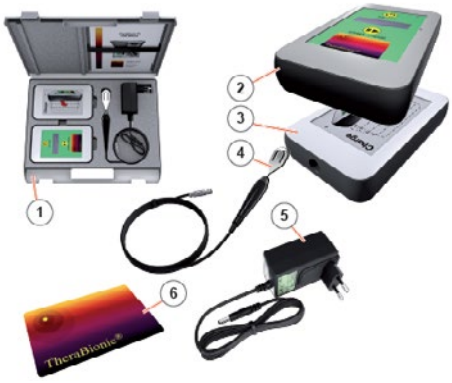
In 2013, Barbault and I founded TheraBionic GmbH in Ettlingen, Germany with the goal to develop and produce a medical device suitable for commercial use in Europe. Following the development of the OncoBionic P1 device, which was used in two clinical studies(*9, 12*), Barbault and I, with the assistance of Hans-Peter Völpel, the engineer who designed the current TheraBionic P1, conducted a critical analysis of our then existing OncoBionic P1 device. As a result of this analysis, the current TheraBionic P1 device was developed. Among the improvements incorporated into the TheraBionic P1 device (Fig. 2) are:

1. Avoidance of missed treatment time when the ohmic contact between the spoon-shaped antenna and the patient's oral mucosa is lost. The new TheraBionic P1 device constantly monitors the impedance of the coaxial cable ending with the spoon-shaped antenna placed on the anterior part of the patient's tongue. The device interrupts treatment and starts beeping whenever it detects a significant change in the impedance of coaxial cable ending with the spoon-shaped antenna. Treatment resumes as soon as the patient places the spoon-shaped antenna back on the tongue. This improvement addresses the need for continuous monitoring of treatment delivery ensuring that physicians will know the exact treatment time delivered between each visit. It also informs the patient if treatment is not being delivered appropriately and that the spoon-shaped antenna needs to be replaced on the patient's tongue.

2. Minimizing the risk of electrocution at all times. The new TheraBionic P1 device is made of two separate units, one docking station connected to the mains, which charges wirelessly the treatment unit. Hence, the risk of electrocution has been markedly reduced as the treatment unit is powered by a 5 V battery, which cannot cause any significant harm to the human body.

3. Optimization of the spoon-shaped antenna: The spoon-shaped antenna of the new TheraBionic P1 is permanently connected to the coaxial cable, which ensures optimal connection between the coaxial cable and the spoon-shaped antenna. The entire spoon-shaped antenna is a barcoded disposable unit, which can only be used by one single patient.

4. Minimizing the risk of uncontrolled treatment and providing accurate monitoring of treatments received. The TheraBionic P1 device is delivered to patients with 20 treatment hours so that treatment can be initiated as soon as prescribed by the physician. Additional treatments can only be received following reloading with an activation chip card, which adds 93 one-hour sessions. This provides a well-defined system to control the number of treatments, which can be traced with chip cards. Indeed, the physician will know exactly how many hours and minutes of treatment have been administered at each return visit. The number of hours and minutes of treatment administered is equal to the number of treatment hours loaded in the device (20 hours at the time of delivery, 113 hours after activation of 93 additional one hour treatment sessions, 206 hours after activation of 186 additional one hour treatment sessions, etc.) minus the number of hours and minutes left, which is displayed on the TheraBionic device whenever it is turned on.



1 **Device carrying case**

2 **Therapeutic device**

3 **Docking station**, for wireless charging of the therapeutic device

4 **Patient spoon**, of stainless steel with connecting cable to the therapeutic device

5 **Power supply**, to power the docking station

6 **Activation card**, for insertion into the docking station, allows activation time upload into the therapeutic device, void after data transfer

Figure 2: Components of the TheraBionic P1 medical device

Following the successful development of the novel TheraBionic P1 device, TheraBionic GmbH began the European registration procedure with Regina Müller overseeing the quality management systems. In July 2018, TheraBionic GmbH received European certification for the TheraBionic P1 device as a class II a (low risk) medical device for unmet medical needs according to the European MDD 93/42/EEC guidelines and ISO 13485:2016 quality managements systems regulatory requirements for medical devices. Production of the certified devices has begun and the first devices will become available for commercial use in Europe in October 2018.

The European regulatory approval is the first step towards the further development and expansion of this novel technology for the diagnosis and treatment of various tumor types using TheraBionic discoveries. Upcoming clinical trials will include randomized studies of the TheraBionic P1 device in the first-line and second-line treatments of advanced hepatocellular carcinoma in combination with current standard of care therapies. Additional studies will be launched to assess the safety and effectiveness of TheraBionic treatment in women with stage IV refractory breast cancer with or without brain metastases. Preliminary data generated by Sambad Sharma in the laboratory of Kounosuke Watabe at Wake Forest Baptist Comprehensive Cancer Center suggest activity in breast cancer brain metastases.

European regulatory approval is only the beginning of the large-scale clinical use of amplitude modulated radiofrequency electromagnetic fields, which may well usher a new era in oncology.

Disclosures: Boris Pasche is the cofounder of TheraBionic LLC, TheraBionic Inc., and TheraBionic GmbH. He holds stocks in TheraBionic Inc. and TheraBionic GmbH.

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