A Phase II Study Of Intrabucally Administered Electromagnetic Fields and Regorafenib as Second-line Therapy For Patients with Advanced Hepatocellular Carcinoma.
Wake Forest Baptist Comprehensive Cancer Center
WFBCCC # 55319

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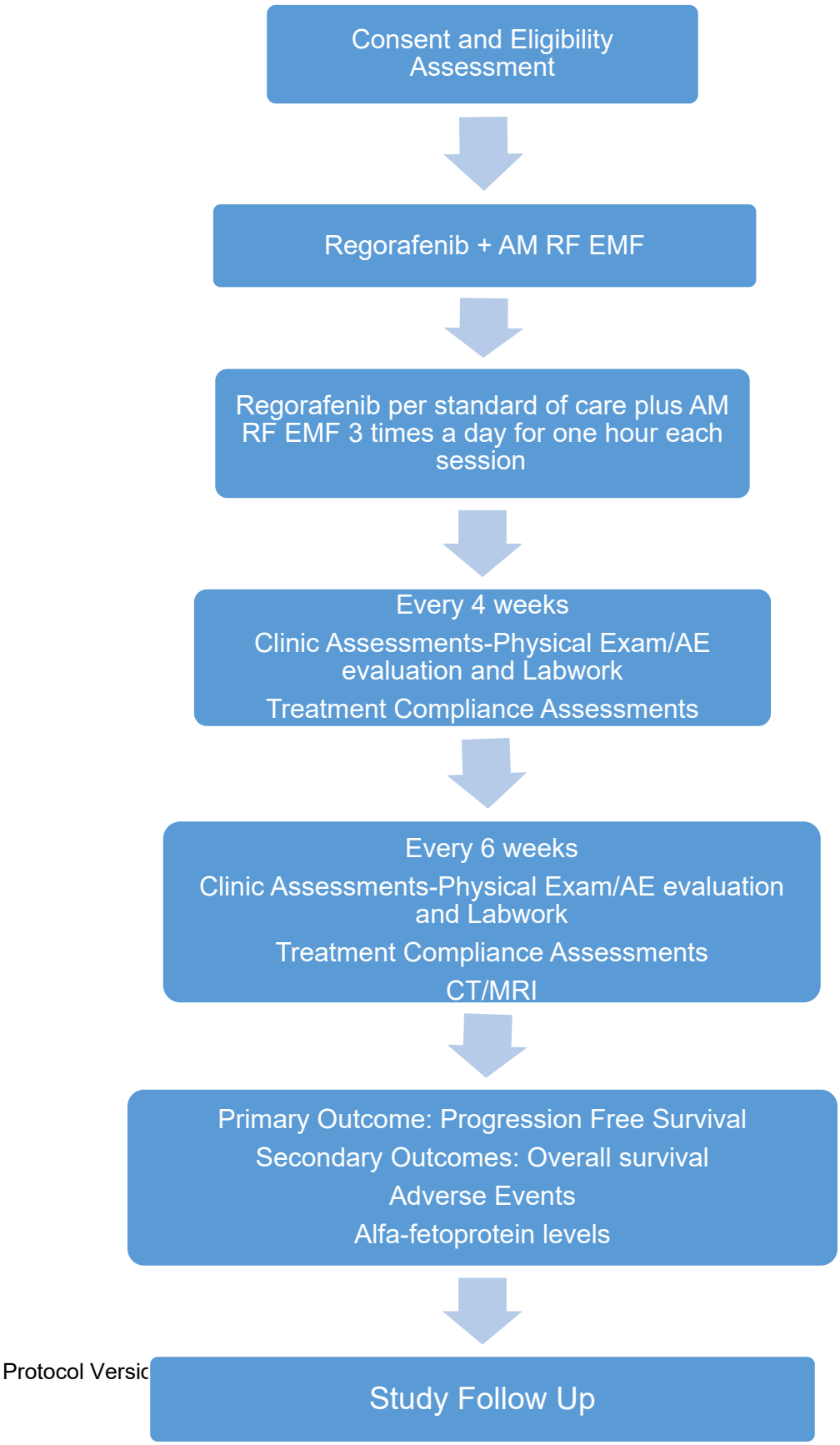

1.0 Introduction and Background

Hepatocellular carcinoma (HCC) is the fourth most common cause of cancer death worldwide [1, 2]. This tumor is the fastest rising cancer in the United States. In 2019, it is estimated that 42,030 new cases will be diagnosed and there will be 26,810 deaths from HCC, i.e. 86% and 73% increase in the number of new cases and deaths since 2009, respectively[3, 4]. The prognosis of patients suffering from advanced HCC is poor with an average survival time of ten months [2]. Factors negatively influencing patient survival include: poor performance status, history of alcoholism, low albumin rate, hepatic cirrhosis, high level of alpha-fetoprotein (AFP), and an obstruction of the portal vein. Therapies for HCC are limited. Resection of the primary tumor is the therapeutic approach of first choice when possible [2]. Although this intervention results in long-term survival for some patients, the majority of patients are not surgical candidates due to tumor size, the patient's overall condition, or presence of hepatic cirrhosis. Until 2017, only two therapeutic interventions had demonstrated survival benefit in such patients: chemoembolization [5] and sorafenib, a multikinase inhibitor [6] [7]. Systemic therapies for patients with advanced hepatocellular carcinoma have recently improved, with several new agents showing clinical efficacy in phase 3 trials: Regorafenib [8], Lenvatinib [9], Cabozantinib [10],and Ramucirumab [11]. Sorafenib, lenvatinib, and atezolizumab + bevacizumab are currently approved for first-line treatment; regorafenib, cabozantinib, ramucirumab, lenvatinib, nivolumab, nivolumab + ipilumab, sorafenib, and pembrolizumab are approved for second-line treatment. While these new therapies provide additional options for patients with advanced hepatocellular carcinoma, the incremental survival benefits are limited to approximately 3 months. Additional therapies for this disease are sorely needed, especially for the large number of patients with advanced disease who progress after first line therapy.

1.1 Current standard of care

The current standard of care is described in the following Figure 1 [2].

Regimens recommended by the NCCN guidelines version 1.2020

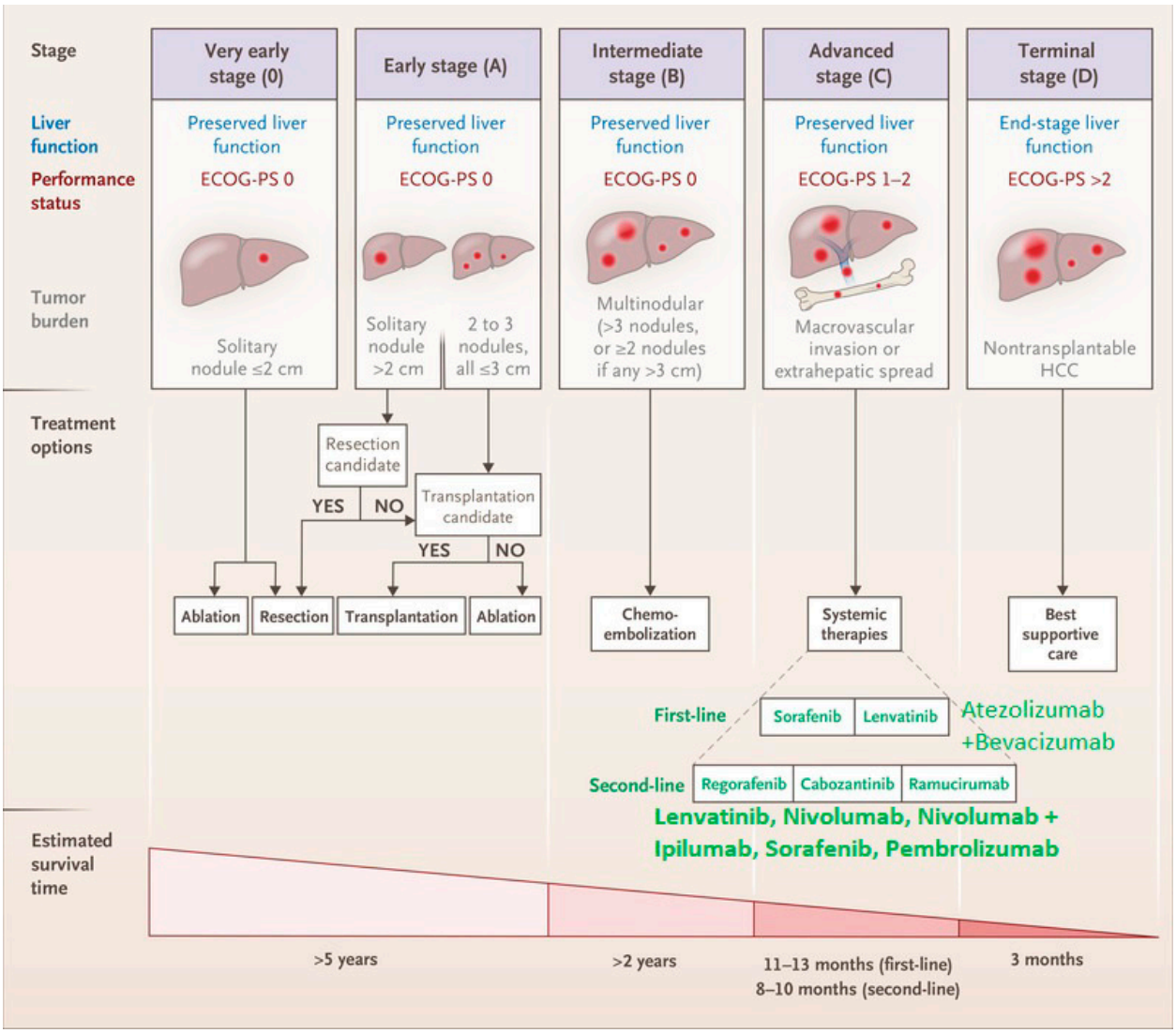

Figure 1: Clinical algorithm for the management of hepatocellular carcinoma
The algorithm is based on the Barcelona clinic liver Cancer (BCLC) algorithm, which classifies patients in one of 5 stages, and European Association for the Study of the Liver guidelines [12] [13]. Depicted is the estimated survival time in each stage once the recommended therapy has been administered or performed. As of March 23, 2020, the combination of atezolizumab together with bevacizumab is an additional preferred regimen recommended by the NCCN Guidelines Version 1.2020. Additionally, nivolumab, nivolumab plus ipilumab, and pembrolizumab are FDA approved second line therapies for advanced hepatocellular carcinoma.

1.2 Administration of amplitude-modulated radiofrequency electromagnetic fields (AM RF EMF) of low intensity

1.2.1 Electromagnetic fields and tumor cell growth

Until a decade ago little was known regarding the kHz to THz electromagnetic interactions with biological systems, which do not result in changes in temperature within tumor tissues or in tumor cell membrane electroporation. Recent studies provide growing experimental and clinical evidence that alternating electric fields (Tumor Treating Fields) and amplitude-modulated radiofrequency electromagnetic fields (AM RF EMF) are capable of blocking tumor growth [14].

In 2001, Pasche and Barbault hypothesized [15] and subsequently provided experimental evidence that intrabuccal delivery of 27 MHz radiofrequency electromagnetic fields, which are amplitude-modulated at tumor-specific frequencies (AM RF EMF), has anticancer activity. Using noninvasive patient-based approaches, Barbault et al. were the first to identify tumor-specific modulation frequencies, i.e., changes in pulse amplitude upon exposure to specific AM RF EM, in patients with cancer that were not found in healthy individuals [16].They subsequently determined that these frequencies had anticancer effects both *in vitro* and *in vivo [17, 18]* They conducted first a feasibility study in 28 patients with advanced cancer and no therapeutic options [19] then a phase I/II in patients with advanced HCC without therapeutic options [20]. These studies showed that treatment with AM RF EMF results in clinical benefits with minimal risks, even after several years of continuous daily use [20] [21, 22]. These clinical and translational findings led to the European regulatory approval of the TheraBionic P1 medical device for the treatment of advanced HCC [15]. The TheraBionic P1 medical device received European approval as a class IIa low risk medical device in 2018 and is indicated for patients with advanced hepatocellular carcinoma who have failed or are intolerant to first line and second lines therapies. In 2019, the FDA granted Breakthrough Designation to the same medical device for use in patients with advanced hepatocellular carcinoma who have failed or are intolerant to first line and second line therapies for HCC.

1.2.2 Administration of electromagnetic fields of low intensity in human beings

Intrabuccal administration of tumor-specific AM RF EMF as a single anticancer treatment modality has yielded objective tumor shrinkage in the femur [16], adrenal gland [16], liver [20], lungs [20] and brain [23] of patients suffering from unresectable or

metastatic cancer, which suggests that intrabuccally-administered 27 MHz AM RF EMF have systemic anticancer effects distant from the point of administration, i.e., the buccal mucosa. Jimenez et al. recently demonstrated that intrabuccal administration of AM RF EMF results in absorption throughout the body because contact between the spoon-shaped “antenna” applicator and the anterior part of the patient’s tongue results in the patient’s body acting as an extension of the antenna. The AM RF EMF reach the whole body because of the 27 MHz carrier frequency, which has a wavelength of 11 m. Optimal antenna absorption by living systems is achieved at one third of the wavelength, i.e., 3.6 m, which is very close to the length of the human body (1.4-2.2 m). Additionally, because the carrier wave is 27.12 MHz and the electrical charge is so low, the signal (AM RF EMF) extends throughout the body and thereby in effect allowing the body to become an extension of the coaxial cable delivering treatment systemically.

The RF output of the TheraBionic P1 medical device is adjusted to 100 mW into a 50 Ω load using a sinusoidal test signal [16, 20, 22]. The Specific Absorption Rate (SAR) level and distribution inside the human body during treatment (Fig. 1a and 1b) was assessed and the variability depending on device positioning and patient posture (Fig. 1c) was quantified. Homogeneous and inhomogeneous model simulations show similar SAR distribution pattern (Fig. 1d and 1e). The whole-body (wbSAR) and the peak spatial SAR (psSAR) over any 10 g (psSAR10g) and 1 g (psSAR1g) of major organs according to IEEE standard[24] are listed in Table 1.

Table 1a: Organ-specific SAR with the device on the thigh or away from the body

Organ-specific SAR	Device on thigh (case 1)			Device away (case 3)		
mW/kg/W	Mean	PS-10 g	PS-1 g	Mean	PS-10 g	PS-1 g
Whole body	13.5	2583	6900	13.4	3399.0	9110.0
Brain grey matter	2.2	10.1	15.7	2.5	14.2	22.1
Brain white matter	1.0	3.0	4.6	1.0	3.5	6.3
Midbrain	3.7	3.9	5.4	3.9	4.2	5.9
Heart	7.7	13.7	15.8	4.9	8.1	9.3
Liver	8.4	16.0	29.2	4.2	7.5	14.7
Lung	13.9	59.7	92.1	11.2	63.7	99.6

Table 1b: Simulation power budget for a homogeneous human model, nominal input power 1 W

Body/device positions	1	2	3	4
Mean SAR (mW/kg/W)	13.400	13.400	13.200	10.100
Std. deviation (mW/kg/W)	44.600	57.400	50.200	61.600
Conductivity loss (W)	0.978	0.977	0.967	0.723
Radiated power (W)	0.003	0.003	0.012	0.262
Total power (W)	0.980	0.980	0.980	0.990

Table 1c: Simulation power budget for rectangular tank phantom, nominal input power 1 W

	case 1	case 2	case 3	case 4
Mean SAR (mW/kg/W)	74.500	75.400	72.600	60.500
Std. deviation (mW/kg/W)	90.300	148.800	129.200	120.800
Conductivity loss (W)	0.993	0.990	0.987	0.807
Radiated power (W)	0.001	0.001	0.004	0.185
Total power (W)	0.990	0.990	0.990	0.990

Table 1d: Variation assessment during the use of the TheraBionic device

Contributions	Unc (dB)	Distr	Div	Std. Unc (dB)
Posture	0.11	R	1.73	0.06
Weight deviation (±10 kg)	0.66	R	1.73	0.39
Applicator spoon matching	3.30	R	1.73	2.19
Combined standard variation (k=1)				2.21

Intrabuccal delivery of 27 MHz AM RF EMF results in whole-body absorption from head to toe and the highest peak spatial SAR (psSAR) is at the interface between the tongue and the spoon-shaped applicator. As shown in Table 1b, there is more radiated power when the device is far away from the body. Conversely, the wbSAR is slightly higher when the device touches the body, regardless of position (Table 1c). Experimental validation measurements based on a tank phantom (Fig. 1f) show that the SAR distribution inside the tank is similar to that of the homogeneous human model.[22]. The wbSAR is 1.35 mW/kg with psSAR over 1 g from 146 to 352 mW/kg when the applicator spoon is perfectly matched, which is significantly below the International Commission on Non-Ionizing Radiation Protection [25] standard safety limits of 80 mW/kg wbSAR, or psSAR of 2,000 mW/kg (Table 2).[25] Organ-specific SAR ranges from 0.02 to 1 mW/kg (Table 2). Liver-specific SAR ranges from 0.05 to 0.5 mW/kg (Table 2).

Table 2: Organ-specific SAR

Organs	Mean SAR (mW/kg)	psSAR 1g (mW/kg)
Whole body	0.20 – 1.00	146.00 – 352.00
Brain grey matter	0.04 – 0.20	0.20 – 0.60
Brain white matter	0.02 – 0.10	0.05 – 0.15
Midbrain	0.06 – 0.20	0.06 – 0.20
Heart muscle	0.05 – 0.50	0.10 – 1.00
Liver	0.05 – 0.50	0.10 – 1.00
Lung	0.20 – 1.00	1.00 – 4.00

Uncertainty and variation assessments were conducted with the patient sitting with the device placed away from the body. The posture variation based on sitting positions as shown in introduces 0.06 dB deviation. A ±10 kg variation in body weight introduces 0.39 dB deviation, the biggest contribution, 2.19 dB comes from the variation of the applicator spoon impedance with posture and device position and its effect on the power delivered to the patient. Therefore, the total variation of 2.21 dB, as shown in Table 1d, mainly depends on the delivered power.

In summary, dosimetry analysis shows that 27 MHz AM RF EMF administered by means of a spoon-shaped applicator results in systemic EMF absorption, which is more than one hundred fold lower than the SAR generated by cell phones and does not result in heating of any body part. In all conditions studied, the device complies with the standard for human exposure to RF EMF. [26] The results also demonstrate that the human body acts as an antenna resulting in head to toe delivery of AM RF EMF. These findings provide a biophysical rationale for the antitumor effects documented in patients with metastases in the femur, liver, adrenal glands, lungs, and brain receiving intrabuccally-administered treatment with the TheraBionic device. Tumor-specific AM RF EMF appear to have a broad therapeutic window as tumor shrinkage was observed in humans,[16] [20] in human xenografts [23], and *in vitro* [17] at SARs ranging from 0.02 mW/kg to 400 mW/kg.

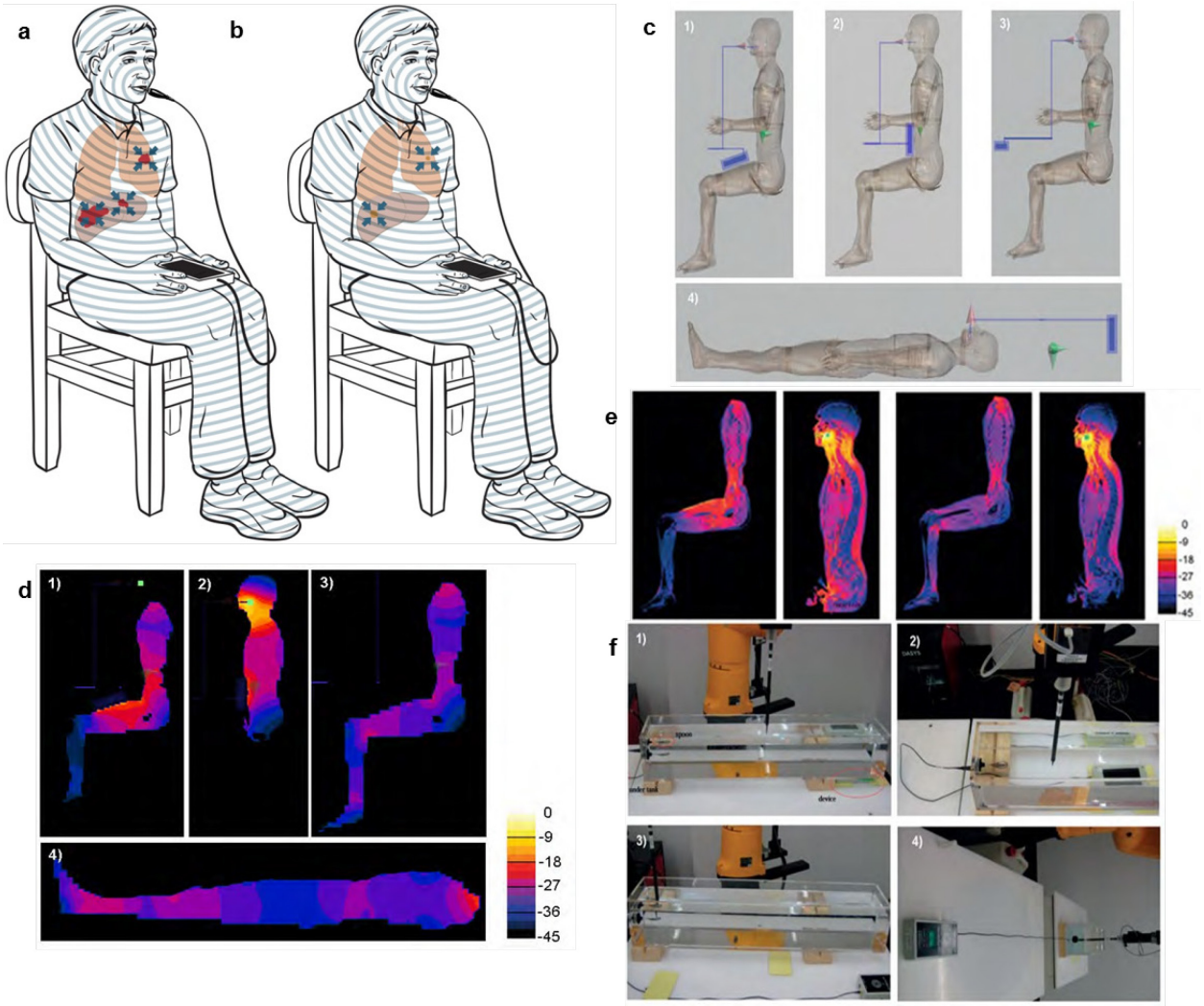

Figure 1. Intrabuccal delivery results in systemic absorption of AM RF EMF**. a** Patient with advanced hepatocellular carcinoma receiving first treatment with the AM RF EMF emitting device. The concentric lines represent AM RF EMF emission from the spoon-shaped antenna placed on the patient's tongue. Red: primary and metastatic tumors. Blue arrows: depiction of antitumor activity location. **b** Same patient several months later with evidence of shrinkage or disappearance of the primary tumor and its metastases [18, 27], Brown: shrunken tumor following AM RF EMF treatment, **c**,1) Patient sitting with device on thigh, 2) Patient sitting with device on the abdomen, 3) Patient sitting with device placed away from the body, 4) Patient in supine position to test the hypothesis that the body can act as one half of a short dipole antenna while the cable and device form the other half, or for treatment lying down while the device is behind the head. The device and coaxial cable are shown in blue. The electric dipole is shown in green. **d**, SAR slice views in the

homogeneous human model: the images are normalized to 5 W/kg/W, with 3 dB/contour. 1) Patient sitting with device on thigh, 2) Patient sitting with device on the abdomen, 3) Patients sitting with device placed away from the body, 4) Patient in supine position. **e**, SAR slice views in the inhomogeneous model: the images are normalized to 5 W/kg/W, with 3 dB/contour. Left panels: patient sitting with device on thigh. Right panels: patient sitting with device located away from the body. **f**, Experimental validation measurements setups and results.1) The device is located at one extremity underneath the tank, 2) The device is in the middle underneath the tank, 3) The device is beside the tank, 4) The device is as far away from the tank as possible.

1.2.3 Clinical effects of amplitude-modulated electromagnetic fields of low intensity (Low Emitting Therapy Emission: LEET) on sleep and insomnia.

In 1986, the Food and Drug Administration authorized the first tests of LEET technology in humans indicating that the device was a nonsignificant risk device (NSR). The emitting device was a precursor of the TheraBionic device, which had identical emitting power as the TheraBionic P1 medical device, which was adjusted to 100 mW into a 50 Ω load using a sinusoidal test signal 100 mW.[28] As a result, clinical studies were allowed to proceed upon local IRB approval. After approval by Swiss and American ethical committees (local IRBs), the first study using LEET was carried out on 104 volunteers at 2 different centers (Montreux, Switzerland and Denver, Colorado, United States). All subjects received in a blinded fashion one 15-minute active treatment and one 15-minute placebo treatment at least one week apart. An analysis of the electroencephalograms obtained immediately after treatment showed that active treatment decreased sleep latency and allowed healthy volunteers to reach a deeper sleep stage following active treatment than following placebo treatment [28, 29]. Active treatment also caused a feeling of relaxation when compared with placebo treatment[30].

The effects of LEET on chronic insomnia were assessed with polysomnography (PSG) on a total of 106 patients at two U.S. sleep disorder centers (Scripps Clinic, La Jolla, CA and University of Colorado Health Sciences Center, Denver, CO) [31] [32]. Active or inactive LEET was administered for 20 minutes in late afternoon three times a week for a total of 12 treatments. There was a significant increase in total sleep time as assessed by polysomnography (PSG) between baseline and post-treatment values for the active treatment group (76.0 ± 11.1 minutes, $p = 0.0001$). The increase in total sleep time for the inactive group was not statistically significant (20.0 ± 13.5, $p = 0.15$). There was a significant decrease in sleep latency as assessed by polysomnography between baseline and post-treatment values for the active treatment group (-21.6 minutes ± 5.9 minutes, p

= 0.0006), whereas the decrease noted for the inactive treatment group was not statistically significant (- 6.0 ± 6.0 minutes, p = 0.32). Interestingly, the number of physiological sleep cycles per night increased by 30 % (0.84 ± 0.19) after active treatment (p = 0.0001), but was unchanged (0.23 ± 0.21) following inactive treatment. Subjects did not experience rebound insomnia, and there were no significant side effects. Lastly, the number of sleep cycles increased by 30% in the active group (0.84 ± 0.19, p = 0.0001), whereas it did not change in the placebo group (0.23 ± 0.21, p = 0.27).(31) Hence, LEET was able to effectively improve the sleep of chronic insomniacs by increasing the number of sleep cycles without altering the percentage of the various sleep stages during the night. The therapeutic action of LEET differs from that of currently available therapies in that the sleep patterns noted in insomniacs following LEET treatment more closely resembles nocturnal physiological sleep. More than 1000 patients were treated with this therapy in Europe and in the United States between 1982 and 2003 [33]. The only recorded side effects reported were an increase in dreams (likely related to the observed increase in the number of physiological cycles of the sleep).

1.2.4 Low levels of amplitude-modulated radiofrequency electromagnetic fields (AM RF EMF) in the treatment of cancer.

Barbault and Pasche have developed a patient-based, noninvasive biofeedback approach to measure physiological reactions upon exposure to high-frequency carrier signals, which are amplitude- modulated at various frequencies. Frequency discovery consists in the measurement of variations in pulse amplitude and blood flow [15, 16]. These measurements are conducted while individuals are exposed to low and safe levels of amplitude-modulated frequencies emitted by a handheld device or while holding the spoon antenna in the mouth. Exposure to these frequencies results in minimal absorption by the human body, which is well below the Institute of Electrical and Electronics Engineers [24] limits [26]. These frequency detection methods allowed them to identify specific modulation frequencies commonly encountered in patients suffering from cancer but not encountered in healthy controls [16]. In collaboration with Professor Niels Kuster and his team from the Swiss Federal Institute of Technology in Zurich, Pasche and Barbault developed new devices (OncoBionic P1 used for clinical trials; TheraBionic P1 device for European registration according to ISO 13485:2016 and 93/42 EWG guidelines) for the administration of radiofrequency electromagnetic fields, which are amplitude-modulated with a precision reaching 10^{-7} Hz. These devices differ from the older Symtonic devices used for the treatment of insomnia in that the precision of the emitted modulation frequencies has been significantly improved [15, 16]. The TheraBionic P1 devices, which received the CE mark in 2018 will be available for the proposed study. Importantly, the emitting power and the levels of absorbed

electromagnetic fields of the TheraBionic P1 and OncoBionic P1 are identical to those of the Symtonic device.

In 2002, after having identified frequencies likely to have a therapeutic effect in the treatment of cancer, Pasche and Barbault initiated a feasibility study to evaluate the tolerability of this approach in the treatment of patients with solid tumors. A total of 28 patients with advanced cancer were offered compassionate experimental treatment [16]. Independent review of the imaging studies by U.S. Board certified radiologists using RECIST criteria [34] showed a complete response lasting 11 months in a patient with breast cancer metastatic to the adrenal gland and bone, and a partial response lasting 13.5 months in another patient with breast cancer metastatic to the liver and bone. The tumor of a patient suffering from pancreatic cancer metastatic to the liver was stable for 4.0 months. The tumor of a patient with stage IV non-small cell lung cancer was stable for 5.1 months. One patient with recurrent follicular thyroid cancer metastatic to the lungs enrolled in August 2006 is still on therapy as of November 2019, more than 13 years and three months after enrollment into the study [21]. None of the patients have experienced any serious side effects as a result of the administration of electromagnetic fields, even after more than 13 years of continuous treatment. These preliminary results indicated that a therapeutic approach based on low intensity electromagnetic fields administered daily for three hours does not entail major health risks, even after several years of exposure, and is potentially effective in the treatment of advanced cancer.

1.2.5 Specific frequencies in HCC

By means of the above described methods and devices, Barbault and Pasche have identified potentially effective frequencies for the treatment of HCC [16]. Given the fact that there was no effective therapy for the treatment of advanced HCC resistant to conventional therapies, Dr. Frederico Costa initiated in 2005 a phase I/II study at the University of Sao Paulo in Brazil [20]. The main goal of this phase I/II study was to assess the safety and effectiveness of low levels of radiofrequency electromagnetic fields amplitude-modulated at HCC-specific frequencies and administered by means of an intrabuccal spoon-shaped probe in patients with advanced HCC. The primary efficacy end point was progression-free survival (PFS) ≥ 6 months. Secondary efficacy end points were progression-free survival and overall survival (OS). Three daily 60 min outpatient treatments were administered until disease progression or death. Imaging studies were performed every eight weeks. From October 2005 to July 2007, 41 patients with advanced HCC and Child-Pugh A or B were enrolled in this study. Thirty-one patients (75.6%) had radiological evidence of disease progression at the time of enrollment as defined by comparison of baseline imaging studies with imaging studies obtained within the previous

six months. Thirty-four (82.9%) patients had received therapy prior to enrollment. The majority of patients had severely impaired liver function as demonstrated by the fact that 22 patients had Child-Pugh B disease. Six of the first 23 patients (26.1%) had progression-free survival ≥ 6 months, which led the study team to continue enrolling patients up to the pre-planned total of 41 patients. In total, fourteen patients (34.1%) had stable disease for more than 6 months. Median progression- free survival was 4.4 months (95% CI 2.1-5.3) and median overall survival was 6.7 months (95% CI 3.0-10.2). One patient with evidence of disease progression at the time of enrollment remained on therapy for 63 months. Estimated survival at 12, 24 and 36 months was 27.9% (SE = 7.1%), 15.2% (SE = 5.7%) and 10.1% (SE = 4.8%). A total of 28 patients were evaluable for tumor response. Four (14.3%) patients had a partial response assessed with CT with or without contrast-enhanced ultrasound. All partial responses were independently reviewed by two radiologists. Serial alpha-fetoprotein (AFP) measurements, which predict radiological response and survival in patients with HCC [20], were available for 23 patients. AFP decreased by 20% or more in four (17.3%) patients following initiation of therapy.

Overall, there were six long-term survivors with an overall survival greater than 24 months, and four long-term survivors with an overall survival greater than three years. Importantly, five of the six (83%) long-term survivors had radiological evidence of disease progression at the time of study enrollment. The two patients with the longest survival (48.4 and 63 months) had BLCL stage C disease and portal vein thrombosis, predictors of short survival [20]. Eleven patients reported pain prior to treatment initiation, three patients reported grade 3, five grade 2, and three reported grade 1. Five patients reported complete disappearance of pain and two patients reported decreased pain shortly after treatment initiation. Two patients reported no changes and two patients reported increased pain. There was no treatment-related grade 2, 3 or 4 toxicities. The only treatment-related adverse events were grade I mucositis (1 patient) and grade I somnolence (1 patient).

1.2.6 *In vivo* effects of amplitude-modulated radiofrequency electromagnetic fields

Having characterized SAR levels delivered to patients during treatment with the AM RF EMF emitting device, Jimenez et al. set out to replicate human exposure conditions *in vivo*. They used a custom-designed small animal AM RF EMF exposure system [35] [18] and exposed tumor-bearing mice with tumors developing in subcutaneous tissue predominantly surrounded by fat. The exposure system RF output was set for delivery of a SAR level of 67 mW/kg. This SAR was selected so that it was 1) within the range of previously demonstrated *in vitro* activity (30 - 400 mW/kg) [36] and 2) within the range of wbSAR1g and psSAR1g in patients receiving treatment with the TheraBionic device (1 -

352 mW/kg) (Table 2). Huh7 and patient-derived tumor cells were used as subcutaneous cellular xenograft models of HCC [23, 37], [38]. Thirty-seven of 40 mice injected with Huh7 cells developed palpable tumors and were exposed to HCC-specific AM RF EMF (HCCMF), randomly chosen AM RF EMF (RCF), or were not exposed to EMF (SHAM) as controls. Both control subsets (RCF and no exposure) were compared to the group exposed to HCC-specific frequencies (N = 20). The treatment by time interaction was highly significant ($p<0.001$) showing that tumor growth curves were different among the three groups. Comparison between the two control subsets showed no statistical difference over the course of six weeks; $P = 0.655$. The two groups were therefore combined as control group. Comparison between the pooled control group (N = 17) and the HCCMF (N = 20) group shows beginning separation between groups at week four ($p = 0.08$), and significant separation at week five ($p = 0.045$), and week six ($p = 0.019$) (Figure 2a). The commonly used RECIST 1.1 criteria [39] were applied to assess response to treatment. At week six, the volume of 8 (42.1%) of the 19 tumors exposed to HCCMF had decreased by 30% or more. At the same time point none of the 17 tumors of the control group had evidence of shrinkage [22].

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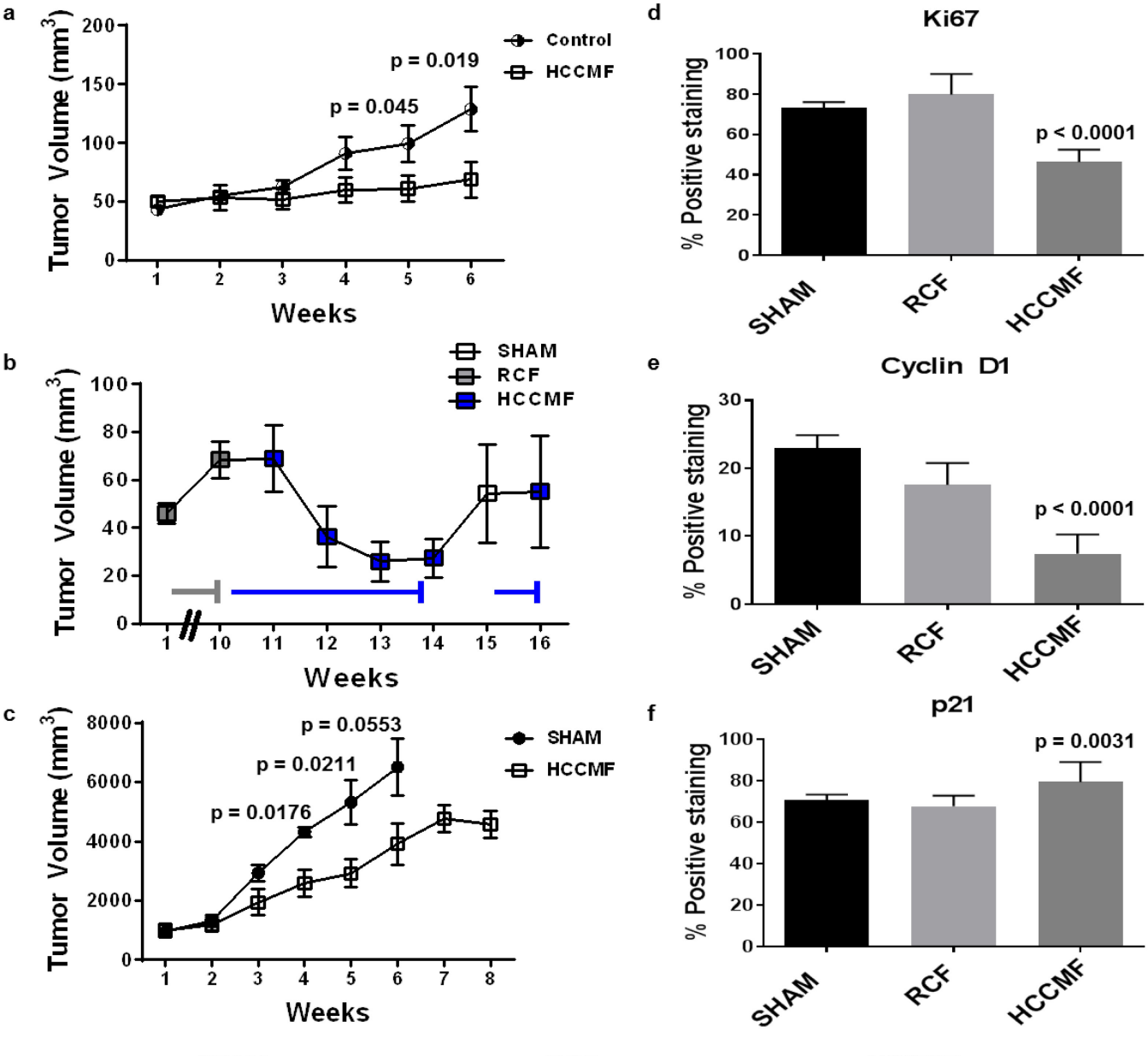

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Figure 2. Antiproliferative effects of HCCMF in vivo**. a**, Mice were exposed to either HCCMF exposure with a xenograft-specific SAR of 67 mW/kg or are assigned to the control treatment group three hours per day. Control group is comprised of mice receiving either randomly chosen frequencies (RCF) with a xenograft-specific SAR of 67 mW/kg and those receiving no exposure (SHAM). Tumor volume is measured three times per week and volume is calculated as (Length x $(Width)^2$) / 2. After six weeks of exposure, statistical significance between treated and control groups has been achieved; [Week five ($p = 0.045$), Week six ($p = 0.019$) Student's two-tailed t-test] and [Test for treatment by time interaction ($p < 0.001$)]. **b**, Sequential exposure to randomly chosen and HCCMF. Five mice carrying Huh7 xenografts were exposed to RCF (grey line) three hours per day for ten weeks during which the average tumor volume increased by 48%. Exposure was switched to HCCMF (blue line) after ten weeks. Exposure to EMF was discontinued for one week after four weeks exposure, then resumed. Mice were sacrificed at the end of week 16. **c**, Patient-derived HCC xenografts (PDX) exposed to HCCMF or not exposed to EMF. Patient derived xenografts from a 63-year-old male with hepatocellular carcinoma. Mice received either HCCMF exposure (HCCMF; N = 6) or received no treatment (SHAM; N =4). Tumor volume measured three times per week and volume is calculated as (Length x Width2) / 2. After eight weeks of exposure, statistical significance had been achieved; [Week four ($p = 0.0176$), Week five ($p = 0.0211$) Student's two-tailed t-test]. At week six, all mice in the Sham group expired and tumor volume was imputed, Week six ($p = 0.0553$) [student's t-test]. [Test for treatment by time interaction ($p = 0.0006$)]. **d**, Ki-67 staining of SHAM, RCF and HCCMF treated tumors. [Anova: $F = (2, 33)$ 67.55, $P = <0.0001$]. [Post-Hoc Tukey Test: Sham vs RCF $p = 0.2067$, SHAM vs HCCMF $p <0.0001$, RCF vs HCCMF $p < 0.0001$.] **e**, Cyclin D1 staining of SHAM, RCF and HCCMF treated tumors. [Anova: $F = (2, 33)$ 23.29, $p < 0.0001$]. [Post-Hoc Tukey test: Sham vs RCF $p = 0.1379$, SHAM vs HCCMF $p <0.0001$, RCF vs HCCMF $p = 0.0005$]. **f**, p21 staining of SHAM, RCF and HCCMF treated tumors [Anova: $F = (2, 33)$ 6.907, $p = 0.0031$]. [Post-Hoc Tukey test: Sham vs RCF $p = 0.7373$, Sham vs HCCMF $p = 0.0411$, RCF vs HCCMF $p = 0.0049$]. **g**, IHC of Huh-7 Xenograft tumors and all images at 20X (Lens; scale bar is 50 um). Tumor and intestinal crypt cell proliferation in mice exposed to HCCMF, RCF, and mice not exposed to EMF. Representative figures and graphs (d-f) represent mean +/- SEM (SHAM: N = 3; RCF: N = 3; HCCMF: N = 6) and three randomly selected fields of view per slide were used to quantify all staining. BRDU staining of SHAM, RCF and HCCMF treated tumors showed positive staining in all crypts; no statistics performed.

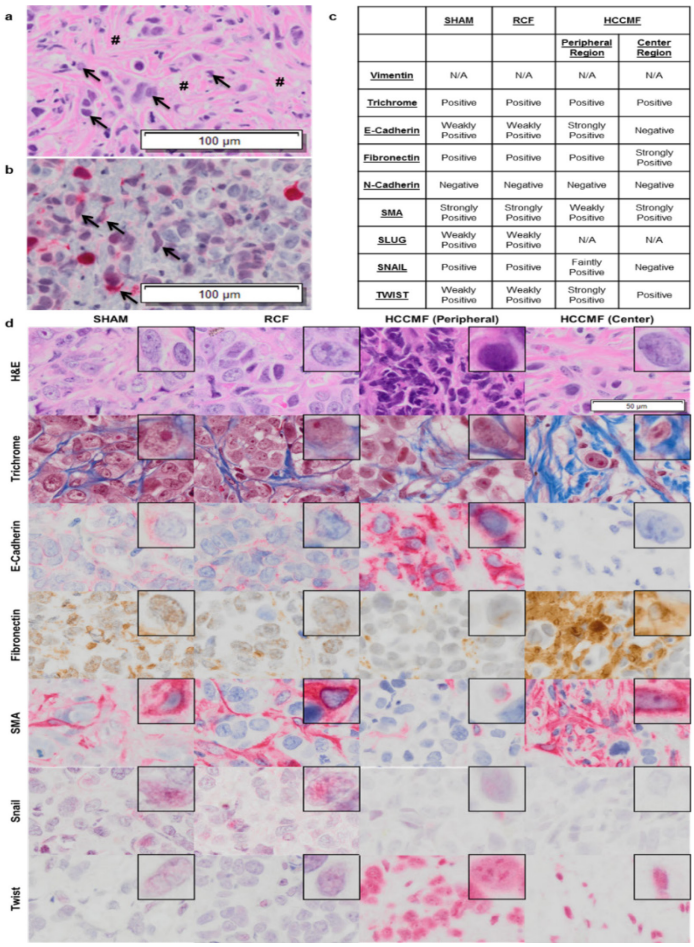
To characterize *in vivo* the temporal relationship between exposure to HCCMF and HCC growth, we randomly selected and studied five mice, which had been exposed to RCF

three hours daily for ten weeks, all of which had evidence of tumor growth while exposed to RCF. As shown in Fig. 2b, the growth of Huh7 xenografts stopped within one week of switching from exposure RCF to exposure to HCCMF (week 11) and the tumors shrank by 62% within three weeks of exposure to HCCMF. To assess the carry-over effect of HCCMF, exposure was stopped at the end of week 14. As shown in Fig. 2b, the average volume of Huh7 xenografts increased by 109% within two weeks. To determine whether repeat exposure to HCCMF could again block tumor growth, mice were re-exposed to HCCMF at week 15. As shown in Fig. 2b tumor growth was, again, effectively blocked within one week of re-exposure. Lastly, to assess *in vivo* the effect of HCCMF on patient-derived xenografts (PDX), 6 PDX mice were exposed to HCCMF. Four PDX mice were observed as controls. As shown in Fig. 2c, tumor growth was significantly inhibited by HCCMF, test for treatment by time interaction ($p = 0.0006$).

In summary, there was significant tumor shrinkage ranging from near complete and partial responses to tumor stabilization in two different mouse models of HCC, which establishes that HCCMF exert sustained control over the growth of HCC tumors *in vivo* at SAR levels corresponding to the levels delivered in patients with advanced HCC. In contrast, there was no shrinkage in any tumors exposed to randomly chosen frequencies or not exposed to any EMF.

1.2.7 Tumor shrinkage is associated with HCC cell differentiation into quiescent cells with spindle morphology

Having demonstrated that exposure to HCCMF results in shrinkage of Huh7 tumor xenografts, similarly to what has been observed in patients receiving treatment with HCCMF, we examined the histology of shrunken tumors and tumors from the control group. The first difference between tumors treated with HCC-specific AM RF EMF compared with control tumors was the absence of necrosis [22] Second, we consistently observed accumulation of fibroblast-like cells around and intermeshed with the shrunken HCC xenografts suggesting that fibroblast-like cells had replaced HCC cells (Fig. 3a). To identify the origin of these cells, we used green-fluorescent protein (GFP) tagged Huh7 cells.



	SHAM	RCF	HCCMF	
			Peripheral Region	Center Region
Vimentin	N/A	N/A	N/A	N/A
Trichrome	Positive	Positive	Positive	Positive
E-Cadherin	Weakly Positive	Weakly Positive	Strongly Positive	Negative
Fibronectin	Positive	Positive	Positive	Strongly Positive
N-Cadherin	Negative	Negative	Negative	Negative
SMA	Strongly Positive	Strongly Positive	Weakly Positive	Strongly Positive
SLUG	Weakly Positive	Weakly Positive	N/A	N/A
SNAIL	Positive	Positive	Faintly Positive	Negative
TWIST	Weakly Positive	Weakly Positive	Strongly Positive	Positive

As shown in Fig. 3b, there was strong GFP staining of the fibroblast-like cells surrounding and intermeshed with residual Huh7 cells demonstrating their HCC origin. To further characterize these cells, we performed immunohistochemistry analysis of several fibroblast cell markers. As shown in Fig. 3c-d, marker analysis identified two separate subpopulations of fibroblast-like cells in shrunken tumors compared to control tumors, a peripheral region surrounding a distinct central region. The peripheral cells were positive for E-cadherin, fibronectin, and TWIST, weakly positive for smooth muscle actin (SMA) and faintly positive for SNAIL while the centrally located cells were more intensely positive for fibronectin and SMA but did not stain for E-cadherin, N-cadherin and SNAIL and had decreased staining for TWIST (Fig. 3c).

Figure 3. Histological analysis of Huh7 xenograft tumors.
Tumors were exposed to either no treatment (SHAM), or randomly chosen frequencies (RCF) or HCC-specific frequencies (HCCMF) three hours daily at a SAR of 67 mW/kg. The selected control tumors (SHAM & RCF) grew in size while tumors exposed to HCCMF shrank by approximately 70%. **a**, Fibroblast-like cells intermeshed with tumor cells following HCCMF mediated tumor shrinkage. Histological analysis 10X (Lens; scale bar is 100um) shows residual tumor cells (→) surrounded by layers of fibroblast-like cells (#) and occasional lipocytes. **b**, Epithelial neoplasm (Huh7-GFP tagged cells) intermeshed with vector red positive cells (→) of spindle morphology demonstrating the same cell of origin for the two morphologically different populations 10X (Lens; scale bar is 100um). **c**, IHC summary of multiple IHC stains for SHAM, RCF, and HCCMF treated tumors. **d**, IHC staining images of Huh7 tumors following treatment. Images at 20X (Lens; scale bar is 50 um) with upper right embedded image at 60X (lens).

These findings suggest that centripetal tumor shrinkage during exposure to HCCMF

results in the differentiation of HCC cells into quiescent cells with spindle morphology at the centre with residual carcinoma-like cells at the periphery of the shrunken tumor.

To test the hypothesis that HCCMF only targets the proliferation of HCC cells in mice carrying tumor xenografts, we assessed tumor Ki67 as well as intestinal crypt BrdU staining in mice in which HCCMF had yielded at least 55% tumor shrinkage as well as in control mice. As shown in Fig. 2d-f, Ki67 and cyclin D1 staining was decreased and p21 increased in the tumors of mice exposed to HCCMF compared to control mice. However, there was no difference in intestinal crypt BrdU staining between mice exposed to HCCMF and control mice (Fig. 2g). Similarly, there were no differences in white blood cells, red blood cells and platelets between HCCMF-exposed and control mice at the time of sacrifice [22]. In summary, HCCMF target HCC cell proliferation *in vivo* without affecting the proliferation of other cells and tumor shrinkage occurs through HCC cells differentiation into quiescent cells with spindle morphology.

1.2.8 HCCMF antiproliferative effects require calcium influx through $Ca_v3.2$ T-type Voltage Gated Calcium Channels (CACNA1H)

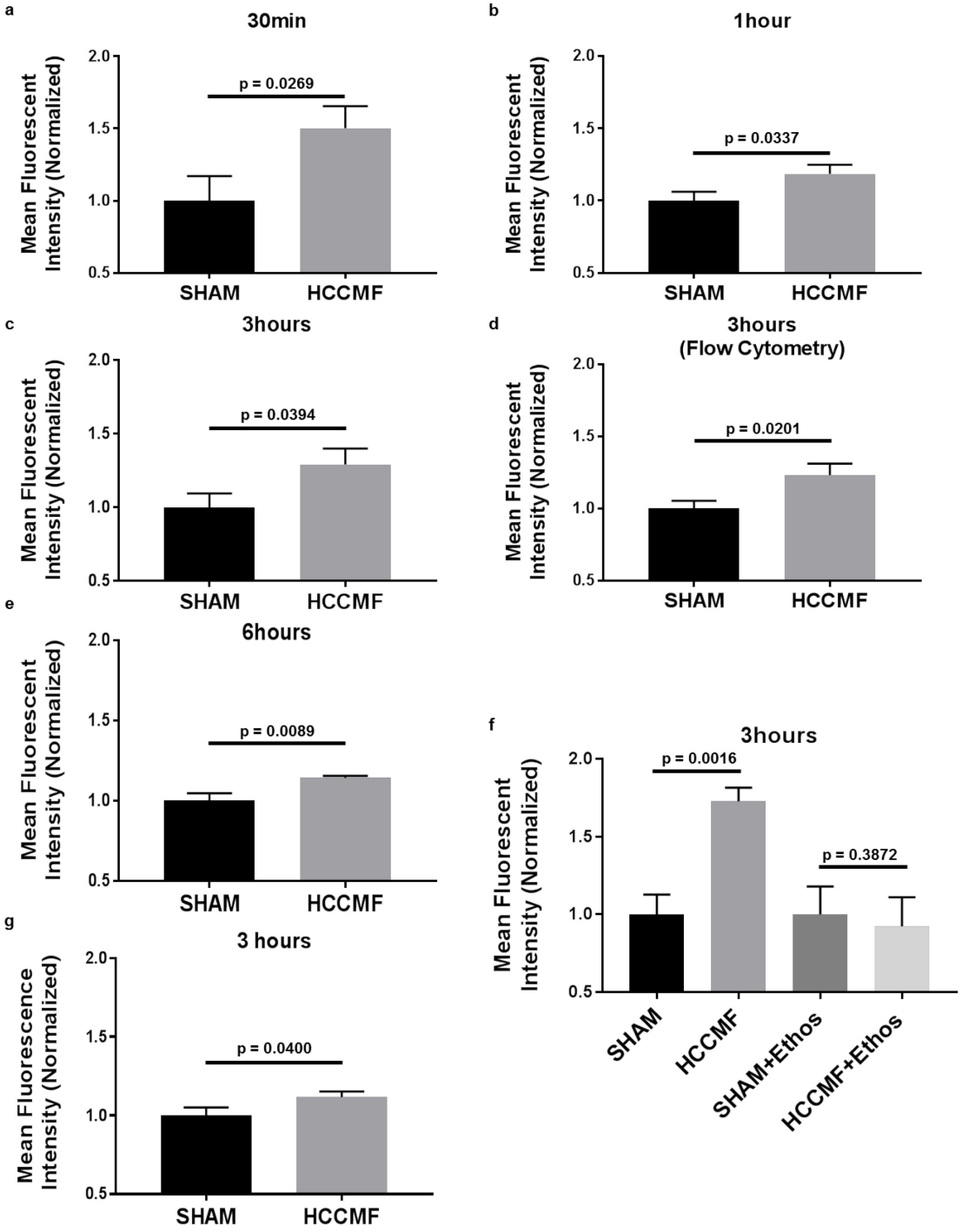
To agnostically assess the impact of HCCMF on HCC, we performed a combined review of the previously published RNA-Seq data [40] and new microRNA array assays. Ingenuity pathway analysis identified the IP3/DAG signalling pathway through differential expression of several genes and microRNAs. Differential expression of several key genes and microRNAs modulating this pathway was confirmed in Huh7 cells (Jimenez, 2019 #217) Ca^{2+} modulates several steps of this pathway,(Albarran, 2016 #280) and several investigators have shown that Ca^{2+} flux from brain tissue is enhanced upon exposure to RF EMF but only when modulated at specific frequencies, irrespective of the carrier frequency used (50, 147, and 450 MHz).[41, 42].We therefore postulated that Ca^{2+} is involved in HCCMF antiproliferative effects on HCC cells. To begin to test this hypothesis, we added BAPTA, a chelator of Ca^{2+}, during each HCCMF exposure. As shown in Fig. 5a, chelation of extracellular Ca^{2+} abrogates HCCMF antiproliferative effects without affecting the proliferation of Huh7 cells exposed to RCF demonstrating that extracellular Ca^{2+} plays a central role in this process.

Next, we sought to determine if extracellular Ca^{2+} enters HCC cells upon exposure to HCCMF. We validated changes in intracellular Ca^{2+} following exposure to HCCMF with Fluo-4 calcium imaging. As shown in Fig. 4, intracellular Ca^{2+} was significantly increased after exposure of HCC cells (Huh7) to HCCMF for 30 min, one hour, three-hour, and six-hour. We additionally validated increased intracellular Ca^{2+} in a second HCC cell line

(Hep3B) at the three-hour time point (Fig. 4). However, no increase in intracellular Ca^{2+} was observed after exposure to either RCF or breast cancer-specific AM RF EMF (data not shown). Moreover, there were no changes in intracellular Ca^{2+} levels when culturing cells in Ca^{2+}-free medium demonstrating that HCCMF-mediated increase in intracellular Ca^{2+} is due to Ca^{2+} influx from the extracellular to the intracellular compartment, not by mobilization of intracellular Ca^{2+} stores. Lastly, HCCMF exposure did not have an impact on intracellular Ca^{2+} levels in human non-malignant hepatocytes (THLE2; ATCC), human microglia cells (HMC3; ATCC) or Rat myocardium cells (H9c2; ATCC) (data not shown) demonstrating that Ca^{2+} influx is both tumor- and tissue-specific as it only occurs when HCC cells are exposed to HCCMF.

Having demonstrated that extracellular Ca^{2+} influx was necessary for HCCMF inhibition of HCC cell proliferation, we sought to identify how Ca^{2+} enters the cells. Ca^{2+} entry is mainly controlled by voltage-gated Ca^{2+} channels (VGCCs) [43-45]. We used inhibitors of L-type and T-type VGCCs to determine whether these VGCCs are involved in HCCMF Ca^{2+} influx and inhibition of cell proliferation. Amlodipine, which blocks L-type VGCCs, did not alter Ca^{2+} influx (data not shown). In contrast, ethosuximide, which blocks all three T-type VGCCs (Ca_v 3.1, 3.2, 3.3), (Gomora, Daud et al. 2001) abrogated HCCMF's Ca^{2+} influx as well as inhibition of Huh7 cell proliferation (Fig. 5b). Having identified T-type VGCCs as the necessary mediators of HCCMF Ca^{2+} influx and inhibition of HCC cell proliferation,we asked which of the three T-type VGCCs isoforms accounted for this effect. While knockdown of $Ca_v3.1$ (CACNA1G) and $Ca_v3.3$ (CACNA1I) did not affect HCCMF-mediated inhibition of HCC cell proliferation, knockdown of $Ca_v3.2$ (CACNA1H) abrogated HCCMF antiproliferative effects in Huh7 and Hep3B cell lines (Fig. 5c).
In summary, HCCMF exert their selective antiproliferative effects on HCC cells by targeting CACNA1H, an event contingent on Ca^{2+} influx into HCC cells, an event restricted to HCC cells.

Figure 4. Intracellular Ca^{2+} measurement in Huh7 and Hep3B cells following exposure to AM RF EMF. **a**, Huh7: 30 min HCCMF exposure results in 50.2% increase in fluorescence (p = 0.0296), N =6 per group. **b**, Huh7: one-hour HCCMF results in 18.6% increase in fluorescence (p = 0.0337), N =5. **c**, Huh7: three-hour HCCMF results in 29.2%



increase in fluorescence (p = 0.0394), N =5 per group. **d**, Huh7: three-hour HCCMF exposure results in 23.2% increase in fluorescence (p = 0.0201), N =5 per group. Measured by Flow Cytometry. **e**, Huh7 six-hour HCCMF exposure of Huh7 cells results in 14.3% increase in fluorescence (p = 0.0089), N =5 per group. **f**, Huh7 three-hour HCCMF results in 72.8% increase in fluorescence (p = 0.0016), N = 6 per group. There was no increase in intracellular Ca^{2+} in the presence of 0.5 mM Ethosuximide (Ethos) (p-value 0.3872). **g**, Hep3B three-hour HCCMF exposure of Hep3B cells results in 11.68% increase in fluorescence (p = 0.0400), N =11 SHAM & N = 10 HCCMF. All fluorescent data was read using a Fluostar fluorescent plate reader (unless otherwise stated i.e. flow cytometry) with 485 excitation/520 emission and calcium staining was accomplished using the Fluo-4 calcium imaging kit (Molecular Probes). All experiments performed at least twice with representative experiments shown. [Student's one-tailed t-test was used to identify statistical significance as Ca^{2+} <u>influx</u> (directionality) was identified and established].

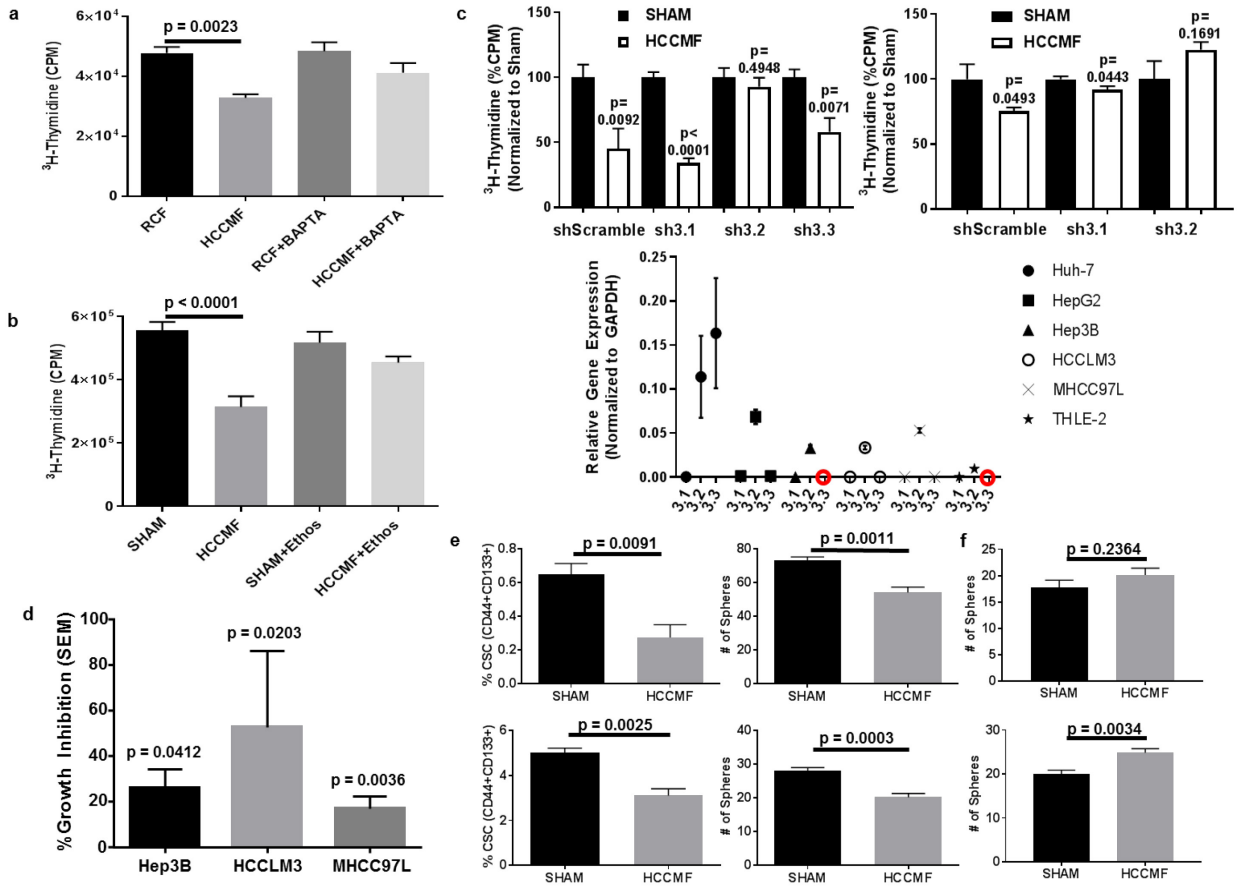


Figure 5. HCCMF antiproliferative effects on HCC cells and downregulation of CSCs are mediated by Cav 3.2 T-type voltage gated calcium channels (CACNA1H). **a**, Ca^{2+} chelation abrogates AM RF EMF-mediated inhibition of Huh7 cell proliferation. Huh7 cells were exposed to either randomly chosen (RCF) or hepatocellular carcinoma-specific (HCCMF) AM RF EMF three hours daily for seven days prior to cell proliferation assays with tritiated thymidine incorporation. ANOVA followed by Tukey post hoc-test: [Anova: F = (3, 20) 8.258, P = 0.0009] showed that proliferation of cells exposed to HCCMF was significantly lower than cells exposed to RCF [Post-Hoc Tukey Test p = 0.0023]. **b**, T-type voltage gated calcium channel blockade with ethosuximide abrogates HCCMF inhibition of Huh7 cell proliferation. Huh7 cells were exposed to HCCMF three hours daily for seven days prior to cell proliferation assays. Huh7 cells not exposed to AM RF EMF (SHAM) were used as controls. Experiments were performed in the presence or absence of ethosuximide (Ethos). ANOVA followed by Tukey Post Hoc-Test [Anova: F = (3, 36) = 13.06, P < 0.0001] indicates that only HCCMF block Huh7 cell proliferation [Post-Hoc Tukey Test: SHAM vs HCCMF p < 0.0001]. **c**, Inhibition of cell proliferation by HCCMF in Huh7 and Hep3B cells with selective knockdown of Cav 3.1, 3.2 and 3.3 T-type voltage

gated calcium channels. The three T-type voltage-gated calcium channels Cav 3.1, Cav 3.2, and Cav 3.3 were selectively knocked down using siRNA targeting the CACNA1G (sh3.1), CACNA1H (sh3.2), and CACNA1I (sh3.3) genes, respectively. CACNA1I (sh3.3) expression in Hep3B cells was not detectable by qPCR, as noted in Cav isoform relative expression graph, hence knockdown was not attempted. Cell proliferation was assessed after exposure to HCCMF three hours daily for seven days. Huh7 data (LEFT): shScramble (N=5 for both groups) [Student's two-tailed T-test p value: 0.0092]; sh3.1 (N=6 for both groups) [Student's two tailed t-test p value: < 0.0001]; sh3.2 (N=6 for both groups) [Student's two-tailed t test p value: 0.4948]; Sh3.3 (N=6 for both groups) [Student's two-tailed t-test p-value: 0.0071]. Hep3B data [46].: shScramble (N=5 for SHAM and N=6 for HCCMF) [Student's two-tailed t-test p-value: 0.0493]; sh3.1 (N=6 for SHAM and N=5 for HCCMF) [Student's two-tailed t-test p-value: 0.0443]; sh3.2 (N=6 for both groups) [Student's two-tailed t-test p-value: 0.1691]. (Lower): Basal expression levels of T-type voltage-gated calcium channels isoforms Cav 3.1, Cav 3.2, and Cav 3.3 in multiple cell lines (RED circles signify targets with no detectable expression via qRT-PCR). **d**, Antiproliferative effects of HCCMF on HBV positive HCC cells. Cell proliferation was assessed in HCC cells exposed to HCCMF using a [^{3}H] thymidine incorporation assay as described before. **e**, Effect of HCCMF on HCC cancer stem cells. The cancer stem cell population of Huh7 (upper panel) and Hep3B (lower panel) cells was assessed after one-week exposure to HCCMF. Control cells were not exposed to EMF. The population of cancer stem cells was significantly lower following exposure to HCCMF. (UPPER PAIR) Huh7 %CSC Data: SHAM (N = 4) and HCCMF (N = 4); [Student's two-tailed t-test p-value = 0.0091]. Huh7 Sphere formation: SHAM (N = 5) and HCCMF (N = 5); [Student's two-tailed t-test p-value = 0.0011]. (LOWER PAIR) Hep3B %CSC Data: SHAM (N = 4) and HCCMF (N = 3); [Student's two-tailed t-test p value = 0.0091]. Hep3B Sphere formation: SHAM (N = 7) and HCCMF (N = 6); [Student's two-tailed t-test p-value = 0.0011]. **f**, Effect of HCCMF on Cav 3.2 knockdown HCC cancer stem cells. The cancer stem cell population of Huh7 Cav3.2 knockdown (upper panel) and Hep3B Cav 3.2 knockdown (lower panel) cells was assessed after one week of exposure to HCCMF. Control cells were not exposed to EMF. The population of cancer stem cells was equal to or greater than the control group following exposure to HCCMF. (UPPER) Huh7 Cav 3.2 knockdown sphere formation: SHAM (N = 5) and HCCMF (N = 5); [Student's two-tailed t-test p-value = 0.2364]. (LOWER) Hep3B Cav 3.2 knockdown sphere formation: SHAM (N = 6) and HCCMF (N = 7); [Student's two-tailed t-test p-value = 0.0034].

1.2.9 HCCMF block HCC cancer stem cells

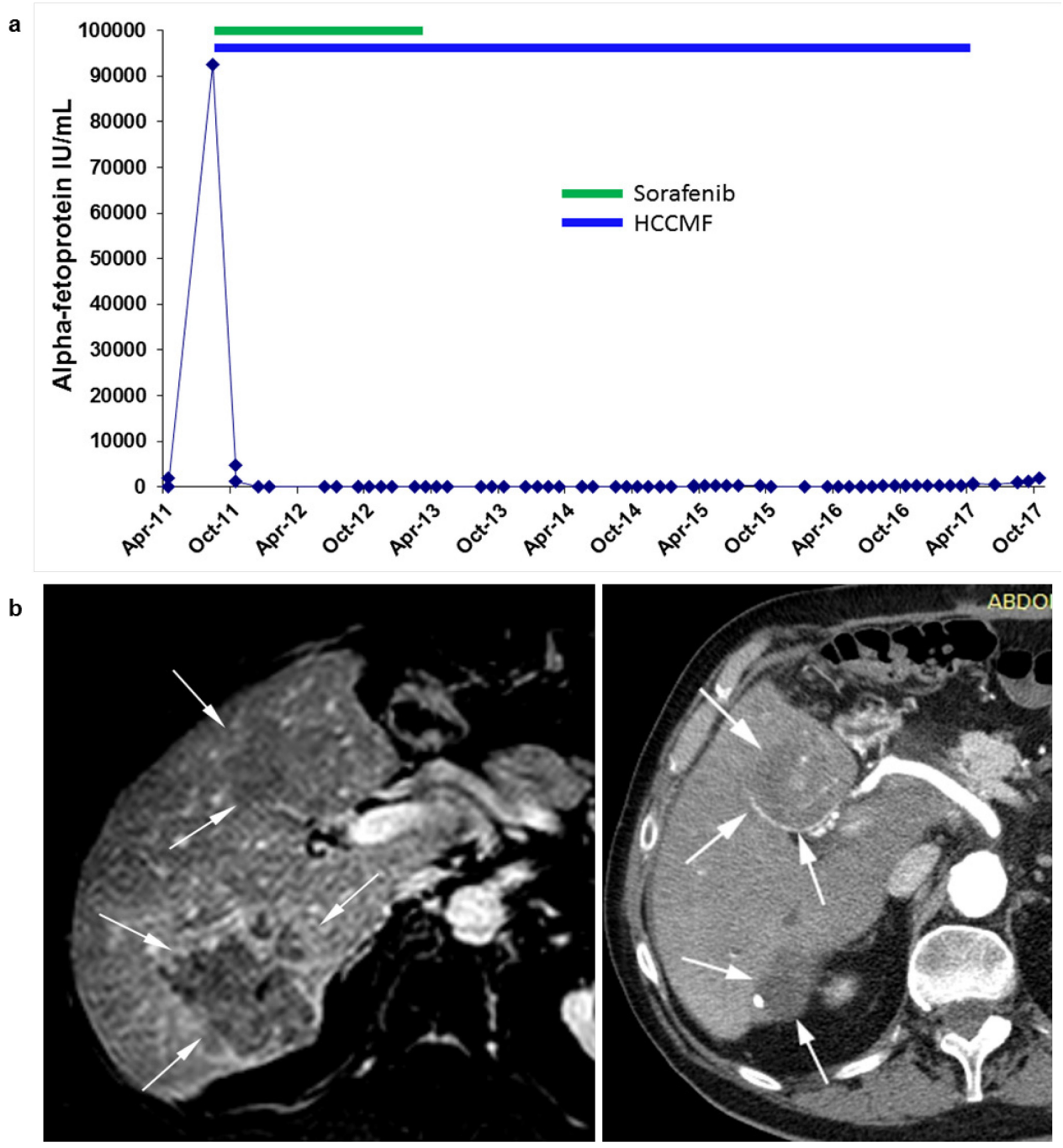
It has been shown that sorafenib and lenvatinib significantly improve the survival of patients with advanced HCC.[6] However, patients invariably develop resistance to both agents [6, 7, 47, 48]. There is growing evidence that HCC cancer stem cells (CSCs) are responsible for tumor recurrence and resistance to sorafenib [49-51] We have previously reported several long-term responses in patients with advanced HCC receiving intrabuccally administered AM RF EMF. Specifically, 6 (14.6%) of the 41 patients receiving treatment with the TheraBionic device, had an overall survival in excess of 26 months, one patient was treated continuously for 44.6 months, and one patient was treated continuously for 62 months without any evidence of disease progression prior to expiring of causes unrelated to her malignancy.[20], [21] One additional off-study patient with rapidly progressive disease received continuous treatment with HCCMF for 74 months prior to expiring with minimal progression of disease (section 1.2.10). These unexpectedly long-lasting responses, which have not been observed with the use of either sorafenib or lenvatinib [48], led us to test the hypothesis that HCCMF target CSCs as therapies affecting CSCs are associated with long-term survival [52].

We first assessed the anti-proliferative effects of HCCMF on hepatitis B virus (HBV) positive cell lines as 53% of HCC cases worldwide are attributable to HBV infection.[53]. As shown in Fig. 5d, the proliferation of HBV positive HCC cell lines from patients of Asian (HCCLM3 and MHCC97L) and African American (Hep3B) ancestry was effectively blocked by HCCMF. Next, we assessed the impact of HCCMF on HBV negative (Huh7) and HBV positive (Hep3B) CSCs. As shown on Fig. 5e, exposure to HCCMF led to 57% and 38% decreases in Huh7 and Hep3B CSCs (CD44+CD133+) cells, respectively. Sphere formation was similarly decreased by 26% and 28% in Huh7 and Hep3B cells, respectively. However, in the presence of ethosuximide, there were no changes in CSCs or sphere formation in either Huh7 or Hep3B cells demonstrating that inhibition of CSCs is also mediated by T-type VGCCs. The experiments were repeated with cells in which CACNA1H had been knocked down. As shown in Fig. 5f, knockdown of CACNA1H abrogated HCCMF downregulation of CSCs in Huh7 as well as Hep3B cells.

1.2.10 Potential synergy between AM RF EMF tyrosine kinase inhibitors

Figure 6: Long-term response in a patient with metastatic hepatocellular carcinoma receiving treatment with HCCMF (a) [22] A 79-year-old man was diagnosed in January 2011 with hepatitis A and B negative hepatocellular carcinoma. He underwent left hepatectomy in February 2011, which revealed the presence of a poorly differentiated hepatocellular carcinoma. The tumors largest diameter was 10 cm and was staged as pT3NxMx. In May 2011, the patient had evidence of disease progression with four new

lesions identified within the right lobe of the liver and new mediastinal adenopathy. Chemoembolization with doxorubicin and lipiodol was performed in June 2011. A follow up MRI performed in July 2011 showed progression of disease. Treatment with sorafenib was initiated in August 2011 when the patient's performance status was KPS 80%, ECOG 1 with a Child Pugh of A6. Treatment with sorafenib was discontinued in February 2013 because of intolerable side effects. Simultaneous compassionate treatment with the TheraBionic device was administered 3 hours a day from September 2011 until April 2017 when the patient became unable to receive treatment on a regular basis because of hip fracture secondary to a fall, generalized weakness, and worsening renal failure. He passed away in October 2017. The patient had complete response by marker as the level of alpha foetal protein (AFP) decreased from 92,620 international units/ml (IU/ml) on August 12, 2011 prior to treatment initiation to 4.18 IU/ml on January 16, 2011. Following discontinuation of Nexavar in February 2013 the patient's AFP level remained below 20 IU/ml until May 2014 when it started to rise slowly and was 553.0 IU/ml in June 2017 prior to admission to the hospital for left femur fracture repair.



The AFP level began to rise more rapidly from the time the patient became unable to receive TheraBionic treatment until October 9, 2017, when it reached 2038.0 IU/ml. **(b)** Key imaging studies of the same patient. Imaging studies obtained prior (left panel: July 2011) and during (Right panel: December 2011) treatment show partial response as assessed by RECIST criteria

2.0 Objectives

The primary goal of this study is to gather efficacy data concerning the progression-free survival rate with EMF + Regorafenib when compared to historical data with Regorafenib alone as a second-line therapy in patients with advanced HCC who have received any first line systemic therapy either standard of care Sorafenib or Lenvatinib or any experimental therapy. Patients who have received any treatment that includes either EMF or Regorafenib will be excluded.

2.1 Primary Objective

2.1.1 To estimate progression-free survival rates according to the RECIST 1.1{Eisenhauer, 2009 #7245} and modified RECIST (mRECIST) for HCC [54]

2.2 Secondary Objectives

2.2.1 To obtain information concerning Overall survival-defined as date of enrollment to date of death; progression-free survival at 4 months, 6- month survival rate, proportion of patients with disease control (complete or partial response or stable disease) according to RECIST 1.1 and modified RECIST (mRECIST) for HCC [54] at 4 and 6 months, and time to radiologic progression.

2.2.2 To evaluate type, incidence, severity (graded by the National Cancer Institute [NCI] Common Terminology Criteria for Adverse Events [CTCAE], version 5.0), timing, seriousness, and relatedness of adverse events and laboratory abnormalities

2.2.3 To evaluate the effect on levels of alpha-fetoprotein.

3.0 Patient Selection

3.1 Inclusion Criteria

- Biopsy-proven HCC that is locally advanced or metastatic. Or

    - Patients without biopsy confirmation are also eligible if they meet the following:
    - Radiologic diagnosis of HCC as per the AASLD guidelines:
    - –liver cirrhosis AND

- –a liver mass confirmed by BICR that shows arterial phase hyperenhancement on triphasic CT or MRI, AND EITHER:
- Is ≥20 mm with either non-peripheral portal washout or an enhancing capsule
- OR is 10-19 mm with non-peripheral portal venous washout AND an enhancing capsule {Marrero, 2018 #302}

• Patients must have been treated with at least one standard systemic treatment modality for advanced HCC such as sorafenib, lenvatinib, atezolizumab plus bevacizumab, or another approved or experimental systemic therapy prior to study entry.
• Measurable disease according to RECIST version 1.1{Eisenhauer, 2009 #7245} and mRECIST for HCC [54].
• At least one target lesion should not have previously received any local therapy, such as surgery, radiation therapy, hepatic arterial embolization, TACE, hepatic arterial infusion, radio-frequency ablation, percutaneous ethanol injection or cryoablation, unless it has subsequently progressed by 20% or more according to RECIST version 1.1{Eisenhauer, 2009 #7245} and mRECIST for HCC [54].
• Patients with Child's Pugh A (at time of enrollment), with compensated cirrhosis, as defined by the parameters contained in the Child Pugh Calculator found in Appendix E [55].
• Performance status ECOG 0-2
• Absence of medical or psychiatric contraindication which, in the opinion of the treating Investigator, would make the patient's participation in this trial inappropriate.
• Patient must not have curative treatment options, including surgery or radiofrequency ablation, available.
• Any extra-hepatic metastases, including treated CNS metastases but patients cannot have leptomeningeal disease.
• At least 2 weeks must have elapsed since administration of any anti-cancer treatment.
• Other anti-cancer treatments are not permitted during this study, including alternative medicine and herbal therapies.
• Patients must be 18+ years old and must be able to understand and sign an informed consent.
• Patient must agree to be followed up according to the study protocol.

3.2 Exclusion Criteria

• Known leptomeningeal disease.
• Fibro lamellar HCC.
• Patients who had surgical resection of the disease and who do not have measurable disease.

• Patients with any of the following history within the 12 months prior to study drug administration: severe/unstable angina, myocardial infarction, coronary artery bypass graft, symptomatic congestive heart failure, cerebrovascular accident, including transient ischemic attack, or pulmonary embolism.
• Pregnant or breastfeeding women
• Patients diagnosed with another type of cancer (excluding basal cell carcinoma) whose cancer diagnosed previously is not in remission.
• Patients receiving calcium channel blockers and any agent blocking L-type or T-type Voltage Gated Calcium Channels, e.g. amlodipine, nifedipine, ethosuximide, etc. are not allowed in the study unless their medical treatment is modified to exclude calcium channel blockers prior to enrollment. **(Refer to Appendix F for a complete list of excluded medications and/or pathways).**
• Patients allergic or intolerant to Sorafenib.

3.3 Inclusion of Women and Minorities

Men and women of all races and ethnicities who meet the above-described eligibility criteria are eligible to participate in this study.
The study consent form will also be provided in Spanish for Spanish-speaking participants. Based on CCCWFU population estimates, we expect approximately 40% of participants to be women. We do not expect the percentage of Hispanic/Latino or racial minority cancer patients eligible for this study to be higher than the percentage of Hispanic or racial minority new cancer patients seen at CCCWFU (1.7% and 14.4%, respectively). Should we not meet or exceed these estimates, the PI will engage the Cancer Center Health Equity Advisory Group to discuss strategies to enhance recruitment in these target populations.

3.4 Life Style Guidelines

Female patients must be surgically sterile or be postmenopausal, or must agree to use adequate contraception during the period of therapy. Male patients must be surgically sterile, or must agree to use adequate contraception during the period of therapy. Adequate contraception is defined as double barrier contraception (i.e., condom plus spermicide in combination with a female condom, diaphragm, cervical cap or intrauterine device). List of adequate contraceptives in accordance with local regulations may be reported in the patient's Informed Consent Document.
If a female patient becomes pregnant while on therapy, treatment will stop immediately. The patient will be assisted by the investigator physician during her pregnancy. If the outcome of the pregnancy meets the criteria for immediate classification as a serious

adverse event, i.e. spontaneous abortion, stillbirth, neonatal death, or congenital anomaly (including that in an aborted fetus, stillbirth or neonatal death), the investigator should follow the procedures for reporting serious adverse events.
In the case of a live birth, the "normality" of the newborn can be assessed at the time of birth. The "normality" of an aborted fetus can be assessed by gross visual inspection, unless pre- abortion test findings are suggestive of a congenital anomaly.
Additional information about pregnancy outcomes that are classified as serious adverse events follows: "Spontaneous abortion" includes miscarriage and missed abortion. All neonatal deaths that occur within 1 month of birth should be reported, without regard to causality, as serious adverse events. In addition, any infant death after 1 month that the investigator assesses as possibly related to the exposure in utero to the investigational treatment should be reported. Further follow-up of birth outcomes will be handled on a case-by-case basis (eg.. follow-up on preterm infants to identify developmental delays)

4.0 Registration Procedures

All patients entered on any WFBCCC trial, whether treatment, companion, or cancer control trial, **must** be linked to the study in EPIC within 24 hours of Informed Consent. Patients **must** be registered prior to the initiation of treatment.

You must perform the following steps in order to ensure prompt registration of your patient:

1. Complete the Eligibility Checklist (Appendix A)
2. Complete the Protocol Registration Form (Appendix B)
3. Alert the Cancer Center registrar by phone, *and then* send the signed Informed Consent Form, Eligibility Checklist and Protocol Registration Form to the registrar, either by fax or e-mail.

   Contact Information:
   Protocol Registrar PHONE (336) 713-6767
   Protocol Registrar FAX (336) 713-6772
   Protocol Registrar E-MAIL (registra@wakehealth.edu)

   *Protocol Registration is open from 8:30 AM - 4:00 PM, Monday-Friday.

4. Fax/e-mail ALL eligibility source documents with registration. Patients **will not** be registered without all required supporting documents.

Note: If labs were performed at an outside institution, provide a printout of the results. Ensure that the most recent lab values are sent.

To complete the registration process, the Registrar will:

- assign a patient study number
- assign the patient a dose
- other appropriate actions
- register the patient on the study

5.0 Study Outcomes and Study Measures

5.1. Primary Outcome

Primary outcome is progression-free survival. This measure will be assessed throughout the trial.

5.2 Secondary Outcomes

5.2.1 Overall survival defined as date of enrollment to date of death; progression-free survival at 4 months, 6- month survival rate, proportion of patients with disease control (complete or partial response or stable disease) according to the modified RECIST (mRECIST) for HCC [54] at 4 and 6 months, and time to radiologic progression using standard RECIST v1.1 criteria.

5.2.2 Type, incidence, severity (graded by the National Cancer Institute [NCI] Common Terminology Criteria for Adverse Events [CTCAE], version 5.0), timing, seriousness, and relatedness of adverse events and laboratory abnormalities

5.2.3 Alfa-fetoprotein levels.

6.0 Study-Related Activities

	Pre-Study [a]	Baseline	Phone call	Clinic Visit	Imaging	End of Treatment Visit	Long Term Follow Up
		(Cycle 1, Day 1)	(Cycle 1, day 15)	Week 5 and on the first day of subsequent cycles	Week 7 & every 6 weeks thereafter	Within 30 days of withdrawal or progression	
		Week 1	Week 3	Week 5	Week 7	At progression	
Informed Consent	X						
Baseline Documentation	X						
Regorafenib		X		X			
Concomitant Medications and Treatments	X					X	
Pre-Toxicity	X						
Medical/Oncologic History and Demographics	X						
Physical Examination, including oral exam	X	X		X	X	X	
Hematology and Blood Chemistry including Alpha-feto protein [d]	X			X		X	
Coagulation	X					X	
Pregnancy Test [b]	X			X		X	
Correlative Blood Studies	X					X	
dynamic multiphase contrast-enhanced CT/MRI Scan [h]	X				X		
ECOG PS, body weight, and vital signs	X			X			
Bone Scan [f]	X				X		
Adverse Events Signs and Symptoms		X	X	X	X	X	
Treatment Compliance Diary[c]			X	X		X	
Device Reconciliation Form			X	X		X	
Post-Study Anticancer Treatment [g]						X	X
Post-Study Survival Status [g]						X	X
EMF [e]		X		X			
a Pre-study requirements listed in table must be completed within 14 days prior to treatment initiation with the exception of hematology and blood chemistry and pregnancy test which should be performed with 7 days prior to treatment initiation.							

b Serum pregnancy test (women of childbearing potential) ± 7 days prior to treatment initiation
c All patients included in this study will be evaluated by a research nurse in order to monitor treatment compliance using a specific device as described in section 7.1., and conduct an initial assessment of symptoms and symptom intensity as per the National Cancer Institute Common Toxicity Criteria (NCI-CTC) version 5.0
d Hematology and blood chemistry should include CBC with WBC differential count, platelet count, sodium, potassium, BUN, creatinine, fasting glucose, bilirubin (direct and indirect), transaminases (ASAT and ALAT), LDH, alkaline phosphatase, gamma glutamyl transferase, albumin, PT and INR, AFP, and hepatitis B and C serology. Hematology and blood chemistry screening assessment is required within 7 days prior to the start of study treatment as is standard of care.
e At baseline visit, subject will receive device education, first 60 minute treatment, and be provided with treatment logs.
f Bone scan will be required only in case of clinical suspicion of central nervous system metastases or bone metastases at screening or subsequently.
g Post-Study Survival Status will be recorded after discontinuation of study treatment Any new post-study anticancer treatment will be recorded. This data will be collected by clinic visit or telephone contact approximately every 2 months until one year after end of treatment visit or death.
h Brain MRI/CT will be required only in case of clinical suspicion of central nervous system metastases or bone metastases at screening or subsequently.

6.1 Description of Procedures

Screening and Consent

- The screening period is planned to last a maximum of 14 days prior to treatment initiation. All study procedures necessary for patient selection, determination of eligibility and treatment initiation should be performed within this period unless otherwise indicated.
- Informed Consent must be obtained prior to undergoing any study procedure and should occur prior to the 14-day screening period. Pre-Study Assessments.
- Medical/Oncology History and Demographics should include age, race, sex, hepatitis virus exposure, Child-Pugh score, Barcelona score (BLCL), CLIP score, information on prior cancer treatments, and histological diagnosis of HCC by previous or recent biopsy.
- Concomitant medications and treatments and the amount per day will be recorded at registration.
- Baseline Signs and Symptoms will include any signs or symptoms experienced within the past 14 days. Assessment of symptoms and symptom intensity will be per the National Cancer Institute Common Toxicity Criteria (NCI-CTC) version 5.0
- Physical Examination should include examination of major body systems, body weight, height, abdominal diameter, ECOG performance status, and vital signs (temperature, blood pressure, resting heart rate, respiratory rate) and an intraoral examination.
- Hematology and blood chemistry should include CBC with WBC differential count, platelet count, sodium, potassium, BUN, creatinine, fasting glucose, bilirubin (direct and indirect), transaminases (ASAT and ALAT), LDH, alkaline

phosphatase, gamma glutamyl transferase, albumin, PT and INR, AFP, and hepatitis B and C serology. Hematology and blood chemistry screening assessment is required within 7 days prior to the start of study treatment.

- Pregnancy Test (Serum or Urine) is mandatory for women of reproductive potential and must be tested within 7 days prior to the start of study treatment.
- Correlative blood tests will involve collection of 8.5 ml blood draw volumes from each patient. The blood samples will be collected into PAXgene Blood DNA Tubes (Qiagen) containing a proprietary blend of reagents that both prevents blood coagulation and stabilizes genomic DNA in white blood cells. The tubes are identified by a blue Hemogard stopper. Samples will be collected and transported at room temperature then refrigerated within 24 hours of collection. Samples will transported by study staff to the Pasche lab, NRC, 441. Samples may be stored at 2-8° C refrigeration for 1-7 days, then moved to -80° C for longer storage as needed for batch DNA processing.
- Tumor Imaging is required within 30 days of treatment initiation and will include dynamic multiphase contrast-enhanced CT with intravenous contrast abdomen, and pelvis, and other applicable sites of disease. If the evaluation by CT-scan does not highlight the lesions in a sufficiently clear way, magnetic resonance imaging studies (MRI) must be obtained within 30 days of treatment initiation. In this case, only MRI studies will be performed to evaluate changes in target lesions.
- Brain CT or MRI Scan and Bone scan will be required only in case of clinical suspicion of central nervous system metastases or bone metastases at screening or subsequently.

During Treatment

• All assessments should be performed prior to cycle initiation unless otherwise indicated. Acceptable time window for performing each assessment is considered a maximum of 3 (three) days.

- Physical Examination should be performed on Day 1 of each cycle. This assessment should include examination of major body systems, ECOG performance status, body weight, abdominal diameter, and vital signs (temperature, blood pressure, heart rate, respiratory rate) and an intraoral examination.
- Hematology and blood chemistry assessments should include: CBC with WBC differential, platelets, sodium, potassium, urea, creatinine, fasting glucose, bilirubin (direct and indirect), transaminases (ASAT and ALAT), LDH, alkaline phosphatase, gamma glutamyl transferase, albumin, PT and INR, and AFP.
- For Cycle 1 Day 1, Hematology and blood chemistry assessments are not required if

acceptable screening assessment is performed within 7 days prior to the start of treatment with study treatment.

- For the subsequent assessments, Day 1 acceptable assessment time window for hematology and blood chemistry is considered a maximum of 3 (three) days prior to starting each treatment follow-up visit.
  - Tumor Imaging will include dynamic multiphase contrast-enhanced CT or MRI scans of the chest, abdomen, and pelvis, and other applicable sites of disease, performed every 6 weeks during the study. The schedule of assessments should be fixed according to the calendar, regardless of treatment delays. Allowable time windows for on study tumor assessments are ±7 days.
- Adverse Events must be followed from the first day of study treatment until at least 28 days after the last on-study treatment administration, or until all serious or study treatment-related toxicities have resolved or are determined to be “chronic” or “stable,” whichever is later. Serious adverse events should be monitored and reported as described in the protocol.

Baseline tumor-related signs and symptoms will be recorded as adverse events during the trial if they worsen in severity or increase in frequency.

- At the beginning of each treatment cycle (Day 1) and at the completion of therapy, accountability and compliance will be verified and documented in CRF or other appropriate documents
- Concomitant medications and treatments will be recorded at study entry and during the study. At the end of the study and at follow-up
- End of Treatment Visit- Device/Withdrawal assessment should be obtained during the last week on study or within 30 days from the patient’s exclusion date. This assessment should include: Physical Examination, including examination of major body systems, ECOG performance status, body weight, abdominal diameter, and vital signs (temperature, blood pressure, heart rate, respiratory rate).Signs and Symptoms as per the National Cancer Institute Common Toxicity Criteria (NCI-CTC) version 5.0. Concomitant medications and treatments should be recorded for 28 days or until all study treatment-related toxicities have resolved, whichever is later.
- Post-Study Survival Status will be recorded after discontinuation of study treatment and any new post-study anticancer treatment will be recorded and post-study survival status will be collected by clinic visit or telephone contact approximately every 2 months until one year after end of treatment visit or death.

6.2 Treatment with Regorafenib

Patients will receive 160 mg regorafenib (four 40 mg tablets) orally once daily for the first

3 weeks of each 4-week cycle per approved prescribing information. Patients may continue on this schedule until they withdraw or experience progression.

6.3 Treatment With TheraBionic

Amplitude-modulated electromagnetic fields will be self-administered and given continuously to patients in three courses of 60-minute treatments per day, administered in the morning, at noon and in the evening at the patient's home, with the exception of the first 60-minute treatment, which will be delivered at the research site. Each 4 week treatment period will be considered a cycle of treatment.

6.3.1 Selection of frequencies

HCC-specific frequencies were identified by Barbault and Pasche using the methods described in section 1.2.4 and 1.2.5. These frequencies have previously been shown to block HCC in vitro,[36] [22] in vivo,[22] as well as in patients with advanced HCC as a single anticancer modality [20] and in combination with a tyrosine kinase inhibitor.

6.3.2 Use of the TheraBionic P1 device

Instructions for patient use: The patients included in the study will receive the TheraBionic P1 device free of charge for home use as well as the accompanying instructions manual. The device will be programmed with HCC-specific modulation frequencies and will be activated for more than 200 one-hour treatment sessions.

Prior to treatment initiation the study coordinator for the study will recharge the device’s batteries for 24 h as described in section 4.2, page 14 of the User Manual (TheraBionic P1 User Manual, IFU_TheraBionic_P1_USEN_rev07_2018-06-20n). The exact number of HOURS:MINUTES available prior to administration of the first treatment will be documented and constitute the pre-treatment baseline. The total number of HOURS:MINUTES appears on the TheraBionic P1 device display after the device is switched on as shown in section 4.6, page 21 of the Instruction Manual and on the TheraBionic.com web site: . https://www.therabionic.com/use-of-the-therabionic-p1/.

6.3.3 Treatment location

With the exception of the first 60-minute treatment, which will be delivered at the clinic, all other treatments will be self-administered at the patient's home. Each treatment day

with consists of three courses of 60-minute treatments per day, administered in the morning, at noon and in the evening. It is not necessary to keep rigid treatment schedules. However, it is recommended to keep schedules consistent over time. Each treatment should be delivered without interruption. Each 4 week treatment period will be considered a cycle of treatment, similar to each regorafenib treatment cycle.

6.3.4 Treatment interruption

Treatment will be allowed to be interrupted temporarily in case of:

- Limited toxicity
- Potentially reversible clinical condition, which temporally prevents continuation of treatment, e.g. gastrointestinal bleeding.
- Hospitalization that prevents continuation of treatment.
- Technical problems related to the TheraBionic P1 device until replacement of a new TheraBionic P1 device. Treatment should be restarted, without modification in the above described treatment plan, if treatment was interrupted for at most 21 days. In case of any treatment interruption of longer duration, the patient should be excluded from the study.

6.3.5 Treatment compliance

At each visit treatment compliance will be assessed by the research nurse. Patients will be asked how many treatments per day they received, and whether or not they completed treatment sessions. Treatment compliance will be further determined by determining the number of HOURS:MINUTES at each return visit. The total treatment time between clinic visit will be the difference in HOURS:MINUTES displayed by the device.

If any technical problem related to the TheraBionic P1 device is suspected, a new TheraBionic P1 device programmed with the same treatment the patient is currently using will promptly replace the TheraBionic P1 device.

If a lack of compliance is identified, the investigator will make every reasonable effort to keep the patient on therapy. The patient should be re-oriented how to use the device. The patient should be oriented that the re-incidence of lack of compliance will require the patient cease treatment. If the patient desires not to continue on therapy, the patient will be removed from the study protocol.

6.4 General Concomitant Medication and Supportive Care Guidelines

No other experimental therapy may be used on this protocol. Simultaneous participation in another clinical treatment study protocol is not allowed. Additionally, as indicated above, the use of calcium channel blockers and any agent that could block calcium channels is strictly prohibited as there is in vitro evidence that calcium channel blockers antagonize the anticancer effect of AM RF EMF.

External beam radiotherapy will be permitted during the study period only for palliative indication, i.e. symptomatic control, if it does not treat all target lesions. Any form of radiotherapy to the hepatic parenchyma (external or internal) will not be permitted during the study period.

Concomitant loco-regional treatment, such as TACE, ethanol injection, microwave ablation, etc. is not allowed while on the study period.

If a patient, while on the study period, receives any concomitant treatment not allowed in this protocol, the patient should come off study as a protocol violation.
Anti-inflammatory or narcotic analgesics may be offered as needed. Packed red blood cell and platelet transfusions may be administered as clinically indicated. Prophylactic use of hematopoietic growth factors to support neutrophil or platelet counts may be used at the discretion of the treating Investigator. Patients may be supported with appropriate hormone replacement therapy in the event they develop adrenal or thyroid insufficiency in the absence of disease progression or unacceptable treatment-associated toxicity.

Medications considered necessary for the patient's well-being may be given at the discretion of the investigator, i.e., chronic treatments for concomitant medical conditions, as well as agents required for life-threatening medical problems, etc. Patients should be advised to contact the physician before starting any new drug. All concomitant medications will be recorded on the appropriate CRF page.

6.5 Duration of Therapy

The Investigator will make every reasonable effort to keep each patient on their study treatment, unless it is in the patient’s best interest to discontinue from study treatment. Patients in the study will be removed from study intervention in the following situations:

- If they received less than 50% of the prescribed AM RF EMF treatments at two consecutive visits (total of 16 weeks) as assessed by patient’s report and

confirmed by objective measurement of treatment compliance.

- If they lost or damaged the equipment provided for this study due to a lack of care.
- If they do not adhere to the treatment protocol and or planned protocol procedures.
- If there is evidence of radiological progression of disease.
- If the patients receive concomitant anti-tumor treatment (chemoembolization or tumor ablation by means of radiofrequencies, chemotherapy or any other anticancer agent) not allowed during the study period.
- If they voluntarily withdraw informed consent.
- If the physician responsible for the study decides to stop the treatment for medical reasons.
- If the treatment is temporally interrupted for more than 21 days in a row.
- In the instance of unacceptable toxicity or if the same serious adverse event recurs following resumption of treatment.
- If the patient dies.
- If the patient was unable to complete at least 4 weeks of therapy from the date of enrollment.

6.5.1 Concomitant therapy exclusion

If a patient, while on treatment, needs palliative radiotherapy for metastatic disease for symptomatic control without documentation of disease progression; the patient should NOT come off study (exception if the radiation is delivered to the only site of measurable disease) or discontinue study treatment. Any palliative radiotherapy given during the study should be recorded on the appropriate CRF page.
If a patient, while on treatment, needs palliative surgery, the patient may stop study treatment at the discretion of the investigator. There is no need to delay the surgery to minimize the risk of impaired wound healing and bleeding. Postoperatively, the decision to reinitiate study treatment should be based upon a clinical assessment of satisfactory recovery from surgery within 21 days after stopping study treatment. If the timing for resuming study treatment is longer than 21 days, the patient should be removed from the study.

6.5.2 Inclusion of discontinued patients

All patients removed from the study after having measurement of efficacy performed will be considered eligible for the measurement of efficacy and should be evaluated regarding the status of disease at the time of exclusion. Those patients in whom measurement of efficacy was not performed prior to treatment discontinuation will be censored for progression free survival (PFS) and time to progression (TTP) at the date of

randomization.

All patients who signed a consent form and were later removed from the study treatment should be classified, at the time of removal, into the following categories:

- No evidence of disease.
- Stable disease.
- Objective response (partial or complete)
- Progression of disease.
- Death from other causes, e.g. hepatic failure, gastrointestinal bleeding.
- Death from tumor progression.
- Lost to follow up
- Screen failure
- Excluded patients classified as progression of disease or death will be considered as an event in the study analysis.
- Excluded patients classified as screen-failures will not be considered for study analysis. All other patients will be included in the analysis.

6.6 Duration of Follow Up

Adverse events monitoring will continue for a minimum of 30 days after removal from study or until death, whichever occurs first. Patients removed from study for unacceptable adverse events will be followed until resolution or stabilization of the adverse event.

After discontinuation of study treatment and the mandated 30-day follow-up period, patients will be followed by phone every 2 months in order to collect information on further anti-tumor therapy and survival. Patients will be followed until death. Patients who discontinue study treatment without documented evidence of radiologic disease progression should continue tumor imaging and quality of life assessment at the same frequency until disease progression, or initiation of another anticancer treatment, whichever is earlier.

7.0 Dosing Delays/Dose Modifications

7.1 TheraBionic Device

7.1.1 There will be no dose modifications of the device. If the device causes severe or unwanted DLTs that cannot be tolerated by the patient, the patient will be removed from the study.

7.2 Regorafenib

7.2.1 The recommended daily dose of Regorafenib is 160 mg orally every day (qd) on days 1-21 of 28-day cycle (i.e. 3 weeks on, 1 week off)

7.2.2 Concomitant strong CYP3A4 inducers

7.2.3 Management of suspected adverse drug reactions may require a dose reduction of Regorafenib. If dose modifications are required, reduce the dose in 40-mg (1 tablet) increments, the lowest recommended daily dose is 80 mg.

7.2.4 If treatment with regorafenib is stopped for any reason, treatment with the TheraBionic device should continue as long as the patient is tolerating the AM RF EMF treatment.

Dose modifications/interruption for ALT and/or AST increases related to study drug Adapted from (Bruix, J., 2016)

NCI-CTCAE v 5.0	First occurrence	Second occurrence	Third occurrence
Grade ≤2	Treat on time and check AST, ALT, and bilirubin weekly for at least 4 weeks	Treat on time and check AST, ALT, and bilirubin weekly for at least 4 weeks	Treat on time and check AST, ALT, and bilirubin weekly for at least 4 weeks
Grade 3	Interrupt treatment. Check AST, ALT, bilirubin until grade ≤2 or baseline. Reduce one dose level and check AST, ALT, and bilirubin weekly for at least 4 weeks*	Interrupt treatment. Check AST, ALT, bilirubin until grade ≤2 or baseline. Reduce one dose level and check AST, ALT, and bilirubin weekly for at least 4 weeks*	Discontinue†
Grade 3 with ALT or AST >8×ULN and a concomitant rise in bilirubin (of any degree) compared with previous bilirubin values	As above. In case of negative risk–benefit assessment, consider permanent discontinuation at the first occurrence†‡	Discontinue†	
Grade ≥4	Discontinue†		

ALT=alanine aminotransferase. AST=aspartate aminotransferase. NCI-CTCAE=National Cancer Institute Common Terminology Criteria for Adverse Events. ULN=upper limit of normal.
*If all values remain stable for two full cycles, dose re-escalation may be considered at the discretion of the investigator. After re-escalation, AST, ALT and bilirubin should be checked weekly for at least 4 weeks.
†In case of discontinuation, check AST, ALT and bilirubin weekly until recovery to baseline or stabilisation.
‡Patients with Gilbert's syndrome who develop elevated transaminases should be managed as per the above outlined recommendations for the respective observed elevation of ALT and/or AST.

Dose modification/delay for toxicities related to study drug (except HFSR, ALT and/or AST increases, and hypertension)* ***Adapted from (Bruix, J., 2016)***

NCI-CTCAE v5.0	Dose interruption	Dose modification	Dose for subsequent cycles
Grade 0–2	Treat on time	No change	No change
Grade 3	Delay until grade ≤2†	Reduce one dose level	If toxicity remains grade ≤2, dose re-escalation can be considered at the discretion of the treating investigator. If dose is re-escalated and toxicity (grade ≥3) recurs, institute permanent dose reduction
Grade 4	Delay until grade ≤2†	Reduce by one dose level. Permanent discontinuation can be considered at the discretion of the treating investigator	

ALT=alanine aminotransferase. AST=aspartate aminotransferase. HFSR=hand–foot skin reaction. NCI-CTCAE=National Cancer Institute Common Terminology Criteria for Adverse Events.
*Excludes alopecia, non-refractory nausea/vomiting, non-refractory hypersensitivity, and asymptomatic laboratory abnormalities. †If no recovery after a 4-week delay, treatment will be permanently discontinued.

8.0 Measurement of Effect - Radiological assessment

Assessment will be made by independent radiologic review using RECIST version 1.1 and mRECIST for HCC.

8.1 Antitumor Effect-Solid Tumors

Disease progression and response to treatment will be assessed using RECIST version 1.1. and mRECIST for HCC [54] and response will be assessed using standard RECIST 1.1 criteria and mRECIST for HCC.T

For the purposes of this study, patients should be reevaluated for response every 6 weeks.

8.2 Definitions for RECIST 1.1

Definitions

At baseline, tumour lesions/lymph nodes will be categorised measurable or non-measurable as follows:

Measurable

Tumour lesions: Must be accurately measured in at least one dimension (longest diameter in the plane of measurement is to be recorded) with a minimum size of:

• 10 mm by CT scan (CT scan slice thickness no greater than 5 mm).
• 10 mm caliper measurement by clinical exam (lesions which cannot be accurately measured with calipers should be recorded as non-measurable).

Malignant lymph nodes: To be considered pathologically enlarged and measurable, a lymph node must be ≥15 mm in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm). At baseline and in follow-up, only the short axis will be measured and followed.

Non-measurable
All other lesions, including small lesions (longest diameter <10mm or pathological lymph nodes with ≥10 to <15mm short axis) as well as truly non-measurable lesions. Lesions considered truly non-measurable include: leptomeningeal disease, ascites, pleural or pericardial effusion, inflammatory breast disease, lymphangitic involvement of skin or lung, abdominal masses/abdominal organomegaly identified by physical exam that is not measurable by reproducible imaging techniques.

8.3 Definitions for mRECIST

- To be selected as a target lesion using mRECIST, an HCC lesion should meet all the following criteria:
- The lesion can be classified as a RECIST measurable lesion (i.e., the lesion can be accurately measured in at least one dimension as 1 cm or more).
- The lesion is suitable for repeat measurement.
- The lesion shows intratumoral arterial enhancement on contrast-enhanced CT or MRI.

It is important to point out that only well-delineated, arterially enhancing lesions can be selected as target lesions for mRECIST. This may not be the case of infiltrative-type HCC. Infiltrative-type HCC should be considered as a nontarget lesion when the mass shows ill-defined borders and therefore does not appear to be suitable for accurate and repeat measurements. HCC lesions previously treated with locoregional or systemic treatments may or may not be considered as suitable to be selected as target lesions for mRECIST: if the lesion shows a well-delineated area of viable (contrast enhancement in the arterial phase) tumor that is at least 1 cm in longest diameter, then it can be selected as a target lesion. In contrast, if the lesion is poorly demarcated or exhibits atypical enhancement as a result of the previous intervention, then it cannot be selected as a target lesion for mRECIST.

8.4 Methods for Evaluation of Measurable Disease

Response Criteria for RECIST version 1.1 and mRECIST

RECIST	mRECIST for HCC
CR = Disappearance of all target lesions	CR = Disappearance of any intratumoral arterial enhancement in all target lesions
PR = At least a 30% decrease in the sum of diameters of target lesions, taking as reference the baseline sum of the diameters of target lesions	PR = At least a 30% decrease in the sum of diameters of viable (enhancement in the arterial phase) target lesions, taking as reference the baseline sum of the diameters of target lesions
SD = Any cases that do not qualify for either partial response or progressive disease	SD = Any cases that do not qualify for either partial response or progressive disease
PD = An increase of at least 20% in the sum of the diameters of target lesions, taking as reference the smallest sum of the diameters of target lesions recorded since treatment started	PD = An increase of at least 20% in the sum of the diameters of viable (enhancing) target lesions, taking as reference the smallest sum of the diameters of viable (enhancing) target lesions recorded since treatment started

AASLD, American Association for the Study of Liver Diseases; JNCI, Journal of the National Cancer Institute; HCC, hepatocellular carcinoma; mRECIST, modified Response Evaluation Criteria in Solid Tumors; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease.

8.5 Evaluation of Best Overall Response

The best overall response should be confirmed within 6 weeks with follow-up radiological assessment. The confirmation criteria described below will be applied:
The serologic marker alpha-fetoprotein (AFP) will not be used primarily to evaluate effectiveness. However, pre-treatment and during treatment AFP measurements will be collected in all patients and changes in AFP constitute a secondary efficacy end point for this study. A rise in AFP will not be used as a criterion for progression of disease.

Best early response (not yet confirmed)	Best late response (confirmed)	Best overall response
CR	CR	CR
CR	No CR or not available	SD
PR	CR or PR	PR
PR	SD or PD or not available	SD
SD	Not applicable	SD
PD	Not applicable	PD

8.6 Disease control

Disease control will be defined as the percentage of patients who are alive and have documented response status of CR, PR or SD at 4 month and 6 months from the date of enrollment.

8.7 Tumor progression

Duration of overall response: The duration of overall response is measured from the time measurement criteria are first met for CR/PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded on study).
The duration of overall complete response is measured from the time measurement criteria are first met for CR until the first date that recurrent disease is objectively documented.
Duration of stable disease: Stable disease is measured from the start of the treatment until the criteria for progression are met, taking as reference the smallest measurements recorded since the treatment started.

8.8 Time to progression (TTP)

Time-to-radiological progression assessment will be recorded in days and will represent the period starting at the date of enrollment date and finishing at the date of radiological assessment documenting tumor progression. Patients who initiate post-study anti-cancer therapy or die without documented progression will be censored at that time point.

8.9 Survival Outcomes

Overall survival assessment will be recorded in days and will represent the period starting at the date of randomization and finishing at the date of patient death. Living patients at the time of analysis will have the date of last contact (consultation visit or phone contact) used to define overall survival.
Progression-free survival assessment will be recorded in days and will represent the period starting at the date of enrollment and finishing at the later of the date of documentation of radiologic tumor progression, date of last follow-up on study treatment or death, whichever comes first. Patients who initiate post-study anti-tumor therapy prior to radiologic progression will be censored for PFS at that date.

9.0 Adverse Events List and Reporting Requirements

9.1 Potential toxicity

The TheraBionic P1 device is a low risk device as it emits and results in the absorption of levels of radiowaves that are more than ten times lower than those of cellphones. The only known risks are irritation of the tongue, fatigue, and increased dreaming.

Only minor adverse effects were observed in two studies of AM RF EMF in patients with cancer [16]. 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. Post Marketing Clinical Follow Up (PMCF) data collected since European Regulatory Approval have not revealed any additional adverse events (data on file, TheraBionic).

9.2 Adverse Events for Regorafenib

Based on the RESORCE study [8], the following AE's were reported and could reasonably be expected in the current study:

	Treatment-emergent						Treatment-emergent drug-related					
	Regorafenib (n=374)			Placebo (n=193)			Regorafenib (n=374)			Placebo (n=193)		
	Any grade	Grade 3	Grade 4	Any grade	Grade 3	Grade 4	Any grade	Grade 3	Grade 4	Any grade	Grade 3	Grade 4
Any adverse event	374 (100%)	208 (56%)	40 (11%)	179 (93%)	61 (32%)	14 (7%)	346 (93%)	173 (46%)	14 (4%)	100 (52%)	31 (16%)	1 (1%)
Hand-foot skin reaction	198 (53%)	47 (13%)	NA	15 (8%)	1 (1%)	NA	196 (52%)	47 (13%)	NA	13 (7%)	1 (1%)	NA
Diarrhoea	155 (41%)	12 (3%)	0	29 (15%)	0	0	125 (33%)	9 (2%)	0	18 (9%)	0	0
Fatigue	151 (40%)	34 (9%)	NA	61 (32%)	9 (5%)	NA	110 (29%)	24 (6%)	NA	37 (19%)	3 (2%)	NA
Hypertension	116 (31%)	56 (15%)	1 (<1%)	12 (6%)	9 (5%)	0	87 (23%)	48 (13%)	1 (<1%)	9 (5%)	6 (3%)	0
Anorexia	116 (31%)	10 (3%)	0	28 (15%)	4 (2%)	0	88 (24%)	10 (3%)	0	12 (6%)	0	0
Increased blood bilirubin	108 (29%)	37 (10%)	2 (1%)	34 (18%)	15 (8%)	6 (3%)	70 (19%)	24 (6%)	1 (<1%)	7 (4%)	4 (2%)	0
Abdominal pain	105 (28%)	13 (3%)	NA	43 (22%)	8 (4%)	NA	34 (9%)	5 (1%)	NA	5 (3%)	0	NA
Increased AST	92 (25%)	37 (10%)	4 (1%)	38 (20%)	19 (10%)	3 (2%)	48 (13%)	16 (4%)	3 (1%)	15 (8%)	9 (5%)	1 (1%)
Fever	72 (19%)	0	0	14 (7%)	0	0	14 (4%)	0	0	4 (2%)	0	0
Nausea	64 (17%)	2 (1%)	NA	26 (13%)	0	NA	40 (11%)	1 (<1%)	NA	13 (7%)	0	NA
Constipation	65 (17%)	1 (<1%)	0	22 (11%)	1 (1%)	0	24 (6%)	0	0	3 (2%)	0	0
Ascites	58 (16%)	16 (4%)	0	31 (16%)	11 (6%)	0	8 (2%)	3 (1%)	0	1 (1%)	1 (1%)	0
Anaemia	58 (16%)	16 (4%)	2 (1%)	22 (11%)	10 (5%)	1 (1%)	23 (6%)	5 (1%)	1 (<1%)	2 (1%)	1 (1%)	0
Limb oedema	60 (16%)	2 (1%)	NA	24 (12%)	0	NA	12 (3%)	1 (<1%)	NA	1 (1%)	0	NA
Increased ALT	55 (15%)	10 (3%)	2 (1%)	22 (11%)	5 (3%)	0	29 (8%)	6 (2%)	2 (1%)	8 (4%)	2 (1%)	0
Hypoalbuminaemia	57 (15%)	6 (2%)	0	16 (8%)	1 (1%)	0	9 (2%)	2 (1%)	0	0	0	0
General disorders and administration site conditions, other	53 (14%)	16 (4%)	2 (1%)	29 (15%)	6 (3%)	3 (2%)	8 (2%)	5 (1%)	0	2 (1%)	1 (1%)	0
Weight loss	51 (14%)	7 (2%)	NA	9 (5%)	0	NA	27 (7%)	4 (1%)	NA	3 (2%)	0	NA
Oral mucositis	47 (13%)	4 (1%)	0	6 (3%)	1 (1%)	0	42 (11%)	4 (1%)	0	5 (3%)	1 (1%)	0
Vomiting	47 (13%)	3 (1%)	0	13 (7%)	1 (1%)	0	27 (7%)	1 (<1%)	0	5 (3%)	0	0
Investigations, other	40 (11%)	4 (1%)	0	11 (6%)	1 (1%)	0	18 (5%)	1 (<1%)	0	0	0	0
Back pain	42 (11%)	6 (2%)	1 (<1%)	17 (9%)	2 (1%)	0	2 (1%)	1 (<1%)	0	2 (1%)	0	0
Thrombocytopenia	39 (10%)	13 (3%)	1 (<1%)	5 (3%)	0	0	19 (5%)	7 (2%)	1 (<1%)	2 (1%)	0	0
Cough	40 (11%)	1 (<1%)	NA	14 (7%)	0	NA	4 (1%)	0	NA	2 (1%)	0	NA
Hypophosphataemia	37 (10%)	30 (8%)	2 (1%)	4 (2%)	3 (2%)	0	22 (6%)	16 (4%)	2 (1%)	2 (1%)	1 (1%)	0
Hoarseness	39 (10%)	0	NA	1 (1%)	0	NA	34 (9%)	0	NA	0	0	NA

Data are n (%). Adverse events were graded using NCI-CTCAE version 4.03. ALT=alanine aminotransferase. AST=aspartate aminotransferase. NA=not applicable. NCI-CTCAE=National Cancer Institute Common Terminology Criteria for Adverse Events. *Events listed are treatment-emergent adverse events occurring in at least 10% of patients in either treatment group.

Table 3: **Treatment-emergent adverse events and treatment-emergent drug-related adverse events* (safety population)**

9.3 Adverse Event Characteristics

- CTCAE term (AE description) and grade: The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 5.0. A copy of the CTCAE version 5.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov).
- 'Expectedness': AEs can be 'Unexpected' or 'Expected' for expedited reporting purposes only.
- Attribution of the AE:
    - Definite – The AE is clearly related to the study treatment.

- Probable – The AE is likely related to the study treatment.
- Possible – The AE may be related to the study treatment.
- Unlikely – The AE is doubtfully related to the study treatment.
- Unrelated – The AE is clearly NOT related to the study treatment.

9.4 DSMC SAE Reporting Requirements

The Data and Safety Monitoring Committee (DSMC) is responsible for reviewing SAEs for WFBCCC Institutional studies as outlined in Appendix D. All Adverse Events that occur during protocol intervention and are coded as either 1) **unexpected grade 4,** 2) **unplanned inpatient hospitalization > 24 hours (regardless of grade)**, or **grade 5 (death)** must be reported to the DSMC using the using the SAE console in WISER. All WFBCCC Clinical Protocol and Data Management (CPDM) staff members assisting a Principal Investigator in investigating, documenting and reporting an SAE qualifying for DSMC reporting are responsible for informing a clinical member of the DSMC as well as the entire committee via the email notification procedure of the occurrence of an SAE.

9.5 WFUHS IRB AE Reporting Requirements

Any unanticipated problems involving risks to subjects or others and adverse events shall be promptly reported to the IRB, according to institutional policy. Reporting to the IRB is required regardless of the funding source, study sponsor, or whether the event involves an investigational or marketed drug, biologic or device. Reportable events are not limited to physical injury, but include psychological, economic and social harm. Reportable events may arise as a result of drugs, biological agents, devices, procedures or other interventions, or as a result of questionnaires, surveys, observations or other interactions with research subjects.

All members of the research team are responsible for the appropriate reporting to the IRB and other applicable parties of unanticipated problems involving risk to subjects or others. The Principal Investigator, however, is ultimately responsible for ensuring the prompt reporting of unanticipated problems involving risk to subjects or others to the IRB. The Principal Investigator is also responsible for ensuring that all reported unanticipated risks to subjects and others which they receive are reviewed to determine whether the report represents a change in the risks and/or benefits to study participants, and whether any changes in the informed consent, protocol or other study-related documents are required.

Any unanticipated problems involving risks to subjects or others occurring at a site where the study has been approved by the WFUHS IRB (internal events) must be reported to the WFUHS IRB within 7 calendar days of the investigator or other members of the study team becoming aware of the event.

Any unanticipated problems involving risks to subjects or others occurring at another site conducting the same study that has been approved by the WFUHS IRB (external events)

must be reported to the WFUHS IRB within 7 calendar days of the investigator or other members of the study team becoming aware of the event.
Any event, incident, experience, or outcome that alters the risk versus potential benefit of the research and as a result warrants a substantive change in the research protocol or informed consent process/document in order to insure the safety, rights or welfare of research subjects.

9.6 Sponsor Reporting Requirements

All serious adverse events will be reported by the investigator to Therabionic Inc. and Bayer within 24 h of the occurrence:

Therabionic Inc. Phone**: 1-312-286-4703**
Fax**: 1-312-342-0578**

Bayer HealthCare Pharmaceuticals Inc, Phone**: 1-888-842-2937.**

In the rare event that the investigator does not become aware of the occurrence of a serious adverse event immediately (e.g., if an outpatient study subject initially seeks treatment elsewhere), the investigator is to report the event within 24 hours after learning of it and document the time of his/her first awareness of the adverse event.
For all serious adverse events, the investigator is obligated to pursue and provide information to Therabionic Inc. and Bayer in accordance with the timeframes for reporting specified above. In addition, an investigator may be requested by Therabionic Inc. and or Bayer to obtain specific additional follow-up information in an expedited fashion. This information may be more detailed than that captured on the adverse event case report form. In general, this will include a description of the adverse event in sufficient detail to allow for a complete medical assessment of the case and independent determination of possible causality. Information on other possible causes of the event, such as concomitant medications and illnesses must be provided. In the case of a subject death, a summary of available autopsy findings must be submitted as soon as possible to Therabionic Inc. or its designated representative.

9.6.1. Conflict of Interest Management Plan Summary

Dr. Richard Zellars, Professor and Chair at the Indiana University Comprehensive Cancer Center, will be appointed as an independent member on the Cancer Center's Safety and Toxicity Reporting Committee, and will provide data oversight by reviewing the adverse events and assessment of the relationship with study drug/device prior to data submission to Bayer Healthcare. If Dr. Zellars expresses concerns about

the safety data he will report his concerns to Dr. Blackstock, and Dr. Blackstock will promptly notify the COI Office. These concerns will be elevated to the I-CIRC.

Dr. Stefan Grant, a member of the Cancer Center's Safety and Toxicity Reporting Committee, has a financial interest in the investigational device and will recuse himself from all adverse event assessments related to this study.

10.0 Pharmaceutical Information

A list of the adverse events and potential risks associated with the TheraBionic device and the regorafenib administered in this study can be found in Section 9.0.

10.1 Device Programming and treatment accountability

A description of the adverse events and potential risks associated with the device administered in this study can be found in Section 9.1.

10.1.1 Programming

The generator of amplitude-modulated electromagnetic fields consists of a battery-driven radiofrequency (RF) electromagnetic field (EMF) generator connected to a 1.2 m long 50 Ohm coaxial cable with a medical grade 316 stainless steel spoon-shaped mouthpiece connected via an impedance transformer. The RF source of the device corresponds to a linear amplifier operating at 27.12 MHz. The carrier frequency is amplitude modulated with a modulation depth of 85%. The resolution of the modulation frequencies is 2-24 (59.6*10-9 in decimal). The output signal is in its entirety synthesized by a Field-Programmable Gate Array (FPGA). The treatment sequence is controlled by a microcontroller (Atmel XMega643U), i.e. duration of session, sequence of modulation frequencies and duration of each sequence are programmed individually during the manufacturing process just before shipment. The RF output is adjusted to 100 mW into a 50 Ohm load, which results in an emitting power identical to the OncoBionic P1 device and that of a similar device previously used for the treatment of patients with insomnia on several hundred patients and healthy volunteers. An activation card allows a defined number of therapeutic sessions, as prescribed by the physician. The docking station has a chip card reader for the activation card as well as a RS-232 interface for monitoring treatment for use by the

physician's office. The power supply for the docking station is an external wall-cube type power supply. The device is built in compliance with European EN ISO 13485:2016 guidelines, and the essential requirements of Annex 1 of the Medical Device Directives MDD 93/42/EEC

10.1.2 Accountability

Each device monitors the number and the total duration of treatments administered, which will be checked by reading the device display. The device is not FDA approved for the treatment of cancer in the United States, but deemed a low risk device [56].

10.2 AM RF EMF Device (TheraBionic® P1 Device)

Product description: The device consists of a battery-driven radiofrequency electromagnetic field generator connected to a 1.2 meter long coaxial cable, to the other end of which a spoon-shaped mouth piece made of steel is connected with the inner conductor.

Spoon antenna cleaning The cable with the spoon-shaped electrode comes into contact with the healthy oral mucosa. As such the spoon-shaped electrode and its holder need to be cleaned with the appropriate agents. It should be cleaned with soap and rinsed under running water between treatments. If desired, the patient spoon can be wiped with an alcohol swab (70% isopropanol or 70% ethanol). It cannot be used in patients with mucositis, thrush or any alteration of the oral mucosa. The spoon-shaped electrode needs to be cleaned prior to the first use and regularly cleaned between uses.

Storage requirements: Store all components in the case provided between treatment sessions

Route of administration: The metal mouth spoon antenna is placed on the anterior part of the tongue during treatment.

Disposal: If the device malfunctions, please inform study staff immediately. Do not dispose of any component of the device

10.3 Pharmaceutical Accountability

Regorafenib is commercially available.

10.4 Regorafenib (STIVARGA)

Product description: Stivarga is a 40 mg, light pink, oval-shaped, film-coated tablet, debossed with 'BAYER' on one side and '40' on the other side.

Storage requirements: Store at a controlled room temperature of 25 degrees C (77 degrees F), with excursions permitted between 15 and 30 degrees C (59 and 86 degrees F). Store in original bottle, do not remove desiccant, and keep bottle tightly closed.

Stability: Stable at room temperature.

Route of administration: Oral

Disposal: Discard any unused tablets 28 days after opening original bottle per local guidelines or per the Wake Forest Baptist Medical Center waste stream.

11.0 Laboratory Correlative Studies

Blood and tissue samples collected at baseline will be assessed for responders and matched non-responders.
Exome-Seq of germline and hepatocellular carcinoma tissues from exceptional responders: Sequence variants associated with HCCMF responsiveness will be assessed in both germline (blood) and somatic (tumor) DNA. These experiments will be conducted by Drs. Lance Miller and Greg Hawkins, co-directors of the CCC Cancer Genomics Core. For germline analysis, 8.5 ml blood draws will be collected from each patient at the time of enrollment into PAXgene Blood DNA Tubes (Qiagen) that contain a proprietary DNA stabilizing agent and coagulation preventative. Additionally, for total RNA isolation, 2.5 mL blood draws will be collected into Paxgene Blood RNA tubes (BD biosciences) that contain proprietary reagent for RNA stabilization. Samples will transported by study staff to the Pasche lab, NRC, 441. Samples will be transported at room temperature then refrigerated within 24 hours of collection. Within 5 days of refrigeration, samples will be moved to -80°C storage. DNA extraction from blood sample

batches will be performed according to the PAXgene Blood DNA System Kit (Qiagen). Total RNA isolation will be performed according to Invitrogen MagMAX for stabilized Blood Tubes RNA isolation kit (including miRNA). Pathologist-verified tumor needle core biopsies (formalin- fixed and paraffin-embedded at the time of biopsy) will be extracted for DNA according to the QIAamp DNA FFPE Tissue Kit (Qiagen). Purified genomic DNA will be assessed for quantity and quality on a Qubit Fluorometer (Life Technologies). Purified total RNA will be assessed for quantity and quality on EPOCH2 microplate reader (BioTek). Indexed DNA libraries will be constructed from 50 ng genomic DNA (Illumina Nextera XT library kit). Enriched libraries (Nextera Rapid Capture Expanded Exome kit) will be sequenced using 150x150 nt paired end sequencing targeting 30X and 100X genomic coverage for germline and tumor DNA, respectively. Samples will be sequenced in a multiplex of 10 libraries on the Illumina NextSeq 500 DNA sequencer with the expectation of >80% of sequence reads at >Q30 Phred quality scores.

Comparison of exceptional responders versus nonresponders:
Exome-Seq data will be analyzed by FastQC (Babraham Bioinformatics) to assess multiple parameters related to sequence quality and reliability. The ExScalibur suite of scalable whole exome sequencing analysis pipelines, designed exclusively for the detection of germline and somatic mutations derived from Illumina exome sequence data, will be used to profile the spectrum of mutations present in responders and non-responders. Importantly, the ExScalibur suite does not rely on a single sequence alignment or variant calling algorithm, but rather incorporates 3 aligners, 6 germline variant callers and 6 somatic mutation callers, then aggregates variants by outputting a collective estimate of concordance of all aligner-caller combinations using a simple multiplicative score. The average non-silent mutation rate in HCC has been estimated at 1.3 to 2.5 per Mb of DNA sequenced equating with approximately 60 protein-altering mutations, on average, per whole exome sequence. Cumulative results of multiple independent whole exome studies indicate that at least 38 genes are significantly recurrently mutated (SNVs, indels) in HCC, with 15 additional genomic loci recurrently amplified or deleted. In our study, we will define: 1) the comprehensive mutation profile (silent, non-silent), and 2) the intrinsic non-silent mutation profile of each patient specimen, and evaluate the significance of disproportionality between responders and non-responders. We will use a Fisher's exact test to compare the proportion of changes in the two groups. With the relatively small sample size available (n=10 per group) we will be able to detect large changes in the loss of driver mutations between groups. For instance, we compute 82% power to detect the difference between groups equal to 55% (10% change in one group versus 65% in the second group) using a Fisher's exact test with alpha=0.05 (2-sided). In a pre-specified sub analysis intended to control for multiple

testing, we will limit significance testing to the previously-discovered 38 driver genes and 15 genomic loci known to be recurrently mutated in HCC. Genes significant at $q<0.05$ after false discovery correction (Benjamini-Hochberg) will be defined as candidate effectors of HCCMF efficacy for downstream studies. miRNA (hsa-Let-7g and hsa-miR-1246) levels will be examined via qRT-PCR (using a Roche Lightcycler II) as potential biomarkers of response. miRNAs will be examined over time and compared to baseline measurements in both responder and non-responder groups. Significance will be assessed via repeated measures mixed models analysis. Group and time will be fixed effects and the patients are treated as random effects. A time by group interaction will be examined to determine if miRNA levels are different between groups. If time by group interaction is found to be significant, mixed models can be fit again stratified by time to allow for groups to be compared at individual time points to determine when the observed effects become statistically significant.

12.0 Data Management

The RED Cap system will be used to prospectively collect the following data:

- Demographic data (age, sex, ethnic status, environmental exposure, habits, origin, etc.)
- Personal and family history (association with other diseases, familial risk, etc.)
- Exposure to medications or infections (risk factors related to treatment)
- Tumor features (staging, pathology, evolution)
- Type and duration of previous treatments
- Status of tumor progression prior to study entry
- Laboratory analysis including AFP and correlative studies
- Imaging data analysis collected as per mRECIST.
- Progression-free survival
- Overall survival – REDCap is a free, secure, web-based application for building and managing online surveys and databases that can be used for data projects spanning multiple institutions. REDCap databases are HIPAA compliant, include audit trails, and data is backed up twice daily.

Informed consent document	EPIC
Protocol registration form (Appendix B)	WISER/OnCore
Off Treatment, Off Study, Survival, and Withdrawal forms	WISER/OnCore
Best Response	WISER/OnCore
Baseline Pre-Toxicity	Forte EDC

Oncologic History	Forte EDC
Current Medications	Forte EDC
Cycle Tracking	Forte EDC
Treatment Response Evaluation	Forte EDC
ECOG PS, body weight, and vital signs	Forte EDC
Adverse Events	Forte EDC
Patient AM RF EMF Diary	Forte EDC
Device Reconciliation Form	Forte EDC
Alpha-Fetoprotein levels	Forte EDC
Post Study Anti-cancer treatment	Forte EDC
Disease status at withdrawal	Forte EDC

This project will also utilize REDCap Clinical Data Interoperability Services. This is a special feature for importing data into REDCap from WakeOne. It provides an adjudication process whereby REDCap users can approve all incoming data from WakeOne before it is officially saved in their REDCap project. REDCap Clinical Data Interoperability Services can only be enabled by a REDCap administrator who serves as an honest broker to PHI. REDCap's Clinical Data Interoperability Services can only be accessed by users with valid WakeOne credentials. Using the Clinical Data Interoperability Service requires using the Medical Record Number (MRN) as a key to automatically gather demographics and laboratory data and reduces data entry errors.

13.0 Statistical Considerations

13.1 Analysis of Primary Objective

The primary objective of this study is to estimate progression free survival rate and compare this with the historical outcomes for patients receiving second line Regorafenib (alone). PFS will be estimated along with estimates of 95% confidence intervals for the treatment group. In addition, we will determine the proportion of patients who are progression free after 12 weeks (after 2nd 6-week visit) and compare this to the proportion of patients that were described as progression free in the RESORCE trial using a Simon's two stage design.

13.2 Analysis of Secondary Objectives

To examine feasibility and compliance data we will assess for each patient the proportion of days that they used the AM RF EMF device within recommendations. We will estimate that this proportion and corresponding 95% Clopper-Pearson exact confidence

intervals. Next, for overall survival we will examine Kaplan Meier curves for patients survival and also estimate the percent of patients who are alive at 6 months and the corresponding 95% Clopper Pearson exact confidence intervals will be calculated. For Disease Control Rates, Objective Response Rates and Response rates we will estimate the proportion of patients who respond and the corresponding 95% Clopper Pearson exact confidence intervals. Based on the RESORCE study, the 6-month Disease control rates (DCR) were 30% and the Objective Response rates (ORR) were 11%. Using these values, we will compare the observed DCRs and ORRs from this study to those fixed values using one-sample exact binomial tests. Patients who are removed from study before the 6-month time point will be considered to not have disease control at that time point.

Descriptive statistics will be estimated for the treatment group for each of the categorical endpoints (see 5.2.2).

Adverse events: Adverse events will be described for each CTL type for this study using counts/percent's. In addition these adverse events will be compared for each CTL type for the treatment group and historical control (from the RESORCE trial), using the Fisher's exact test (two-sided) at level 0.05. These comparisons will be made to compare events of grade greater than or equal to 3 between each group.

AFP levels: We will examine whether these is any association between the AFP levels (potential biomarker for response) and the objective response observed for each patient. Average AFP levels will be examined over time, and these changes in AFP rates after 6 months will be examined for each Response category (CR/PR/SD/PD) and tested using a 1-way ANOVA to see if the change in AFP level differs by response category.

13.3 Power and Sample Size

The RESORCE trial reported a median PFS rate of 3.1 months by mRECIST in the regorafenib arm of the trial. Using the survival distribution parameter conversion tool in PASS version 13 [57]. We estimated that this would correspond to expecting 54% of patients being free of progression at 12 weeks post treatment (46% are expected to have progressed). Using this as our historical PFS rate (54% at 12 weeks), we designed a Simon's two stage design [58] to examine our primary objective. The null hypothesis that the true 12-week PFS rate is 54% will be tested against a one-sided alternative. In the first stage, 11 patients will be accrued. If there are six or fewer responses in these 11 patients, the study will be stopped. In other words, if there are six

or fewer patients who are *not* progression free at 12 weeks then the trial will be stopped. Otherwise, 14 additional patients will be accrued for a total of 25. The null hypothesis will be rejected if 17 or more responses (patients who do not progress after 12 weeks) are observed in 25 patients. This design yields a type I error rate of 9.8% and power of 80% when the true response rate (proportion of patients who do not progress by 12 weeks) is 75%.

13.4 Interim Analysis

As described above, after 11 patients are accrued an interim analysis will be performed to determine the proportion who have progressed within 12 weeks. If there are 6 or fewer responses in these 11 patients, the study will be stopped. In other words, if there are six or fewer patients who are *not* progression free at 12 weeks then the trial will be stopped.

13.5 Estimated Accrual Rate

We anticipate that there will be approximately 25 patients enrolled over the course of 18 months (1-2 patients per month). This will allow accrual to be completed within 1.5 years.

Appendix A – Eligibility Checklist

IRB Protocol No.	WFBCCC Protocol No. 55319
Study Title: Phase II Study Of Intrabucally Administered Amplitude-Modulated Electromagnetic Fields and Regorafenib as Second-line Therapy For Patients with Advanced Hepatocellular Carcinoma.	
Principal Investigator: A. William Blackstock, M.D.	

Inclusion Criteria (as outlined in study protocol)	Criteria is met	Criteria is NOT met	Source Used to Confirm * (Please document dates and lab results)
-Biopsy-proven HCC that is locally advanced or metastatic. -Patients without biopsy confirmation are also eligible if they meet the following: Radiologic diagnosis of HCC as per the AASLD guidelines : –liver cirrhosis AND –a liver mass confirmed by BICR that shows arterial phase hyperenhancement on triphasic CT or MRI, AND EITHER: ☐ Is ≥20 mm with either non-peripheral portal washout or an enhancing capsule ☐ OR is 10-19 mm with non-peripheral portal venous washout AND an enhancing capsule	☐	☐	
Patients must have been treated with at least one standard systemic treatment modality for advanced HCC such as sorafenib, lenvatinib, atezolizumab plus bevacizumab, or another approved or experimental systemic therapy prior to study entry.	☐	☐	
Measurable disease according to RECIST 1.1 and modified RECIST (mRECIST) for HCC [54]	☐	☐	
At least one target lesion should not have previously received any local therapy, such as surgery, radiation therapy, hepatic arterial embolization, TACE, hepatic arterial infusion, radio-frequency ablation, percutaneous ethanol injection or cryoablation, unless it has subsequently progressed by 20% or more according to RECIST 1.1 and mRECIST for HCC	☐	☐	

Patients with Child's Pugh A (at time of enrollment), with compensated cirrhosis, as defined by the parameters contained in the Child's Pugh Calculator found in Appendix E	☐	☐	
Performance Status ECOG 0-2	☐	☐	
Absence of medical or psychiatric contraindication which, in the opinion of the treating investigator, would make the patient's participation in this trial inappropriate.	☐	☐	
Patient must not have curative treatment options, including surgery and radiofrequency ablation, available.	☐	☐	
Any extra-hepatic metastases, including treated CNS metastases but patients cannot have leptomeningeal disease.	☐	☐	
At least 2 weeks must have elapsed since administration of any anti-cancer treatment.	☐	☐	
Other anti-cancer treatments are not permitted during this study, including alternative and herbal therapies.	☐	☐	
Patients must be 18+ years old and must be able to understand and sign an informed consent.	☐	☐	
Patient must agree to be followed up according to the study protocol.	☐	☐	
Exclusion Criteria (as outlined in study protocol)	**Criteria NOT present**	**Criteria is present**	**Source Used to Confirm * (Please document dates and lab results)**
Known leptomeningeal disease.	☐	☐	
Fibro lamellar HCC	☐	☐	
Patients who had surgical resection of the disease and who do not have measurable disease.	☐	☐	
Patients with any of the following within the 12 months prior to study drug administration: severe/unstable angina, myocardial infarction, coronary artery bypass graft, symptomatic congestive heart failure, cerebrovascular accident, including transient ischemic attack, or pulmonary embolism.	☐	☐	
Pregnant or breastfeeding women	☐	☐	

Patients diagnosed with another type of cancer (excluding basal cell carcinoma) whose cancer diagnosed previously is not in remission.	☐	☐	
Patients receiving calcium channel blockers and any agent blocking L-type or T-type Voltage Gated Calcium Channels, e.g. amlodipine, nifedipine, ethosuximide, etc. are not allowed in the study unless their medical treatment is modified-prior to enrollment to exclude calcium channel blockers prior to enrollment. **(Refer to Appendix F for a complete list of excluded medications and/or pathways).**	☐	☐	
Patients allergic or intolerant to Sorafenib.	☐	☐	

This subject is ☐ eligible / ☐ ineligible for participation in this study.

OnCore Assigned PID: ___ ___ ___ ___ ___ ___ ___ ___

Signature of research professional confirming eligibility: _____

Date: ___ ___ / ___ ___ / ___ ___

Signature of Treating Physician: _____

Date: ___ ___ / ___ ___ / ___ ___

Signature of Principal Investigator**: _____

Date: ___ ___ / ___ ___ / ___ ___

* Examples of source documents include clinic note, pathology report, laboratory results, etc. When listing the source, specifically state which document in the medical record was used to assess eligibility. Also include the date on the document. Example: “Pathology report, 01/01/14” or “Clinic note, 01/01/14”

**Principal Investigator signature can be obtained following registration if needed

Appendix B – Protocol Registration Form

DEMOGRAPHICS

Patient: Last Name: _____ First Name: _____

MRN: ___ ___ ___ ___ ___ ___ ___ ___ DOB (mm/dd/yy): ___ ___ / ___ ___ / ___ ___

ZIPCODE: ___ ___ ___ ___ ___

SEX: ☐ Male ☐ Female Ethnicity (choose one): ☐ Hispanic ☐Non-Hispanic

Race (choose all that apply): ☐ WHITE ☐BLACK ☐ ASIAN ☐ PACIFIC ISLANDER ☐ NATIVE AMERICAN

Weight: ___ ___ ___.___lbs.(actual)

Height: ___ ___.___ inches

Surface Area: ___ ___.___m^2

Primary Diagnosis: _____

Date of Diagnosis: ___ ___ /___ ___/___ _

Performance Status: ___ ☐ ECOG

PROTOCOL INFORMATION

Date of Registration: ___ ___ / ___ ___ / ___ ___

MD Name (last) : _____

Date protocol treatment started: ___ ___ / ___ ___ / ___ ___

Informed written consent: (consent must be signed prior to registration) ☐ YES ☐ NO

Date Consent Signed: ___ ___ / ___ ___ / ___ ___

PID # (to be assigned by OnCore): ___ ___ ___ ___ ___ ___ ___ __

Protocol Registrar can be contact by calling [redacted] between 8:30 AM and 4:00 PM, Monday – Friday.

Compete the eligibility checklist in WISER and then give the completed Eligibility Checklist and Protocol Registration Form must be hand delivered, faxed or e-mailed to the registrar at [redacted] or [redacted], respectively.

Appendix C - Race & Ethnicity Verification Form

Thank you so much for helping us to verify your race and ethnicity to ensure the quality of our information. As a brief reminder, the information you provide today will be kept confidential.

1. Are you:
   - ☐ Hispanic or Latino/a
   - ☐ Not Hispanic or Latino/a

2. What is your race? One or more categories may be selected.
   - ☐ White or Caucasian
   - ☐ Black or African American
   - ☐ American Indian or Alaskan Native
   - ☐ Asian
   - ☐ Native Hawaiian or Other Pacific Islander
   - ☐ Other, Please Specify: _____

Internal use only:

Name: _____ MRN#: _____

Was the self-reported race and ethnicity of the participant verified at the time of consent?
☐ **Yes** ☐ **No**

Was a discrepancy found? **Yes** ☐ **No** ☐
If yes, please provide what is currently indicated in the EMR:
Ethnicity: _____ Race: _____

Additional comments:
_____
_____
_____
_____

Appendix D Safety and Toxicity Review Committee Requirements

Data and Safety Monitoring (DSMC) Serious Adverse Event (SAE) Notification SOP	**Date: 02/11/2021**

Mandatory DSMC SAE Reporting Requirements in WISER

This document describes reporting requirements of adverse events from **WFBCCC Investigator Initiated interventional trials to the Data and Safety Monitoring Committee (DSMC).** A trial is considered a **WFBCCC Investigator Initiated interventional trial** if the following criteria are met:

1) The Principal Investigator (PI) of the trial is a member of a department at the Wake Forest University Baptist Medical Center.
2) WFBCCC is considered as the primary contributor to the design, implementation and/or monitoring of the trial.
3) The trial is designated as "Interventional" using the Clinical Research Categories definitions provided by the NCI in the Data Table 4 documentation. (https://cancercenters.cancer.gov/GrantsFunding/DataGuide#dt4)

There are two distinct types of WFBCCC Investigator Initiated interventional trials based on where patient enrollment occurs. These include:

1) Local WFBCCC Investigator Initiated interventional trials defined as trials where **all patients are enrolled from one of the WFBCCC sites.** These include the main outpatient Cancer Center clinics (located in Winston-Salem) as well as WFBCCC affiliate sites located in Bermuda Run (Davie Medical Center), Clemmons, Lexington, High Point, or Wilkesboro.
2) Multi-Center WFBCCC Investigator Initiated interventional trials defined as trials where patients are enrolled from other sites in addition to WFBCCC sites.
   There are three types of trials that are included in this category:
   a. Trials sponsored by the NCI Community Oncology Research Program (NCORP) that are conducted at multiple sites where the PI is a member of a department at the Wake Forest University Baptist Medical Center.
   b. Trials sponsored by Industry that are conducted at multiple sites and the PI is a member of a department at the Wake Forest University Baptist Medical Center.
   c. Trials sponsored by WFBCCC that are conducted at multiple sites and the PI is a member of a department at the Wake Forest University Baptist Medical Center.

All Adverse Events (AEs) and Serious Adverse Events (SAEs) that occur on any patients enrolled on WFBCCC Investigator Initiated Interventional trials must be entered into the WISER system. The only exception to this requirement is for patients enrolled on NCORP trials at non- WFBCCC sites. AEs and SAEs for NCORP patients enrolled at WFBCCC sites must be entered into the WISER system. Once these

AEs and SAEs are entered in WISER, certain actions must be taken regarding the reporting of specific Adverse Events to the DSMC.

All Adverse Events that occur during protocol intervention (defined below) and are coded as either 1) **unexpected grade 4,** 2) **unplanned inpatient hospitalization > 24 hours (regardless of grade)**, or **grade 5 (death)** must be reported to the DSMC using the using the SAE console in WISER.

A research nurse or clinical research coordinator when made aware that an adverse event meets one of the above criteria has occurred on a WFBCCC Investigator Initiated interventional trial, is responsible for informing a clinical member of the DSMC by phone (or in-person) about the adverse event. The nurse/coordinator should contact the treating physician prior to calling the DSMC clinical member to obtain all details of the SAE, as well as all associated toxicities to be recorded along with the SAE. In addition, this nurse or coordinator is responsible for entering the adverse event information into the SAE console in WISER. Once the adverse event has been entered into the SAE console an email informing the entire DSMC will be generated.

THESE REPORTING REQUIREMENTS APPLY TO any staff member on the study team for a WFBCCC Institutional Interventional trial. Ultimately, the protocol PI has the primary responsibility for AE identification, documentation, grading and assignment of attribution to the investigational agent/intervention. However, when an AE event as described above is observed, it is the responsibility of the person who observed the event to be sure that it is reported to the DSMC.

What is considered during protocol intervention?
During protocol intervention is considered to be the time period while a patient is on study treatment or during the time period within 30 days of last study treatment (even if patient begins a new (non-study) treatment during the 30 days). This window of 30 days should be the standard window to be used in all protocols unless a specific scientific rationale is presented to suggest that a shorter window can be used to identify events. If it is a trial sponsored by Industry and the sponsor requires a longer window for monitoring of SAEs, then the longer window of time specified by the sponsor should be followed.

What is considered as an Unexpected Grade 4 event?
Any grade 4 event that was not specifically listed as an expected adverse event in the protocol should be considered as unexpected. A grade 4 adverse event can be considered to be unexpected if it is an event that would not be expected based on the treatment being received or if it is unexpected based on the health of the patient. In either case, if there is any uncertainty about whether a grade 4 adverse event is expected or unexpected it should be reported to DSMC.

DSMC notification responsibilities of the person (e.g., nurse) handling the reporting/documenting of the SAE in WISER:

1. Make a phone call (or speak in person) to the appropriate clinical member of the DSMC according to the schedule as listed below (page if necessary).
2. Enter a new SAE into the SAE module that is located in the Subject>> CRA Console inWISER WITHIN 24 HOURS of first knowledge of the event. Information can be entered and saved, but the DSMC members will not be notified until a date is entered into the DSMC Notification Date Field. This will ensure that all persons that need to be made aware of the event (i.e., PI, study team members and DSMC members) will be notified; remember to file a copy of the confirmation.
3. Document that the appropriate person(s) on the DSMC has been contacted. Indicate the name of the DSMC clinician that was contacted and the date and time contacted in the Event Narrative field in the SAE console of the particular subject.
4. Document whether or not the protocol should be suspended based on the discussion with the DSMC clinician. This is the major function of the email notification. Enter whether the protocol should be suspended in the Event Narrative Field.
5. Follow up/update the clinical member(s) of DSMC regarding any new developments or information obtained during the course of the SAE investigation and reporting process.

Elements needed to complete the SAE form in the Subject Console in WISER (see Screen Shot 3):

1. Event Date
2. Reported Date
3. Reported by
4. If Grade 5, enter Death Date
5. If Grade 5, enter Death occurred: within 30 days
6. Event Narrative: Brief description (include brief clinical history relevant to this event, including therapies believed related to event). Begin narrative with the DSMC clinician who was notified and Date/Time notified. In addition, state attribution by DSMC clinician as either “Unrelated”, “Unlikely”, “Possibly”, “Probably”, or “Definitely”. Always include the following here:
    i. DSMC clinician name, date/time contacted and comments
    ii. Date of last dose before the event
    iii. Is suspension of the protocol needed? Y/N
7. Treating Physician comments
8. PI comments, if available
9. Protocol Attribution after discussion with DSMC clinician
10. Outcome (Fatal/Died, Intervention for AE Continues, Migrated AE, Not Recovered/Not Resolved, Recovered/Resolved with Sequelae, Recovered/Resolved without Sequelae, Recovering and Resolving)
11. Consent form Change Required? Y/N
12. SAE Classification ***This is required in order for the email notification to be sent***
13. Adverse Event Details – Enter all details for each AE associated with the SAE.
    a. Course start date
    b. Category
    c. AE Detail

d. Comments
e. Grade/Severity
f. Unexpected Y/N
g. DLT Y/N
h. Attributions
i. Action
j. Therapy
k. Click ADD to attach the AE Detail to the SAE.

14. Enter Date Notified DSMC -- ***This is required for the email notification to be sent***
15. Click Submit. The auto-generated notification email will disseminate within 5 minutes. If you do not receive an email within 5 minutes, check that you have entered the "Date Notified DSMC" and the "SAE Classification". If these have been entered and the email still has not been received, take a screen shot of the SAE in WISER and immediately email it out to all of the DSMC members listed in this SOP. In the subject line, indicate that this is a manual transmission of the SAE in lieu of the auto-generated email. It is required that a notification goes to the DSMC members immediately so that their assessment can be obtained within the 24 hour period requirement. Contact the Cancer Center Programmer/Analyst to alert that there is an issue with the auto-generated email.

The Clinical Members of DSMC to Notify by Phone or Page:

Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
Lesser	Hughes	Goodman	Reed	Porosnicu	Seegars	Lesser
Hughes	Goodman	Reed	Porosnicu	Seegars	Lesser	Hughes
Goodman	Reed	Porosnicu	Seegars	Lesser	Hughes	Goodman
Reed	Porosnicu	Seegars	Lesser	Hughes	Goodman	Reed
Porosnicu	Seegars	Lesser	Hughes	Goodman	Reed	Porosnicu
Seegars	Lesser	Hughes	Goodman	Reed	Porosnicu	Seegars

Definition of Unavailable:

As a general guideline if the first clinician that is contacted does not respond to the phone call or page within 30 minutes, then initiate contact with the next DSMC clinician listed in the table above on the particular day the SAE is being reported. Allow up to 30 minutes for the new DSMC clinician to respond to a phone call or page before contacting the next member in the table. These times (30 minutes) are a general guideline. Best judgment as a clinical research professional should be used giving considerations of the time of day, severity of the SAE, and other circumstances as to when it is appropriate to contact backup

clinicians. If the event occurs near the end of day, then leave messages (voice or email) as appropriate and proceed with submitting the DSMC notification form. It is important to take reasonable steps and to document that some type of contact has been initiated to one or more of the clinical members of DSMC.

DSMC CLINICAN RESPONSIBILITY:

It is the responsibility of the DSMC clinician to review all reported events, evaluate the events as they are reported; and communicate a response to the Investigator, event reporter and the members of DSMC. The review will include but not be limited to the information reported; there may be times when additional information is needed in order for an assessment to be made and further communication directly with the investigator may be warranted. DSMC reserves the right to disagree with the Investigator's assessment. If DSMC does not agree with the Investigator, DSMC reserves the right to suspend the trial pending further investigation. If there is any immediate danger or harm that could be present for a future patient based on the information provided in the DSMC report then an immediate suspension of enrollment shouldbe considered.

AMENDMENTS TO PREVIOUS REPORTS

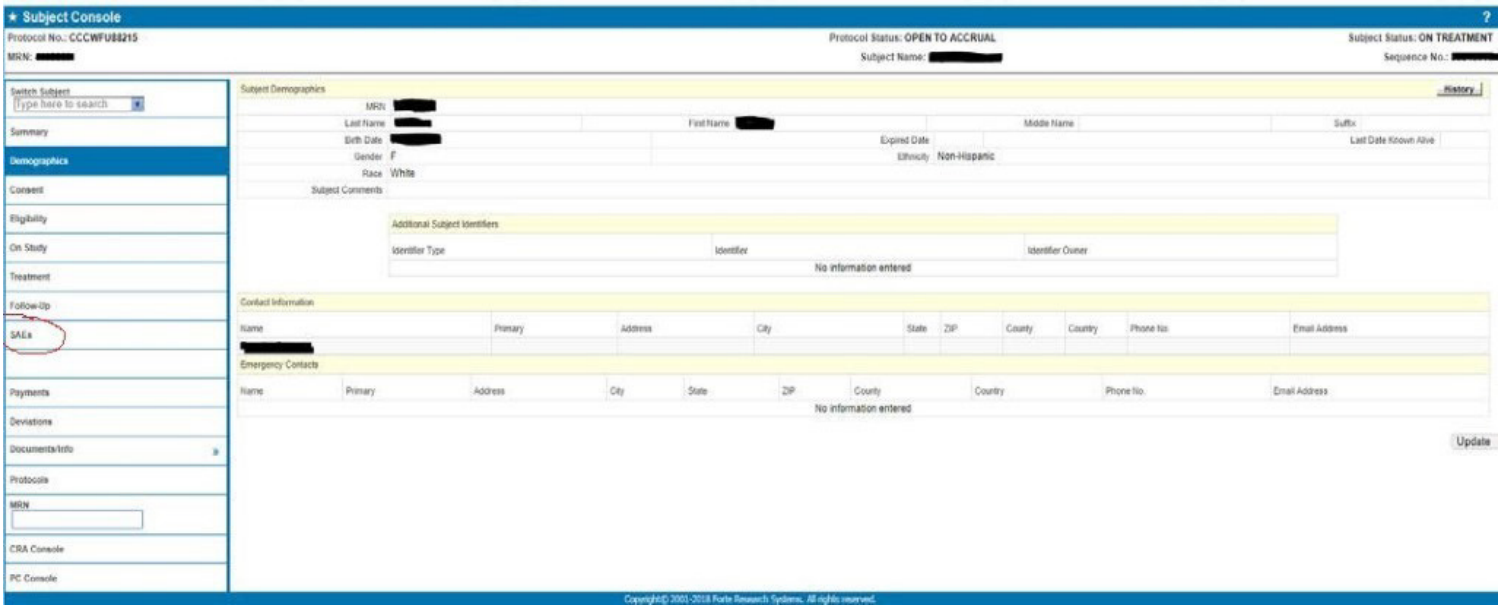
If all pertinent information is unavailable with the initial submission, once the additional information is available **do not submit a new report**. Rather, go to the original email that was sent to the DSMC and using that email "reply to all". Entitle this new email "**Amendment** for (list date of event and patient ID)" this will avoid duplications of the same event. List the additional information being reported. This information needs to be entered into WISER as well. To do this, go to the Subject console and click SAEs on the left column. Click on the appropriate SAE number that needs updating. Then click Update. This will allow additional information to be added.

Acronyms

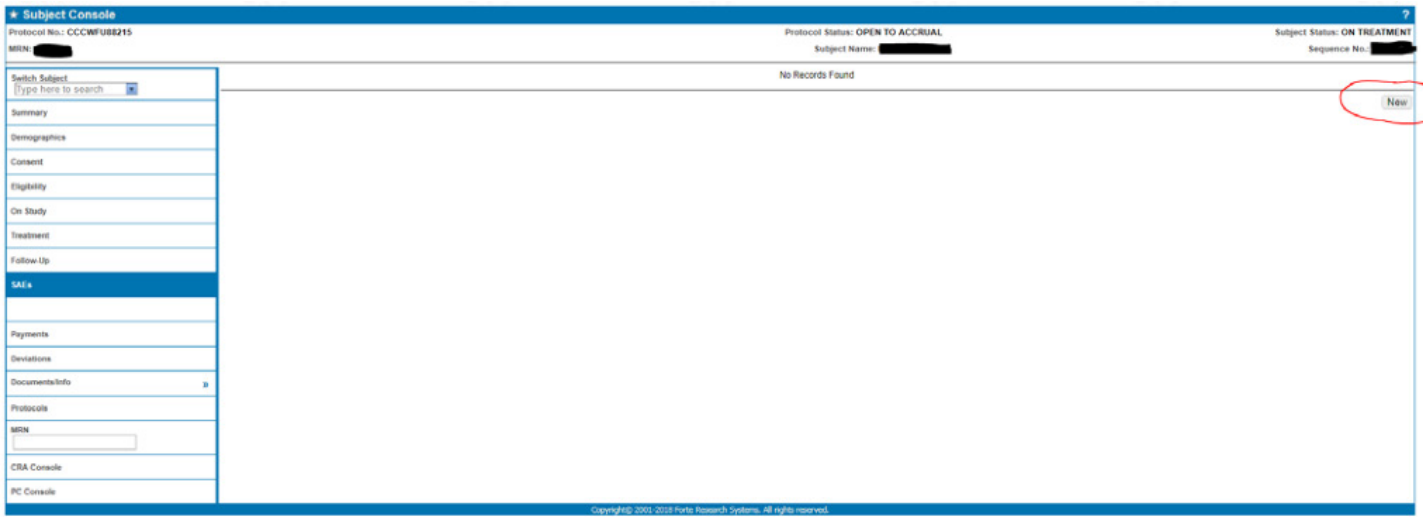
AE – Adverse Event
DSMC-Data and Safety Monitoring Committee
SAE-Serious Adverse Event
WFBCCC – Wake Forest Baptist Comprehensive Cancer Center
NCI-National Cancer Institute
WISER –Wake Integrated Solution for Enterprise Research
Screen Shots:

The following screen shots come from the SAE Console within the Subject Console in WISER.

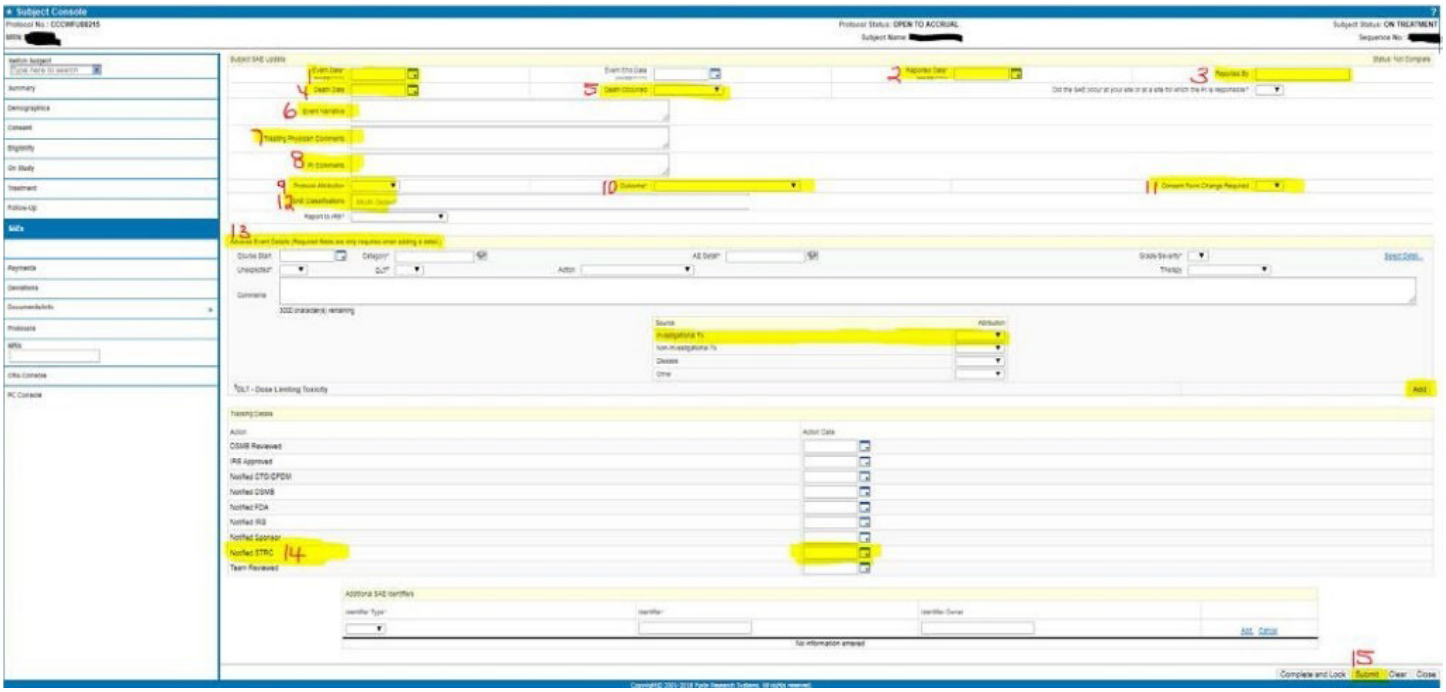
Screen Shot 1:



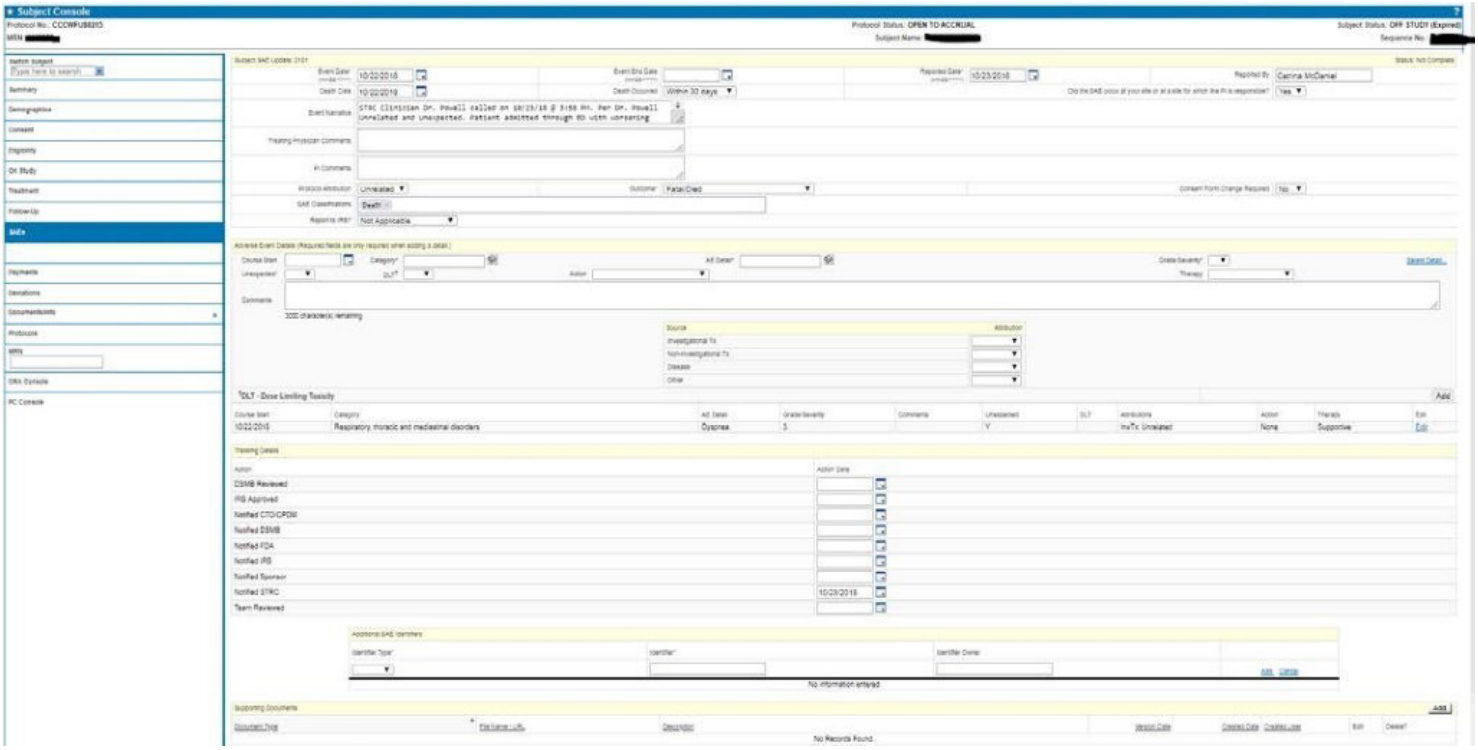
Screen Shot 2:



Screen Shot 3:



Screen Shot 4



Appendix E Calculator: Child Pugh score for severity of liver disease (SI units)

(adapted from UptoDate 2019 (2019))

Encephalopathy

- None (1 point)
- Grade 1: Altered mood/confusion (2 points)
- Grade 2: Inappropriate behavior, impending stupor, somnolence (2 points)
- Grade 3: Markedly confused, stuporous but arousable (3 points)
- Grade 4: Comatose/unresponsive (3 points)

Ascites

- Absent (1 point)
- Slight (2 points)
- Moderate (3 points)

Bilirubin

- <34.2 mcmol/L (1 point)
- 34.2 to 51.3 mcmol/L (2 points)
- >51.3 mcmol/L (3 points)

Albumin

- >35 g/L (1 point)
- 28 to 35 g/L (2 points)
- <28 g/L (3 points)

Prothrombin time prolongation

- Less than 4 seconds above control/INR <1.7 (1 point)
- 4 to 6 seconds above control/INR 1.7 to 2.3 (2 points)
- More than 6 seconds above control/INR >2.3 (3 points)

Total criteria point count:

Child Pugh score interpretation

5 to 6 points:	Child class A
7 to 9 points:	Child class B
10 to 15 points:	Child class C

Notes The Child Pugh score is a modification of the Child Turcotte score.
The terms Child Pugh classification and Child Turcotte Pugh classification are used interchangeably by some clinicians.

Appendix F- List of items/agents/compounds that block T-type channels (Cav 3.1/2/3)

Antihypertensives
Mibefradil
flunarizine
Nicardipine
Nifedipine
Nimodipine
Methoxyverapamil
Diltiazem
Felodipine
Amlodidpine
Aranidipine
Azelnidipine
Barnidipine
Benidipine
Efonidipine

ANTIEPILIPETICS:
Ethosuximide
Methyl-phenylsuccinimide….the active metabolite of the antiepileptic drug methsuximide
Phenytoin (blocks t-type channels but is thought to work by blocking Na+ channels)
Zonisamide
Valproate
Phenobarbital
Trimethadione

Anesthetics / Barbiturates:
Isoflurane
Halothane
Nitrous Oxidie –selective for Cav 3.2
Octanol
Ketamine
Propofol
Etomidate
Thiopental

Antipsychotics:
Penfluridol
Thioridazine
Clozapine
Haloperidol
fluspiriline

pimozide
flunarzine

Vitamins/supplements
Nickel (100uM Ni^{2+})
Vitamin C

Other compounds/agents:
Amiloride
Tetrandrine
Lomerizine

Drug Candidate Pathways Inhibited IC50 -T channel Neuroprotection
A1048400 T-Type and N-Type 1.2-4.6μM
KYS05044 T-Type (CaV3.1, CaV3.2) 1μM
ML218 T-Type (CaV3.1-CaV3.3) 150-310nM
NNC 55-0396 T-Type 7μM
RQ-00311610 T-Type 110-170nM
TTA-A2 T-Type (CaV3.1-3.3) 50-100nM
TTA-P2 T-Type 22nM
VH04 T-Type (CaV3.1) 100nM
Z941/944 T-Type 50-160nM

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