Diagnosis and treatment of cancer with very low levels EMFs modulated at tumor-specific frequencies

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COI: Boris Pasche has filed applications for patent protection and holds patents related to electromagnetic fields amplitude-modulated at tumor-specific frequencies as they relate to the diagnosis and treatment of cancer. He hold stocks in TheraBionic LLC and TheraBionic GmbH.
Patient-based discovery of tumor-specific frequencies

Clinical results

In vitro results

In vivo results

Conclusions
DISCOVERY OF CANCER-SPECIFIC MODULATION FREQUENCIES

Biofeedback methods identified changes in pulse pressure upon exposure to specific frequencies.

Pulse pressure changes occur at identical frequencies for patients with the same type of cancer.

Frequencies eliciting the best biofeedback responses, defined by the magnitude of increased amplitude and/or the number of beats with increased amplitude, were selected as tumor-specific frequencies.

Control individuals without a diagnosis of cancer do not exhibit changes in pulse pressure upon exposure to frequencies identified in patients with a diagnosis of cancer.

Patient with hepatocellular carcinoma – changes in pulse amplitude
# DISCOVERY OF TUMOR-SPECIFIC FREQUENCIES

Frequency discovery in 163 patients with a diagnosis of cancer

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>Number of patients</th>
<th>Number of frequency detection sessions</th>
<th>Number of frequencies</th>
<th>Tumor-specific frequencies Nb and (%)</th>
<th>Frequencies common to two or more tumor types</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain tumors</td>
<td>8</td>
<td>22</td>
<td>57</td>
<td>41 (71.9)</td>
<td>16</td>
</tr>
<tr>
<td>Hematologic malignancies</td>
<td>7</td>
<td>13</td>
<td>56</td>
<td>44 (78.6)</td>
<td>12</td>
</tr>
<tr>
<td>Colorectal cancer</td>
<td>19</td>
<td>40</td>
<td>99</td>
<td>67 (67.7)</td>
<td>32</td>
</tr>
<tr>
<td>Hepatocellular carcinoma</td>
<td>46</td>
<td>63</td>
<td>170</td>
<td>144 (84.7)</td>
<td>26</td>
</tr>
<tr>
<td>Pancreatic cancer</td>
<td>6</td>
<td>44</td>
<td>162</td>
<td>125 (77.2)</td>
<td>37</td>
</tr>
<tr>
<td>Ovarian cancer</td>
<td>10</td>
<td>66</td>
<td>278</td>
<td>219 (78.8)</td>
<td>59</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>32</td>
<td>93</td>
<td>188</td>
<td>141 (75.0)</td>
<td>47</td>
</tr>
<tr>
<td>Prostate cancer</td>
<td>17</td>
<td>80</td>
<td>187</td>
<td>150 (80.2)</td>
<td>37</td>
</tr>
<tr>
<td>Lung cancer</td>
<td>6</td>
<td>17</td>
<td>80</td>
<td>57 (71.3)</td>
<td>23</td>
</tr>
<tr>
<td>Renal cell cancer</td>
<td>2</td>
<td>3</td>
<td>36</td>
<td>33 (91.7)</td>
<td>3</td>
</tr>
<tr>
<td>Thyroid cancer</td>
<td>1</td>
<td>14</td>
<td>112</td>
<td>89 (79.5)</td>
<td>23</td>
</tr>
<tr>
<td>Neuroendocrine tumor</td>
<td>5</td>
<td>5</td>
<td>30</td>
<td>17 (56.7)</td>
<td>13</td>
</tr>
<tr>
<td>Bladder cancer</td>
<td>2</td>
<td>4</td>
<td>31</td>
<td>25 (80.6)</td>
<td>6</td>
</tr>
<tr>
<td>Leiomyosarcoma</td>
<td>1</td>
<td>2</td>
<td>36</td>
<td>31 (86.1)</td>
<td>5</td>
</tr>
<tr>
<td>Thymoma</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>0 N/A</td>
<td>2</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>163</strong></td>
<td><strong>467</strong></td>
<td><strong>1524</strong></td>
<td><strong>1183 (77.6)</strong></td>
<td><strong>341</strong> (224)</td>
</tr>
</tbody>
</table>

* 224 frequencies were common to at least two different tumor types. The total of 341 reflects the sum of all common frequencies identified in all tumor types. The following frequencies were common to most patients with a diagnosis of breast cancer, hepatocellular carcinoma, prostate cancer and pancreatic cancer: 1873.477 Hz, 2221.323 Hz, 6350.333 Hz and 10456.383 Hz
Gaps and challenges regarding tumor-specific frequencies: WM proposal WG1

• What are tumor-specific frequencies identified through changes in pulse pressure in patients with a diagnosis of cancer? Can similar frequencies be identified in animals (Dr. Percherancier, Bordeaux)?

• Can tumor-specific frequencies be used to diagnose specific forms of cancer (Dr. Costa, Sao Paulo)?

• Can tumor-specific frequencies be used to assess response to cancer therapy?
Patient-based discovery of tumor-specific frequencies

Clinical results

*In vitro* results

*In vivo* results

Conclusions
RF AMPLITUDE-MODULATED ELECTROMAGNETIC FIELDS FOR THE TREATMENT OF CANCER

- Output power: 100 mW
- Carrier frequency: 27.12 MHz
- Amplitude modulation (sine wave) with at least one or several of the following frequencies: 1873.477 Hz, 2221.323 Hz, 3669.513 Hz, 4486.384 Hz, 5882.292 Hz, 6350.333 Hz, 8452.119 Hz, 10456.383 Hz
- Frequencies are emitted sequentially for 3s and the sequence is repeated for 60 min
- Emitting power and absorbed doses of electromagnetic fields are identical to those of the device previously presented to the FDA and used in several IRB-approved U.S. and European clinical trials

*J Exp Clin Cancer Res* 2009, 28:51
Tumor-specific modulation frequencies range between 410 Hz and 20 kHz.
The device delivers whole body mean SAR in the range of only 0.2 to 1mW/kg, with a 1g peak spatial SAR between 150 and 350mW/kg.
Compassionate treatment of patients with cancer: a feasibility study

Eligibility: Patients with advanced cancer and no curative treatment options. Patients with measurable disease either by imaging studies or tumor markers. Patients may not receive other anti-cancer therapies

Pre-treatment examination: In order to identify tumor-specific frequencies, each patient was examined using biofeedback methods

Baseline exams: Imaging studies and/or tumor markers were obtained within four weeks prior treatment initiation

Treatment: tumor-specific frequencies administered sequentially for 60 min, three times a day

Study registration: clinicaltrials.gov identifier NCT00805337

59 yo postmenopausal female with ER/PR positive, ERBB2 negative breast cancer with biopsy confirmed metastasis to the left ischium and right adrenal gland.

A) Baseline PET MIP image
B) PET MIP image four months after baseline
C) Baseline PET/CT (left panel)
D) Baseline PET/CT (right panel)
E) Follow-up PET/CT: The fused PET/CT four months after baseline

*J Exp Clin Cancer Res* 2009, 28:51
64 year old man with recurrent thyroid cancer metastatic to the lungs: stable disease at + 8 years and 2 months (Br J Cancer 2011, 105:640-648; Chinese J Cancer, 2013, 32:573-581)
Overall results from compassionate use

1 complete response (CR): complete disappearance of adrenal metastasis confirmed by CT-scan and PET-scan in one patient with breast cancer and disappearance of bone metastasis-related pain. **CR lasted 11 months**

1 partial response (PR): more than 50% decrease liver metastases in one patient with breast cancer metastatic to liver and bone and significant improvement in bone metastasis-related pain. **PR lasted 13.5 months**

5 cases with stable disease (SD): thyroid cancer metastatic to lungs (on therapy since August 20, 2006: +8 years), mesothelioma metastatic to the abdomen (6.0 months), unresectable non-small cell lung cancer (5.1 months), pancreatic cancer metastatic to liver (4.1 months), and unresectable abdominal leiomyosarcoma (4 months)

9 progression of disease (PD): 3 patients with ovarian cancer, 2 patients with prostate cancer, 1 patient each with pancreatic cancer, bladder cancer, breast cancer, and small cell lung cancer

12 not evaluable for response assessment: patients did not complete two months of treatment and/or did not have follow-up imaging studies

Treatment of advanced hepatocellular carcinoma (HCC)

- **HCC diagnosis**
  - *Confirmed histology*
  - *Clinical presentation*

- **BLCL C (advanced stage)**
  - *Portal vein thrombosis*
  - *Extra-hepatic metastasis*
  - *Symptoms*

- **Progression post conventional therapy**

- **Advanced cirrhosis (B8-9)**

- **Progressed after sorafenib or systemic chemotherapy**

- **Study registration:** clinicaltrials.gov identifier NCT00534664

*Br J Cancer 2011, 105:640-648*
Long term near complete response in a patient with biopsy-proven hepatocellular carcinoma

76 year old woman with hepatitis C and Child-Pugh A5, BCLC C, biopsy-proven hepatocellular carcinoma with lung metastases who had evidence of disease progression (+36% by RECIST criteria) between May 3, 2006 (first column) and July 26, 2006 (second column). Treatment with amplitude-modulated electromagnetic fields was initiated on August 9, 2006. The patient had a partial (near complete) response of intrahepatic tumor (white arrows) and left lower lobe lung metastasis (black arrows fourth row), with stable size of right lower lobe lesion (black arrows third row) that persists as she continued receiving treatment for more than five years (fourth column).

## Objective responses

<table>
<thead>
<tr>
<th>Type of response</th>
<th>#</th>
<th>eligible for response (28 patients)</th>
<th>intent to treat analysis (41 patients)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Partial response</td>
<td>4</td>
<td>14%</td>
<td>10%</td>
</tr>
<tr>
<td>Stable disease</td>
<td>16</td>
<td>57%</td>
<td>39%</td>
</tr>
<tr>
<td>Progression</td>
<td>8</td>
<td>29%</td>
<td>N/A</td>
</tr>
</tbody>
</table>

*Br J Cancer* 2011, 105:640-648
Comparison of TheraBionic AM EMF with sorafenib (Nexavar\textsuperscript{R}) in Child-Pugh A and B patients

None of the 137 patients treated with Nexavar had stable disease lasting longer than 15 months. Four of 41 (9.8\%) patients treated with AM EMF had stable disease for more than 15 months, three (7.3\%) had stable disease for more than 27 months, although several of them had received prior systemic treatment.

\textit{J Clin Onc 2006, 24:4293}
\textit{Br J Cancer 2011, 105:640}

TheraBionic response rate (9.8\%) is fourfold higher than that of sorafenib (2.2\%).
Clinical results in patients with advanced HCC as of October 2014

41 patients treated in phase I/II study (Costa et al, Br J Cancer 2011, 105:640)

1 patient treated in feasibility study (Barbault et al., J Exp Clin Cancer Res 2009, 28:51)

6 patients treated off protocol (Brazil and France)

1 patient treated with FDA compassionate use approval (USA)

Partial responses and/or long-term survival in excess of 24 months observed in 11 (22.4%) of 49 patients
78 year old man with hormone-refractory disease since January 2014
Treatment with amplitude-modulated electromagnetic fields: 259 frequencies

9 patients with prostate cancer treated thus far, four with PSA response

TheraBionic treatment initiation on May 8, 2013
CA 19-9 in 61 yo patient stage IV (lungs, bones) pancreatic cancer after FOLFIRINOX and progression on gemcitabine/abraxane

Treatment with pancreatic cancer specific frequencies as of May 5, 2014

7 patients with stage IV pancreatic cancer treated to date, 4 patients with CA 19-9 and/or PET/CT responses
Gaps and challenges regarding clinical efficacy: WM proposal WG1

• What genetic features predict response and duration of response to tumor-specific frequencies?

• Phase III study in advanced hepatocellular carcinoma

• Phase II studies in breast cancer, pancreatic cancer, prostate cancer, ovarian cancer

• Other tumor types?
Patient-based discovery of tumor-specific frequencies

Clinical results

*In vitro* results

*In vivo* results

Conclusions
In Vitro Exposure Equipment and Conditions

- **Cells:** HepG2, Huh7, THLE2, MCF7, MCF10A
- **Exposure:** 3 hours per day for 7 days (21 total hours)
- **Initial Seeding:** 40,000-50,000 cells
- **Exposure Dose (SAR):** 0.03 W/kg to 0.4 W/kg
- **Medium Exchange:** every 48 hours
- **Comparison:** Treated cells to cells not receiving exposure
- **Subculturing (long-term):** every 7 days (1:15)
HCC, BREAST CANCER AND RANDOMLY-CHosen MODULATION FREQUENCIES

Br J Cancer 2012, 106:307-313
Inhibition of cancer cell proliferation by specific modulation frequencies

HCC-specific modulation frequencies

Breast cancer-specific modulation frequencies

HCC-specific modulation frequencies

p=0.001
p=0.01
p=0.02

N=6

Br J Cancer 2012, 106:307-313
Inhibition of cancer cell proliferation by specific modulation frequencies

HepG2

Percent growth inhibition (%)

N=6

1 Hour/day 7 days

3 Hours/day 7 days

6 Hours/day 7 days

3 Hours/day 3 days

Hours of exposure per day and days treated

Br J Cancer 2012, 106:307-313
Inhibition of cancer cell proliferation by specific modulation frequencies

Br J Cancer 2012, 106:307-313
qPCR validation of differentially expressed genes as assessed by RNA Seq

**HepG2**

<table>
<thead>
<tr>
<th></th>
<th>PLP2</th>
<th>XCL2</th>
</tr>
</thead>
<tbody>
<tr>
<td>No exposure</td>
<td>![Graph]</td>
<td></td>
</tr>
<tr>
<td>HCC-specific exposure</td>
<td>![Graph] <strong>p=0.009</strong></td>
<td>![Graph] <em>p=0.02</em></td>
</tr>
</tbody>
</table>

N=4

*p=0.009*

*p=0.02*

*Br J Cancer 2012, 106:307-313*
Fluorescence microscopy assessing the effects of HCC-specific frequencies on microtubule dynamics and cell division in HepG2 cells

A HepG2 cells are able to efficiently assemble a bipolar mitotic spindle, allowing cells to pass through the mitotic assembly checkpoint and successfully progress from metaphase to anaphase.

B HepG2 cells exposed to HCC-specific frequencies display microtubule-associated errors such as tripolar spindle formation, potentially resulting in aneuploid cellular states. Successive rounds of unsuccessful mitosis could be a major determinant in cell survival. (Cyan=DAPI; Gray=Microtubules; Arrows=mitotic spindle)

Br J Cancer 2012, 106:307-313
Galaxy Evaluation of RNA-Seq Data

- **NFAT5** (nuclear factor of activated T-cells 5)
- **MBOAT1** (membrane bound O-acyltransferase domain containing 1)
- **ARHGDIB** (Rho GDP dissociation inhibitor (GDI) beta)
- **S100B** (S100 calcium binding protein B)
- **ANXA1** (Annexin I)

**Goal:** Use more comprehensive software technology to build a pathway based on changes in gene expression

Source: NCBI Gene
MicroRNA (miRNA) Screen

- miRNA sequences are short beyond the scope of RNA-Seq sensitivity
- **Hsa-mir-1246**
  - NFAT5
  - PLCB1
- **Hsa-mir-148a**
  - PLCXD3
  - PLCB4
- **Hsa-let-7g**
  - PLCB2
  - PLCD4

**Common link:** signal transduction through IP3/DAG

Calcium is a necessary mediator of RF EMF-mediated inhibition of cell proliferation.

Huh7 cell proliferation assay following exposure to HCC-specific or random-chosen frequencies for 3h/day for seven days.

- Random Frequencies
- Random Frequencies + BAPTA 100 µM
- HCC-specific Frequencies
- HCC-specific Frequencies + BAPTA 100 µM

P < 0.01
Patient-based discovery of tumor-specific frequencies

Clinical results

*In vitro* results

*In vivo* results

Conclusions
In Vivo Experimental Methods

**AM EMF exposure system:** uses transverse electromagnetic (TEM) wave in a stripline integrated into a ring resonator. Dosimetry analysis confirmed that exposure remained homogenous regardless of mouse posture. Both computer simulations and actual exposures using “phantom” anatomical models were used for verification. The TEM equipment is capable of exposing 16 mice simultaneously to each treatment and control conditions.

- Euthanize upon reaching excessive tumor burden (>2cm)
- Harvest tumor tissue and normal organ tissue
- Evaluate tumor doubling time as well as treatment time to euthanasia
In Vivo Experimental Methods

**NOD scid mice**

1x10⁷ cells: HCC, breast and ovarian cancer cell lines derived from patient tumors

Injections

1x10⁷ cells

Palpable Tumor Formation

**AM RF EMF Exposure**

0.4 W/kg, three one hour treatments daily
Subcutaneous HepG2 Cellular Xenografts Treated with AM RF EMF – Complete response

Each volume is an average of 3 weekly measurements.
Subcutaneous HepG2 Cellular Xenografts Treated with AM RF EMF – Residual Encapsulated Tumor

Magnification: 200x
Subcutaneous Huh7 Cellular Xenografts Treated with AM RF EMF – Change in Total Volume

Model: Subcutaneous Cellular Xenograft in NOD SCID Mice
Cells: Huh7 hepatocellular carcinoma

Total Change in Tumor Volume ($\Delta \text{mm}^3$)

- **Control**: n = 4
  - p = 0.0106

- **Treated**: n = 5

Week

1  2  3  4  5  6
Proliferative Impact on Normal Tissue Following RF EMF Exposure

- Ki-67 stain identifies proliferation in the crypts of the small intestine
- There are no differences in proliferation in small intestine tissue following exposure to HCC-specific RF EMF
Gaps and challenges regarding mechanism of action: WM proposal WG1

- Replication of growth inhibition of Huh7 cells *in vitro* and *in vivo* by 194 hepatocellular carcinoma-specific frequencies (Prof. Schär, Basel)
- Dissection of the molecular mechanism underlying cancer cell growth inhibition
- Identification of novel pathways implicated in cancer growth
FUTURE DIRECTIONS

• The TheraBionic device will become commercially available for the treatment of advanced hepatocellular carcinoma and breast cancer as a class IIa device in Europe in 2015 (DIN ISO 9001 certified, ISO 13485 certification in progress). Five European centers will perform a phase IV study starting in 2015.

• A randomized study assessing pulse pressure changes in 60 individuals with hepatocellular carcinoma, breast cancer and healthy individuals exposed to cancer specific frequencies and randomly chosen frequencies (PI Dr. Frederico Costa, Hospital Sirio-Libanes, Sao Paulo, Brazil; clinicaltrials.gov # CT01688412).

• The FDA has reviewed and approved a phase III protocol in which patients with advanced hepatocellular carcinoma who have failed sorafenib will receive treatment with hepatocellular carcinoma-specific frequencies or randomly chosen frequencies.

• A phase II study in advanced breast cancer will start in early 2015.
CONCLUSIONS

• Intrabuccally-administered 27 MHz electromagnetic fields, amplitude-modulated at tumor-specific frequencies elicit objective responses in patients with advanced breast cancer and hepatocellular carcinoma.

• Several patients experience long-lasting responses or disease-stabilization without significant side effects.

• Tumor-specific frequencies block proliferation of cancer cells both in vitro as well as in vivo at levels of exposure similar to those yielding therapeutic responses in humans (< 0.4 W/kg).

• Tumor-specific frequencies do not affect the proliferation of normal hepatocytes or other tumor types, which suggest a cell-specific resonance mechanism for specific frequencies.
CONCLUSIONS

- Hepatocellular carcinoma-specific frequencies downregulate the expression of the PLP2 and XCL2 genes in hepatocellular carcinoma cells. Expression of the same genes is downregulated in ovarian cancer cell lines, which suggests a common mechanism of action for certain tumor types.

- Changes in siRNA suggest that the IP3/DAG signaling pathway is modulated by AM EMF, affecting calcium efflux as originally reported by Ross Adey and Carl F. Blackman.

- Calcium chelation with Bapta confirms the central role of Calcium in AM EMF targeted antiproliferative effects.

- Hepatocellular carcinoma-specific frequencies disrupt the mitotic spindle in HepG2 cells.

- The experimental findings suggest the existence of a novel receptor mechanism through which cancer growth can be effectively targeted, thus offering a new therapeutic approach.
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