Official Title:	A Single-Institutional, Phase 1 Trial of Repeated Oxygen Measurements in Subcutaneous Tumors by EPR Oximetry using an Implantable Oxygen Sensor (OxyChip)
NCT number:	NCT02706197
Document Type:	Study Protocol
Date of the Document:	July 10, 2018

Clinical Research Protocol

A Single-Institutional, Phase 1 Trial of Repeated Oxygen Measurements in Subcutaneous Tumors by EPR Oximetry using an Implantable Oxygen Sensor (OxyChip)

Principal Investigators	Clinical Scientific	Philip E Schaner, MD PhD Periannan Kuppusamy, PhD
Co-Investigators	Clinical	Lesley A Jarvis, MD PhD Eunice Y Chen, MD PhD Rebecca A Zuurbier, MD Roberta M DiFlorio-Alexander, MD David A Pastel, MD Jason R Pettus, MD
	Scientific	Benjamin B Williams, PhD Dan Tse, MD PhD Eugene Demidenko, PhD
IDE Holder **IDE Number**	Periannan Kuppusamy, PhD G130260	
Funding Sponsor	Sponsor Address Award Number Award Title Award Period	National Cancer Institute BG 9609 MSC 9760, 9609 Medical Center Drive, Bethesda, MD 20892-9760 P01 CA190193 Direct and Repeated Clinical Measurement of pO_2 for Enhancing Cancer Therapy Project 2: Tumor oximetry using OxyChip 07/01/2015 – 06/30/2020
Protocol Numbers	CCRC D14007 CPHS 28499	

Version *Date* *Modification(s)*

Version History			
	V1	6/16/2015	Initial submission
	V2	10/23/2015	Update information regarding tumor stage for inclusion criteria, remove platelet count and ANC from inclusion criteria, Co-I changes
	V3	3/7/2016	Clarification of study procedures, update to specify that tumors can be benign
	V4	1/19/2017	PI change, expansion of Phase 1A and 1B cohorts (justification, results for 6 Patient 1A patients), Increase implant duration to 52 weeks (justification), increase tumor location to 3 cm to skin surface
	V5	3/29/2017	Add ultrasound to OxyChip implantation procedure
	V6	8/28/2017	Clarification of study procedures, clarification of eligibility (no concurrent enrollment in other clinical research studies, add platelet count and ANC to exclusion criteria), details added about OxyChip storage
	V7	5/7/ 2018	Add ultrasound to EPR measurements
	V7.1	7/10/2018	Co-I changes
	V8	**10/10/19**	**Co-I changes, evaluation points in Phase 1B updated, adding information on adding fiducials to OxyChips and OxyChip removal, inclusion criteria revised to include minimum tumor size**

Table of Contents

List of Abbreviations

BOLD MRI	-	blood oxygen level-dependent MRI
CCRC	-	Clinical Cancer Review Committee
CO_2	-	carbon dioxide
CPHS	-	Committee for the Protection of Human Subjects (Dartmouth's IRB used by DHMC)
CRF	-	case report form
CT	-	computerized tomography
DHMC	-	Dartmouth-Hitchcock Medical Center
DSMAC	-	Data Safety Monitoring and Accrual Committee
DWI	-	diffusion-weighted imaging
EPR	-	electron paramagnetic resonance
FDA	-	Food and Drug Administration (US Dept of Health and Human Services)
FTIR	-	Fourier transform infrared spectroscopy
GC-MS	-	Gas chromatography - mass spectrometry
GHz	-	gigahertz
Gy	-	gray
HIF-1alpha	-	hypoxia-inducible factor-1 alpha
HIPAA	-	Health Insurance Portability and Accountability Act of 1996
ICP-MS	-	Inductively coupled plasma – mass spectrometry
LiNc	-	lithium naphthalocyanine
LiNc-BuO	-	lithium octa-*n*-butoxynaphthalocyanine
LiPc	-	lithium phthalocyanine
MRI	-	magnetic resonance imaging
MHz	-	megahertz
NCCC	-	Norris Cotton Cancer Center
NIR	-	near-infrared
NMR	-	nuclear magnetic resonance
O_2	-	molecular oxygen
OxyChip	-	oxygen-sensing probe made by embedding LiNc-BuO in PDMS
PDMS	-	polydimethylsiloxane
PHI	-	patient health information
pO_2	-	partial pressure of oxygen
UADE	-	unanticipated adverse device effect

Study Summary

Title	A Single-Institutional, Phase I Trial of Repeated Oxygen Measurements in Subcutaneous Tumors by Electron Paramagnetic Resonance (EPR) Oximetry using an Implantable Oxygen Sensor (OxyChip)
Short Title	EPR Oximetry using OxyChip
Protocol Number	D14007, CPHS 28499
Phase	First-in-humans, early feasibility study for OxyChip implantation with subsequent expansion to a larger Phase I trial. Phases: **1A** (tumor with planned surgical resection only and consequently with an expected duration of implantation for up to 4 weeks); and **1B** (tumor with neoadjuvant radiation therapy or chemotherapy followed by planned surgical resection, with expected duration of implantation of up to 12 weeks for radiation and 24 weeks or more for chemotherapy.) OxyChip implant duration for both phases can be extended to 52 weeks excluding the first 6 patients enrolled into Phase 1A where implant duration is up to 4 weeks.
Methodology	Phase I (first-in-human, early feasibility trial)
Study Duration	5 years
Study Center(s)	Single institution: Dartmouth-Hitchcock Medical Center (DHMC)
Objectives	Primary: Establishment of safety of OxyChip implantation in humans; Secondary: establishment of feasibility of OxyChip for measurements of oxygen using EPR oximetry in humans.
Number of Subjects	60 total evaluable subjects planned: 3 subjects in Phase1A will be evaluated and reviewed before enrolling another 3 subjects. After completion of the first 6 subjects in Phase 1A, if there are no unacceptable toxicities from the OxyChip, that cohort will be expanded to a total of 30 subjects. Phase 1B will consist of one cohort of 3 subjects receiving chemotherapy and one cohort of 3 subjects receiving radiotherapy. Once a cohort has enrolled 3 subjects and there are no unacceptable toxicities from the OxyChip, the completed cohort will be expanded to enroll additional subjects. No more than 30 subjects will be enrolled in Phase 1B. Phase 1A and Phase 1B will be evaluated separately with regards to this expansion; *i.e.,* Phase 1A may expand to 30 subjects while Phase 1B is still accruing.
Diagnosis and Main Inclusion Criteria	Tumors, located within 3 cm of skin or mucosal surface AND tumor resection is planned.
Study Product, Dose, Route, Regimen	OxyChip containing lithium octa-*n*-butoxynaphthalocyanine (LiNc-BuO) oxygen-sensing crystals embedded in polydimethylsiloxane (PDMS) polymer to be used in recording oxygen levels in tumors using EPR oximetry.
Duration of Administration	Implantation time up to 4 weeks for the first six Phase 1A patients, but ≤52 weeks for the additional 24 patients. The duration of the course of radiation therapy or neoadjuvant chemotherapy for phase 1B patients, but ≤52 weeks.

Reference therapy	N/A
Statistical Methodology	This is a pilot study designed to (1) test the safety of the OxyChip, (2) test the feasibility of taking EPR measurements repeatedly with the OxyChip, and (3) help design larger studies to evaluate oxygen adaptive therapies. We will proceed by initially enrolling 3 patients in a cohort and evaluate the initial (and each subsequent) cohort of 3 patients for adverse events prior to proceeding to the next cohort. As a secondary goal, we will investigate the feasibility of repeated oxygen measurements using EPR oximetry.

1 Introduction

This document is a protocol for a human research study. This study is to be conducted according to US and international standards of Good Clinical Practice (International Conference on Harmonization ICHE6), the Code of Federal Regulations Title 21 parts 803 and 812, and other applicable government regulations and Institutional (Dartmouth's Committee for the Protection of Human Subjects, CPHS) research policies and procedures.

1.1 Background

Tumor hypoxia (low oxygen levels) confers a poor prognosis and resistance to standard radiotherapy in the treatment of many tumor types. Based on oxygen measurements using polarographic electrode and hypoxia markers, several research groups[1,2] have shown that tumor hypoxia is a poor prognostic factor; hypoxic tumors are more resistant to radiotherapy and chemotherapy and have increased malignant potential. Oxygen measurements using a polarographic electrode have been performed pre-treatment but are difficult to perform repeatedly during or after treatment to assess tumor or tissue oxygenation. Hypoxia markers (exogenous substances such as misonidazole and pimonidazole, or endogenous markers such as hypoxia-inducible factor-1 alpha (HIF-1 alpha) and carbonic anhydrase IX (CA IX)) are used to visualize hypoxic areas in histological specimens. The histologic methods only provide a static picture of oxygenation which, *in vivo*, is likely to be in constant flux[3-5]. Knowing the fluctuations in tissue oxygenation may be critical for determining the optimal treatment and the risk of post-treatment complications, thereby optimizing the therapeutic ratio. Such an assessment requires the means to make repeated direct measurements of oxygen in tissue at specific sites, a capability that is not available at present. Thus, there is an unmet need for a device capable of monitoring tumor tissue oxygenation. Achieving this capability would have very significant implications in cancer treatment.

Electron paramagnetic resonance (EPR) oximetry has the potential to fulfill this clinical need. EPR oximetry has been established in both animal models and initial clinical studies at Dartmouth to be a very effective method for measuring oxygen in tissues or tumors directly, repeatedly, and non-invasively following an initial introduction of the paramagnetic EPR reporter material[6]. However, the existing approach for the clinical use of EPR is limited due to the unavailability of a sensitive and reliable oxygen-sensing probe. New oxygen-sensing probes have shown superior characteristics in pre-clinical models, opening up the clinical use of this technology more broadly and importantly to tumors in need of hypoxic monitoring and intervention. We are developing a translational program to apply EPR oximetry more broadly in clinical practice to improve radiation therapy. The first step in this development work is to bring the paramagnetic oxygen-sensing crystals, lithium octa-*n*-butoxynaphthalocyanine (LiNc-BuO), into clinical use.

1.2 Investigational Device: Category A Device: OxyChip as an oxygen sensor

The Category A investigational device that we are proposing to use is a small cylindrical piece (0.6 mm diameter x 3-5 mm length) of implantable material, called **OxyChip**. A summary of the components of the OxyChip is as follows:

Materials in OxyChip	**Prior Clinical Use**
1. Lithium octa-*n*-butoxy-naphthalocyanine (LiNc-BuO) (oxygen-sensing crystal)	This trial represents the first clinical use of LiNc-BuO in humans. The LiNc-BuO will be embedded in a biocompatible polymer, polydimethylsiloxane (PDMS), which is approved by the FDA for clinical use.
2. PDMS polymer (embedding material)	Biocompatible and in clinical use*

*PDMS has been in use in blood pumps, cardiac pacemaker leads, mammary prostheses, drainage implants in glaucoma, artificial skin, maxillofacial reconstruction, replacement esophagus, contact lenses, oxygenators, medical adhesives, finger joints, coating for cochlear implants, catheters, drug-delivery systems and denture liners.

OxyChip is a Category A device (see definition below), as it is an innovative device being tested for the first time in human subjects and the absolute risk of the device has not been established. This study will establish the safety and efficacy of the device for use in humans. The device is Class III (see definition below) only because it is implanted. It is not a life-supporting or life-sustaining device.

Category A Device

A Category A device refers to an innovative device believed to be in Class III for which the "absolute risk" of the device type has not been established (that is, initial questions of safety and efficacy have not been resolved, and the FDA is unsure whether the device type can be safe and effective).

Note: Category A devices are NOT eligible for Medicare coverage.

Class III Device

A Class III device has/requires the following:

Risk: High; devices that present serious risk; most typically these are implanted and are life-supporting or life-sustaining devices.

Special FDA controls through pre-market review: The pre-market review involves a comprehensive evaluation, including data from clinical studies, and is required to ensure safety and efficacy prior to marketing of the devices. This involves bench and animal tests, clinical trials, and the submission of a Premarket Approval (PMA) application.

1.3 Preclinical Data

Extensive preclinical data obtained using animal models and clinical studies in humans have established the efficacy and safety of EPR oximetry. The use of the OxyChip as a probe for EPR oximetry has been studied extensively in animals, which justifies the translation of this technique to clinical trials in humans. This section (1.3) focuses on the pre-clinical data for oximetry using the OxyChip as a probe. In addition, the clinical data for EPR oximetry in tissues with probe other than the OxyChip are reviewed below in Section 1.4.

1.3.1 Tumor oxygenation is a critical factor in malignant cancer progression and therapy

Based on data from the use of the Eppendorf technique and other studies, it has been concluded that the partial pressure of oxygen (pO_2) in solid tumors is the most important parameter affecting clinical outcomes. Pretreatment pO_2 level in solid malignancies, measured by oxygen electrodes, has been shown to be the most important determinant of the therapeutic response, especially to radiation therapy[7-12]. This dependency on oxygen levels appears to be more significant than stage, tumor morphology, or tumor size. There are also data that indicate oxygen levels are very important factors in the response to surgical and chemotherapeutic approaches[13,14] in addition to radiation therapy. For example, in squamous cell carcinomas (SCC) of both the head and neck[1,15-17] and cervix[18,19]. The oxygen level in tumors not only affects the response of tumors to therapy but also the malignant progression of tumors. This dependency of outcomes on oxygen levels may be related to the well-established observation that the level of oxygen can significantly affect the nature of the tumor, with hypoxia leading to more aggressive behavior of tumors through mechanisms that are being increasingly understood[12,20,21].

In addition to influencing treatment efficacy, oxygen levels of the tissues in the tumor bed can influence outcomes related to the morbidity of therapy. Information about pO_2 at critical sites will be useful for enhancing treatment options to facilitate wound healing, and the utility of reconstructive tissue transplantation, especially when surgery and radiation are used in combination.

Oxygen levels in tumors change with time. These changes include those occurring across very short time scales related to underlying pathophysiology, those induced by tumor growth, and those induced by treatment itself. Therefore, it is very desirable to monitor the oxygen level in tumors before, during, and after therapeutic interventions. This requires a means to make direct and repeated measurements of oxygen in tissue at specific sites, a capability that is not available in the clinic. Achieving this capability would have very significant implications for improving cancer therapy across a patient's clinical course, from initial diagnosis to primary treatment to post-treatment morbidity.

1.3.2 Heterogeneity of oxygen distribution in tumors

The basis for the above conclusions about what is needed clinically to be useful, at first consideration, appears to ignore the well-known heterogeneity of oxygen in tumors. There are two principal aspects in our consideration of this important topic. First, heterogeneity of oxygen in tumors has been fairly well characterized[22-24] and second, there is now general acceptance that the amount and sites of hypoxia change both with time and spatially over distances as small as the cell[5,25-33]. Temporal changes in flow and in the number of red cells in capillaries have been shown to vary over seconds[34,35]. Point measurements of oxygen and distributions of markers of blood flow have indicated that, at the level of the circulatory unit and larger aggregates of cells, the local pO_2 can vary significantly within time constants of minutes to days[28,29]. It also has been argued that chronic hypoxia, which does not vary with time, is not especially clinically relevant because of the known effects of prolonged hypoxia on tumor cell viability. There is also good evidence that there are significant gradients in oxygen over cellular dimensions and that, within an individual cell, there can be significant oxygen gradients that include the nucleus. The practical implication of this evidence is that it probably will not be possible, by any technique, to achieve sufficient resolution of heterogeneity to carry out therapy based on the specific location of the hypoxic regions. This is because these hypoxic regions will be changing within timeframes too short to be used to deliver radiation selectively to the hypoxic areas. It will, however, be useful to determine if the tumor has hypoxic areas and especially whether procedures designed to increase oxygenation are effective and the time period over which they are effective. This requires repeated measurements; however, it does not require (or indeed does not benefit from) spatial resolution of the heterogeneity.

Experimental results from our lab and other labs indicate that the ability to monitor the tumor pO_2 repeatedly and directly does provide information on the extent and time dependency of overall oxygenation in the tumor, information that is needed for utilization in clinical decision making[36,37]. These empirical results support the conceptual premise that, while the pO_2 within the tumor will vary, factors that affect oxygenation will affect most or all parts of the tumor, and therefore monitoring at several sites will provide the information that is needed for clinical decision-making. While we believe that it is reasonable to expect that this result, as obtained in animal models, will translate to the clinical setting, we also recognize that one of the key aspects of our proposed research is to test this hypothesis in human subjects, as well as to demonstrate that this information can then be used to affect treatment outcomes.

In summary, considering the fact that local hypoxia is too dynamic to pinpoint spatially in a clinically useful manner, although certainly an important factor in the response to therapy and clinical progression, the key to clinical success will likely be finding a method to follow changes in overall oxygenation and to ascertain responsiveness to interventions to modify oxygenation. We believe that there is an excellent opportunity for our approach using EPR oximetry to provide the information needed to do this. At the present time there is no other method available clinically to make repeated direct measurements of tumor pO_2, with or without the ability to resolve regional hypoxic areas, and *in vivo* EPR oximetry has a very plausible capability to make these measurements in several sites simultaneously and repeatedly over time.

1.3.3 Critical role of oxygen and issues in radiation therapy

Recent developments in radiation oncology have further increased the need to measure tumor pO_2 accurately and repeatedly. Changes in the radiation fraction per dose, especially hypofractionation with single doses of up to 20 or more gray, have led to unexpected clinical effectiveness, raising the need to understand the basis of these effects. Changes in oxygenation are among the most likely factors that are involved. The results from another new clinical approach, modification of angiogenesis, have also been surprising and puzzling. Implementation of clinical protocols based on the excellent preclinical studies that

indicated a window of opportunity with the "normalization" of the tumor vasculature has been disappointing[38,39]. An adequate understanding of the clinical results would be greatly advanced by the ability to repeatedly monitor the oxygen levels in tumors.

1.3.4 Hyperoxygenation as radiation sensitizer - mixed results

Perhaps the most effective and most readily implementable modification of radiation therapy would be to monitor tumor pO_2 to facilitate hyperoxygenation regimes in order to enhance the response of tumors to radiation. Because of the well-known effect of oxygen on radiation response, there have been many attempts to sensitize tumors by increasing their oxygen levels at the time of treatment. Although some positive effects have been found, in general, the results have been disappointing. Our initial clinical measurements with EPR oximetry suggested an explanation for some of the disappointing results of hyperoxic therapy. Using a very simple and readily implementable test regime we have found profound differences in the response of tumors to a simple and readily implementable hyperoxygenation treatment, which is breathing oxygen-enriched gases. In our preliminary studies in a limited number of patients with various superficial malignancies, we found marked differences in the response of tumors when the subject breathed oxygen-enriched gas mixtures. Some tumors showed large responses in tumor pO_2, while others had little or no changes. It is likely that this is a general phenomenon, whose basis is readily understood because of the complex and fragile nature of the blood supply to tumors. This leads to a simple, testable hypothesis that could be definitively tested by EPR oximetry within the usual clinical setting. We hypothesize that by monitoring the changes in tumor oxygen while breathing gas mixtures containing higher oxygen content (e.g., 100% oxygen or carbogen, a mixture of 95% O_2 and 5% CO_2), we can assess the timeframe of hyperoxygenation and thereby administer radiation therapy at times when tumors will respond maximally. We could accomplish the determination of likely responders and of the window for treatment (which may vary among patients) by using EPR oximetry to make repeated non-invasive measurements of tumor pO_2.

The benefit of EPR oximetry will thus apply in both a general manner, by establishing improved standard protocols (e.g., improved guidance regarding time between fractionated doses of radiation therapy, optimized timing of combined therapy involving radiation and chemotherapy and/or surgery), and on a patient-specific basis to enhance individualized therapy (e.g., following the oxygen levels and altering the timing of fractions to maximize therapeutic effect for a specific patient). For the individual patient this would enable us to deliver therapies at times when the tumors have more optimum oxygen concentrations, which in turn could significantly improve the therapeutic ratio of the treatments, and which should lead to improved outcomes.

1.3.5 Methods for oximetry

While a few techniques are available that can provide direct measurements of tumor pO_2 (especially oxygen polarographic electrodes, the OxyLite fluorescence-quenching technique and direct injection of oxygen-sensitive NMR probes based on fluorine[40-43]), these techniques have the disadvantage of not being able to be used repeatedly. This is a significant limitation because the most valuable clinical usefulness depends on obtaining repeated measurements. There are also some concerns about the potential for significant disadvantages if the invasiveness of these techniques causes some artifacts precisely at the time that the measurements are being made[44-48]. To date, only the polarographic method has been systematically used in humans. Some widely available non-invasive techniques to assess oxygenation provide data on parameters that, while related to tissue pO_2, do not measure pO_2 directly. This especially includes techniques such as blood oxygen level-dependent magnetic resonance imaging (BOLD MRI), nuclear magnetic resonance (NMR) proton spectroscopy, diffusion-weighted imaging (DWI) MRI, duplex Doppler ultrasound,[18] F-Miso PET, and near-infrared (NIR) measurements of hemoglobin. These techniques can provide information on the saturation of hemoglobin within the vascular system or redox-related metabolites or reactions, but, while this can be valuable to know, it does not provide direct quantification of the pO_2 in the tumor and surrounding tissues. The clinical importance of direct measurement of pO_2 is especially high in tumors, where typically the microvasculature is complex and irregular, making it very difficult to extrapolate these indirect measurements to the pO_2 in the tumor[49-51]. EPR oximetry could provide the data to facilitate relating these indirect methods to the actual tumor or tissue pO_2, and thereby clarifying the conditions under which these other methods can provide the desired clinically relevant information on tumor pO_2.

1.3.6 EPR oximetry

EPR oximetry refers to the measurement of oxygen concentration by EPR spectroscopy[52]. The basis of EPR oximetry is the physical interaction between molecular oxygen (O_2) and paramagnetic material probes that are introduced into the tissue of interest. As O_2 has two unpaired spins in the ground state, it can have an especially strong effect on the EPR spectra of the introduced probes. Discussion of interactions between the specific probes and O_2 are given in several manuscripts devoted to characterization of chars[53,54] and LiPc and derivatives[55,56]. Briefly, if O_2 approaches the probe within such proximity that there is appreciable overlap of the quantum mechanical wave functions of their unpaired electrons, then a spin-exchange interaction will occur. Under certain conditions which are met with the oxygen-sensitive materials, this exchange results in broadening of the spectral features through shortening of the relaxation times of the introduced paramagnetic material or reduction of existing exchange-narrowing, depending on the probe. When this broadening is calibrated against a range of gases of known pO_2, the resulting calibration curve can be used to provide data on the oxygenation of the tissue surrounding the material. The fact that the widths of EPR resonance lines correlate with oxygen concentration has been used in a variety of biological settings[57-70].

The development of low-frequency EPR instrumentation at L-band (1-2 GHz) and even lower frequencies (600 MHz or 300 MHz) has made it possible to perform EPR oximetry measurements in animals and isolated functioning organs[58,71,72]. Unfortunately, clinical EPR must confront the fact that the depth of penetration will continue to be a limiting factor, unless further advances in hardware design and their applications are successful. Generally speaking, noninvasive EPR measurements are limited to a penetration depth at the range of 1–2 cm at L-band (~1.2 GHz). Penetration depth can be increased to 8 cm or more by lowering the frequency to the 300-600 MHz range. The tradeoff, however, is that the signal-to-noise ratio significantly decreases at these lower frequencies, causing a decrease in spatial resolution and accuracy of pO_2 measurements.

1.3.7 Paramagnetic probes for use in clinical EPR oximetry

Measurement of oxygen-induced EPR line-broadening requires the presence of a suitable paramagnetic probe in the tissue region of interest. Two types of probes are used: (i) soluble probes that report the concentration of dissolved oxygen and (ii) particulate probes that measure partial pressure of oxygen (pO_2) in the *milieu*. Considerable progress has been made in the development and use of both types of probes. The soluble probes provide the potential for imaging but have potential limitations in their sensitivity and ability to repeat the measurements. Nevertheless, they have been very successful in a number of applications in preclinical models.

There are certain advantages in using particulate probes including: (i) high resolution in the range of 0.1 torr (mmHg); (ii) suitability for repeated measurements *in vivo* without reintroduction of the probe into the tissue; (iii) non-invasive measurement – after a one-time introduction of the probe, subsequent measurements are performed under noninvasive conditions; (iv) accuracy - highly reproducible measurements correlate closely with those made by other methods; (v) localized measurements are made from a single voxel/region containing the particulate – thus the spatial resolution is the size of the particulate deposit; (vi) insolubility in aqueous solvents; (vii) no effect of the various biological oxidoreductants, pH, temperature etc.; (viii) nontoxic - the probes are inert in biological systems; (ix) temporal response is very good, usually less than a second; and finally, (x) probes respond to partial pressure of oxygen (pO_2), rather than concentration of oxygen, as the latter may be quite heterogeneous in cellular/tissue environments due to the solubility of oxygen and hence calibration can be difficult. The use of both the naturally occurring (coals, fusinite)[54,73-80], semisynthetic (India ink, sugar chars, carbon blacks)[81-86] and purely synthetic materials (e.g., lithium phthalocyanine and derivatives)[55,56,63,64,68,87-99] have been reported for EPR oximetry.

The particulate probes typically are introduced into tissues using a needle syringe, often several days prior to measurement in order to minimize trauma and allow for healing and incorporation of the material into the tissue prior to pO_2 measurement. An advantage of the particulate approach is that once the probe has been introduced, we can make repeated measurements at the same location without the need to introduce additional material or wait for introduced material to be cleared. This characteristic is particularly useful for the monitoring of tissue oxygenation during the development of disease or in response to therapy, as is needed for the optimal treatment of solid tumor cancers and peripheral vascular disease.

India ink was the first probe used for oxygen measurements in humans[81,82]. India ink has a long history of clinical use as an anatomic marker for surgery and radiotherapy and, fortuitously, the suspended carbon black particles contain stable radical species at sufficient concentrations with EPR spectra that are highly sensitive to the presence of oxygen[82,85]. Charcoal, a naturally occurring carbonaceous material, has been extensively characterized for EPR oximetry in animal models[73,78,100]. Charcoal has been approved for clinical use (as a tissue marker) in Europe. Despite their availability for clinical use, the carbon-based oxygen sensors have some limitations including limited sensitivity to oxygen and, if used as suspensions, the potential to diffuse. Alternatively, purely synthetic materials, such as lithium phthalocyanine[68] or naphthalocyanine[101] and derivatives[56,99], are ideally suited for clinical EPR oximetry.

1.3.8 Polymer-embedded high-sensitive crystalline probes for use in humans

The most notable drawback and potential limitation to the use of the crystalline materials for clinical applications is the need to leave them permanently in the tissue, which may present practical barriers for obtaining approval for use in human subjects. To address this concern, we have developed an alternative approach for their clinical use. The raw particulates (usually 10-100 micron size) are embedded in biocompatible materials that have high oxygen permeability[73,77,102-105]. The probes are effectively shielded from interaction with the biological *milieu* that could result in biochemical degradation and breakdown, as well as limit the probability of local and/or systemic toxicity effects from interactions of the probe with the tissues. The implants could be left in the tissue or removed when no longer needed. We have used polydimethylsiloxane (PDMS), a silicone polymer with properties desirable for holding of oximetry probes. PDMS is biocompatible, highly flexible, oxygen permeable, and has been used in a wide range of medical device and health-care applications. Furthermore, PDMS has been approved for use in human subjects and is one of the reference materials provided by the National Heart Lung and Blood Institute for standardized biocompatibility testing. We have used PDMS to embed/hold LiNc-BuO crystals and developed the implants (OxyChip) in the form of easily implantable shapes and sizes. The efficacy and safety of the OxyChip has been well characterized in animal models[102-105].

1.3.9 Clinical potential of EPR oximetry for direct and repeated measurement of tissue pO_2 in humans

Although several other existing methods are utilized to measure oxygen concentrations *in vivo* in human subjects, a suitable technique for noninvasive and repeated direct measurements of oxygen in the same tissue or cells on a temporal scale is not available currently. EPR oximetry enables reliable and accurate measurements of the pO_2[52]. EPR oximetry can measure pO_2 directly in the tissue and can be performed at the actual site(s) of interest. The placement of the paramagnetic material directly at the site of interest, where it remains and can be interrogated after any temporary perturbation has resolved, also avoids potential concerns about damaging the tissue at the time of measurement[112]. The ability of EPR oximetry to make repeated measurements from localized sites provides a very important capability that can support critical aspects of a number of biomedical applications. The value of EPR oximetry based on stable oxygen-sensitive paramagnetic materials has been shown in animal models to be a very effective method to measure oxygen in tissues directly, repeatedly, and non-invasively. Ongoing clinical studies at Dartmouth have confirmed that the method can be extended to human subjects very effectively, meeting a currently important but unmet need - the ability to make repeated, direct measurements of oxygen (pO_2) in tissue at specific sites[6,85,86,112-114]. These studies also have provided very useful information as to what further developments are needed to achieve the full clinical potential of the technique and its use to enhance cancer therapy. This capability would be a new addition to clinical medicine that would immediately and significantly enhance treatment of malignancies.

1.3.10 General use of *in vivo* EPR oximetry in pre-clinical models

EPR oximetry, based on particulate probes, has been used to study oxygenation in numerous pre-clinical models to obtain oxygen concentration in different anatomic locations, including the brain[46,47,62,68,113,115-144], heart[64-66,89,90,145-154], tumors[24,36,50,62,63,88-90,123,129,134,135,139-144,155-180], gastrointestinal tract[76,181], muscle [182,183], liver[82-84,184], kidney[185], skin[186], and wound healing[112,187-190]. Of note, there are over 350 peer-reviewed publications on EPR oximetry, underscoring the extensive pre-clinical development that has been accomplished to prepare this technique for clinical use.

1.3.11 Advantages of LiNc-BuO oxygen-sensing crystals for in vivo oximetry

EPR oximetry relies on a paramagnetic spin probe that physically interacts with oxygen in tissue. We will use lithium octa-*n*-butoxynaphthalocyanine (LiNc-BuO) crystals (**Figure 1**) as the oxygen probe. The EPR line-width of LiNc-BuO is highly sensitive to oxygen. LiNc-BuO has good stability and rapid response to changes in oxygen concentration (pO_2) in the tissue[56,99]. It has also been tested in numerous pre-clinical models to confirm efficacy and lack of toxicity[65,66,145-150,181,191-194] (see toxicity in next section). In summary, LiNc-BuO crystals exhibit excellent EPR detection and oxygen sensitivity with a stable linear relationship of EPR line-width vs pO_2, and above all biocompatibility in tissues for extended periods of time.

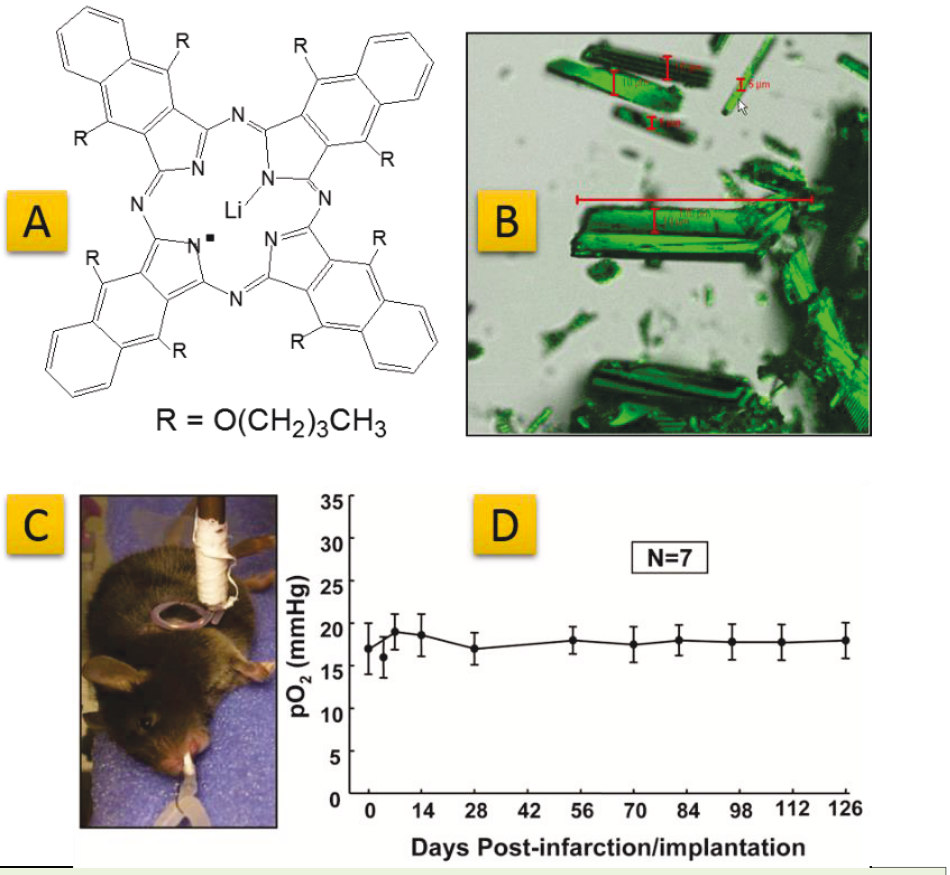


Figure 1. LiNc-BuO oxygen-sensing crystal for *in vivo* oximetry. (**A**) Molecular structure of lithium octa-*n*-butoxynaphthalocyanine (LiNc-BuO). (**B**) Crystals of LiNc-BuO showing needle-shaped microcrystals. (**C**) EPR measurement of LiNc-BuO crystals implanted in the left ventricular wall of a mouse heart. The measurements were done in a closed-chest mouse *in vivo* by EPR spectroscopy using a surface-loop resonator at L-band frequency. (**D**) Repeated measurements of myocardial tissue pO_2 measured from mice (N=7) over a period of > 3

1.3.12 Embedding of LiNc-BuO crystals in a biocompatible polymer, polydimethylsiloxane (PDMS) for safe implantation and retrieval

To avoid any contact between the LiNc-BuO crystals and the tissues, and to minimize any unknown side effects, the LiNc-BuO crystals are embedded in PDMS[102]. PDMS is a silicone polymer and has properties desirable for embedding of oximetry probes. PDMS is a biocompatible, highly flexible, highly oxygen permeable polymer, which has been used in a wide range of medical-device and health-care applications[195]. PDMS has been approved for use in human subjects and is one of the reference materials provided by the National Heart Lung and Blood Institute for standardized biocompatibility testing[196]. **Figure 2** shows photographic images of several PDMS-embedded LiNc-BuO crystals (OxyChip). The OxyChips can be prepared in a variety of shapes and sizes, as well as different amounts (1–40% w/w) of LiNc-BuO[102].

1.3.13 *In vitro* studies established OxyChip as non-toxic to cells

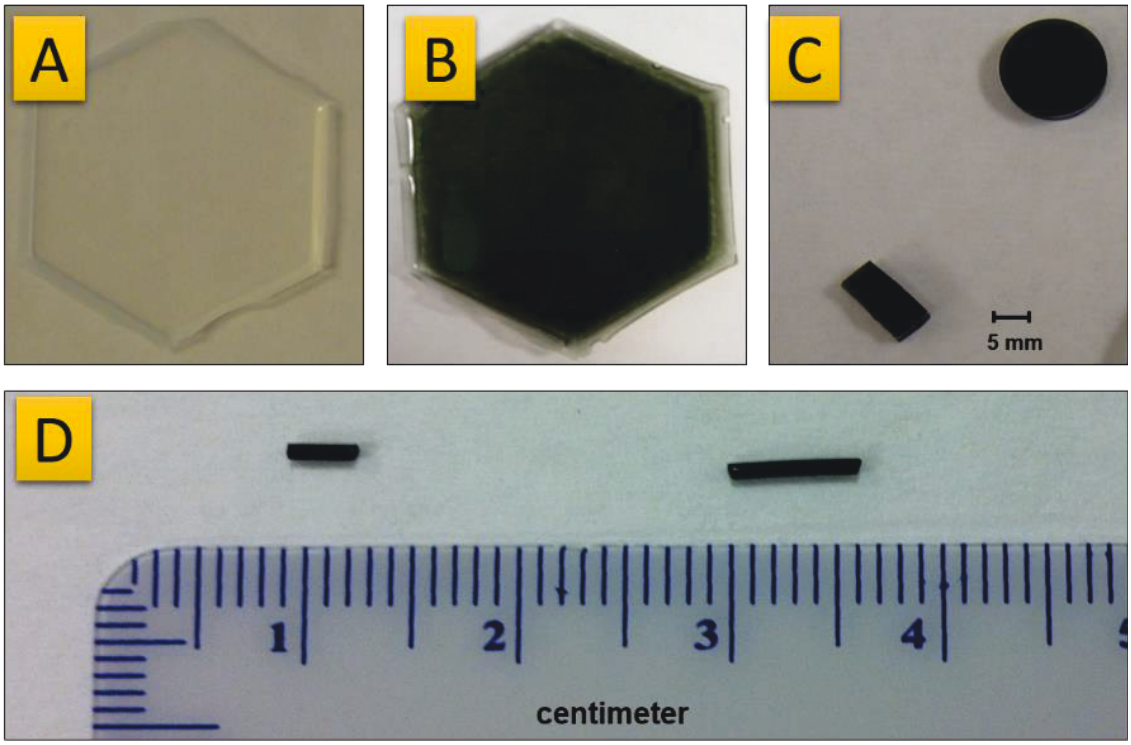


Figure 2. OxyChips fabricated by the embedding of microcrystalline LiNc-BuO in PDMS polymer. (**A**) Pure PDMS polymer without LiNc-BuO. (**B**) A large block (25x25x1 mm) of OxyChip prepared using LiNc-BuO in PDMS (40 mg/5.5 g, w/w). A uniform dispersion of LiNc-BuO is seen in the PDMS film. (**C**) Smaller blocks of LiNc-BuO in the PDMS chip. (**D**) OxyChips prepared in the form of thin wires (0.8 diam. x 3–8 mm are shown). We propose to use OxyChips with the following dimensions and composition for the clinical testing: cylindrical shape (0.6 mm diameter X 3-5 mm length; 40% LiNc-BuO in PDMS.

The OxyChip (LiNc-BuO:PDMS) has been tested for biocompatibility in multiple tests[103]. Initial *in vitro* biocompatibility tests were performed by co-incubating cultured Chinese hamster ovary (CHO), A2780 human ovarian cancer, A2780-CR cisplatin-resistant human ovarian cancer, and human smooth muscle (SMC) cells with autoclaved OxyChips for 48 hours, and subsequently determining cell viability and cell proliferation using colorimetric assays. Autoclaving was performed using a bench-top analog sterilizer (Tuttnauer® by Brinkmann Instruments, New York) at the following settings: 121°C for 1 hour at 1 atm pressure (wet cycle using steam), followed by exhaust-drying for 15–20 min. Cell-viability results did not reveal any significant difference between cells that were cultured without chips in the culture media

(control), and cells that were co-incubated with chips (data not shown). In addition, exposure of the cells to autoclaved OxyChips, by co-incubation in their culture medium for 48 hours, did not have any significant effect on the cell proliferation (**Figure 3**)[103].

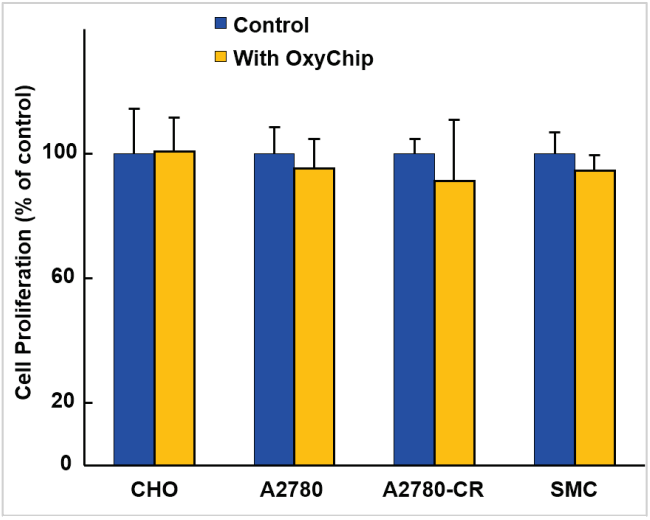


Figure 3. *In vitro* biocompatibility of OxyChip. *In vitro* biocompatibility was evaluated by assessing proliferation of cultured cells, using anti-BrdU colorimetric immunoassay, after co-incubation with OxyChip. Chinese hamster ovary cells (CHO), cisplatin-sensitive human ovarian cancer cells (A2780), cisplatin-resistant human ovarian cancer cells (A2780-CR), and human smooth muscle cells (SMC) were co-incubated with small pieces of OxyChip, in their culture media for 48 hours, after which proliferation was assessed. Data (Mean ± SD, n = 6) from cells exposed to chips, normalized to respective contro values, showed no significant difference in cell proliferation (compared to control). Results indicate that the OxyChips exhibited excellent *in vitro* biocompatibility.

1.3.14 Long-term oxygen-sensing response of OxyChip

OxyChips were implanted subcutaneously in the gastrocnemius muscle of the right hind limb of female C3H mice. A small flap of skin was cut and an OxyChip (~2 mm × 2 mm) was inserted into the muscle tissue, before the flap was sutured back in place. The sutured skin was allowed to heal for at least 24 hours prior to making EPR measurements. For RIF-1 tumors, OxyChips (~3 mm × 1 mm) were implanted into the tumors. In each animal, OxyChips were also implanted in the gastrocnemius muscle of the hind limb contralateral to the tumor (internal control). The muscle pO_2 data, repeatedly measured every week for a total of 10 weeks, demonstrated the ability of the implanted OxyChip to provide repeated measurements of *in vivo* tissue oxygenation over the 70-day duration[103]. Further, in order to verify that the implanted chip was responsive to changes in tissue pO_2, blood flow to the muscle was temporarily constricted using an elastic band and subsequently released. The decrease in blood flow during the constriction would lead to a decrease in oxygen supply to the muscle tissue. Hence, we hypothesized that the implanted chip would be able to sense the change in muscle pO_2 during constriction. The implanted OxyChips reported an average muscle pO_2 of 15.6±2.9 mmHg, which is within the range of mouse gastrocnemius muscle pO_2 values reported previously, using raw LiNc-BuO crystals (19.6±2.1 mmHg)[99], LiNc (17.6±2.5 mmHg)[101], LiPc (18±4 mmHg) [92-94], and TAF-coated LiPc (15.5±1.5 mmHg)[197]. This observation serves to validate the accuracy of the muscle pO_2 information obtained using OxyChips.

1.3.15 Lack of toxicity in pre-clinical models with the OxyChip

In order to assess the long-term *in vivo* biocompatibility of the LiNc-BuO:PDMS chip, we performed histological analysis of excised muscle tissue surrounding the implant.[103]. At the end of the long-term (70 days) implantation in muscle, animals were sacrificed and muscle tissues with implanted OxyChips were excised. Tissues, with embedded chips, were fixed in paraformaldehyde, and then sectioned in paraffin (slice thickness: 4 µm). Tissue slices were mounted on glass slides and stained with hematoxylin and eosin (H&E) stain. Images of the slides were acquired using an inverted light microscope (Nikon TE2000-U) and analyzed using MetaMorph software (Molecular Devices). It was observed that the implanted chip elicited a characteristic wound-healing response, with the recruitment of inflammatory cells and the formation of a thin fibrous capsule (**Figure 4**). Numerous dark nuclei at the interface represent polymorphonuclear leukocytes (PMNs). The presence of PMNs demonstrates that the implant triggered an acute inflammatory response, which is normally expected of any foreign material that is implanted. The pink band of tissue

(indicated by the 'F' in the images), between the inflammatory cells and the underlying muscle tissue, represents the fibrous capsule. The average thickness of the capsule was 34.89 ±11.05 µm and indicated that the fibrous encapsulation was minimal. It must be noted that the acute inflammatory response and subsequent formation of a fibrous capsule did not have any significant effect on the *in vivo* oxygen-sensing ability of the chip[103]. The calibration remained linear, with no significant difference in oxygen sensitivity, compared to a control chip that was not implanted in the animal. Overall, the results demonstrated the biocompatibility and biostability of the chip, with almost no effect on the intended functionality of the chip due to extended *in vivo* residence.

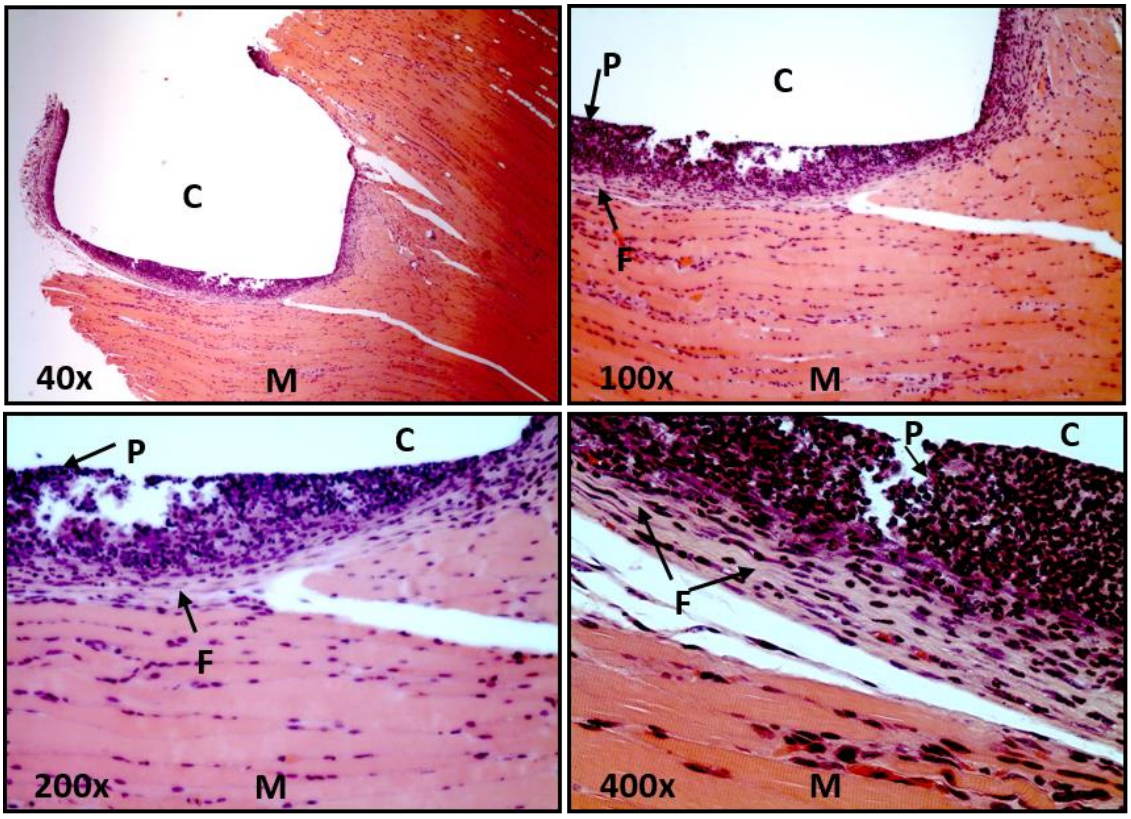


Figure 4. Biocompatibility of OxyChip. Images of the tissue sections, stained with hematoxylin and eosin, obtained using a light microscope at four different magnifications (40x, 100x, 200x, and 400x). The region where the implant was present is indicated as 'C' in the images, while the normal muscle tissue is depicted as 'M'. The letters 'P' and 'F' denote the dark nuclei of polymorphonuclear leukocytes (PMNs), and the pink band of fibrous tissue encapsulating the implant area, respectively. Images demonstrate that the implant triggered an acute inflammatory response, with minimal fibrous encapsulation. Average capsule thickness, estimated using MetaMorph software, was 34.89 ± 11.05 µm (n = 56 measurements). However, any fibrous capsule formation observed did not affect the *in vivo* oxygen-sensing ability of the implanted chip. The results show that OxyChip exhibited good *in vivo* biocompatibility and biostability.

1.3.16 Biocompatibility and biosafety evaluation of OxyChip using ISO 10993-12 guidelines

Since PDMS is established as a safe material for use as implants in humans, and the only other material that is part of the chip is LiNc-BuO, which is embedded in PDMS, we sought to determine that LiNc-BuO

crystals are held within the polymer and that there are no debris or leachables released from the implant upon long-term implantation in tissues. We performed chemical characterization analysis on the final sterilized OxyChips according to ISO 10993-12 specifications to ensure no other residues are detected and that the only material detectable in the device is PDMS. The chemical characterization analysis included Gas Chromatography Mass Spectroscopy (GC-MS) to detect the presence of organic leachables, Inductively Coupled Plasma Mass Spectrometry (ICP-MS) to detect the presence of heavy metals and inorganic leachables, and Fourier Transform Infrared Spectroscopy (FTIR) to identify the presence of the PDMS material in the extracts. The extraction conditions were as recommended in ISO 10993-12:2012 guidelines for a device that is <0.5-mm thick with both polar and non-polar solvents and at 50°C. A brief summary and conclusion of the analysis is given below (Please see **Attachment 10: Report of ISO 10993-12: 2012 Chemical characterization analysis** for a complete report of the study).

GC-MS: According to the putative identifications, the solvent extraction did not contain any of the organic volatile impurities listed in USP Guidance 467 (*United States Pharmacopeia and National Formulary (USP 37-NF 32). Vol 1. Rockville, MD: United States Pharmacopeia Convention; 2014:215-227*) above their defined concentration limits.

ICP-MS: Inductively Coupled Plasma- Mass Spectrometry (ICP-MS) was used to quantify and qualify the extractable elemental impurities from the component (OxyChip device). The samples were measured for heavy metals and elemental impurities using an external calibration approach against calibration solutions prepared in the same diluent as samples. The results showed 399 parts per billion (ppb) of lithium and 53 parts per billion of copper (Cu) in the device water extract samples. The device was found to contain low levels of low risk excipients.

FT-IR: The identity of polydimethylsiloxane (PDMS) material in the OxyChip was tested and confirmed by comparing the Infrared (IR) Spectra of the OxyChip sample with a PDMS control (OxyChip without the sensor, i.e. PDMS alone) and a PDMS reference standard (USP Lot J0L500). Based upon the maxima exhibited, as compared to the USP reference standard (±10 cm^{-1} as per USP <851> Spectrophotometry and Light-Scattering), the identity of PDMS in sample was confirmed.

In conclusion, the chemical characterization analysis of sterilized OxyChips did not show any organic volatile impurities above USP Guidance or metals of high risk, suggesting that the device is biocompatible and thus safe for implantation.

1.3.17 EPR oximetry in animal models of cancer

Repeated oxygen measurements with EPR oximetry have been used in a mouse model system of soft tissue sarcoma, using radiation-induced fibrosarcoma tumor (RIF-1) cells. Most importantly, it was found that when oxygen levels were used to guide delivery of radiation, there was a delay in tumor growth[139,169], and oxygen levels were found to increase in RIF-1 tumors with carbogen breathing[63]. Similar oxygen measurements and oxygen response were measured in RIF-1 tumors using either the OxyChip[102,174] or in TLT tumors using charcoal powder[161,174] and as well in experiments using orthotropic 9L and C6 gliomas implanted in the brain of rat[138]. These studies suggest that there is a potential for improved tumor control by delivering radiation when tumors are well-oxygenated. In some of these studies, either the timing of radiation delivery or administration of carbogen was used to increase tumor oxygenation during therapy, yielding two clinically-feasible approaches to improve the efficacy of radiotherapy.

1.4 EPR oximetry in human subjects using ink

We have demonstrated that oxygen measurements can be made in human subjects in superficial tumors using India ink. EPR oximetry measurements in human subjects were performed using a whole-body EPR spectrometer consisting of a 420-Gauss permanent magnet, magnetic field modulation and sweep coils, RF detection system including surface-loop resonators, and computer control. The spectrometer operates at an L-band frequency (~1.2 GHz), where a practical compromise between RF penetration depth and EPR sensitivity is achieved. At this frequency, RF penetration depths of approximately 10 mm are achieved. We have obtained pO_2 data in superficial tumors at different sites in cancer patients and healthy volunteers. Our initial measurements in human subjects used India ink as an oxygen sensor and under conditions that could readily be incorporated into normal clinical processes. The ink was inserted into the tumors or other

tissues of interest in the outpatient clinic, with optional local anesthesia and using sterile procedures. A 21–23G hypodermic needle and 30G tuberculin syringe were used to insert 10–25 microliters of sterilized India ink (a slurry of 200 mg/ml of Printex-U carbon black in 0.9% NaCl and 1.25% carboxymethylcellulose), whose response to oxygen had been well calibrated *in vitro* and *in vivo* in animals. One to five days later, a baseline measurement was made and then the subject was given 100% oxygen to breathe through a regular simple face mask. EPR measurements were made continuously while breathing 100% oxygen and again when the patient returned to breathing room air. For foot measurements in normal subjects, an additional period was included during which compression was applied to temporarily suspend blood supply to the tissue and induce hypoxia. These procedures took less than an hour in total and were very well tolerated by all subjects. The data obtained from the small number of tumor types led to the following conclusions: (i) the tumors varied considerably in their baseline pO_2, which ranged from 0 to 10 mmHg (Torr); (ii) the subjects varied considerably as to whether their tumors responded to increased oxygen in the breathing gas (4 out of 14 did not respond); (iii) the amount of the increase in tumor oxygen varied widely among the responders (3 to 100 mmHg). Even with such a limited amount of data, these results indicate that the ability to make repeated measurements of oxygen in tumors could be quite useful clinically. These studies demonstrate that the measurements can be made under conditions compatible with usual clinical practice. The observation that a number of tumors did not respond at all to increases in the amount of oxygen in the breathing gas illustrates how misleading it could be to try to evaluate the ability of hyperoxygenation strategies to improve outcomes without being able to detect whether the treatment did actually change pO_2 in the tumor.

1.5 EPR oximetry using OxyChip in cancer patients (Progress to-date)

1.5.1 Enrollment, implantation, and explantation of OxyChip

To-date, we have enrolled a total of 24 patients. Where needed, the OxyChips were implanted using ultrasound guidance (**Figure 5**) for precise placement within deep tumors. Out of the 24 patients, 18 patients did not receive any treatment prior to surgery; 5 patients received neoadjuvant chemotherapy and 1 patient received neoadjuvant radiation therapy while the OxyChip was implanted. All patients underwent standard of care resection with intent to remove the OxyChip (**Figure 6**). The maximum duration of the OxyChip in tumor prior to resection was 141 days. Of 24 OxyChips implanted, 22 have been recovered. In one patient (1750) the OxyChip was never detected by EPR despite multiple attempts. It was placed in a very necrotic scalp malignancy. When it was not found in the pathological specimen after resection, it was assumed to have fallen out prior to surgery. The second OxyChip, in patient 1787, was detected two days prior to surgery via EPR oximetry. It was placed in a necrotic basal cell carcinoma on the face. It was not found after exhaustive examination of the pathology specimen after resection, and the surgeon did not see it prior to placement of a myocutaneous graft. Given concerns it was still present within the patient, an MRI

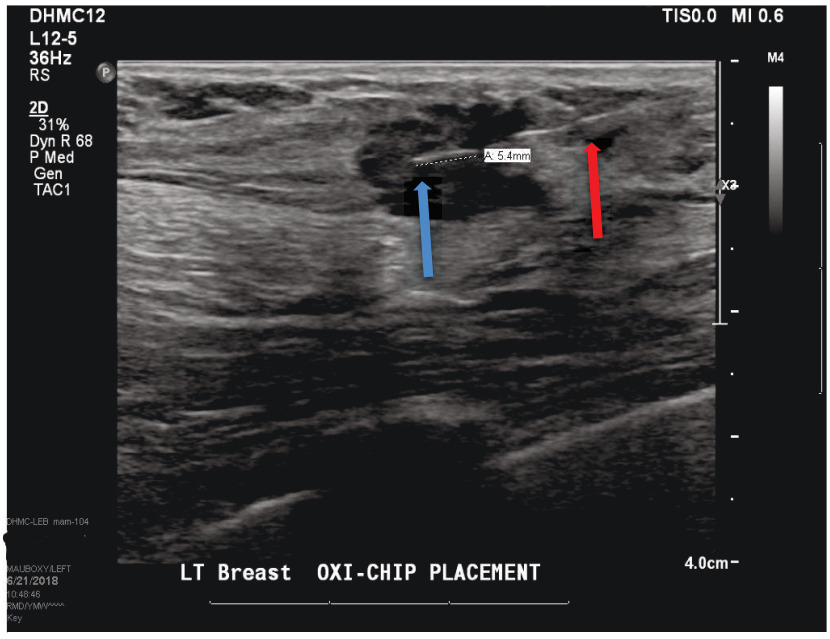


Figure 5 A typical ultrasound-guided implantation of an OxyChip. This OxyChip was inserted into the breast tumor of patient (#1782). The OxyChip (blue arrow) is within the hypodense tumor. The needle (red arrow) is being retracted.

of the face was obtained. Signal voids consistent with surgical clips were seen but no findings consistent with the OxyChip were evident (based on prior MRI imaging of the OxyChip in a phantom). Consequently it is assumed to have fallen out during surgery. In order to prevent further risk of "lost" OxyChips patients will now be implanted with an adjoining fiducial (see 1.6.3), with planned visualization of fiducials prior to surgical opening and closure.

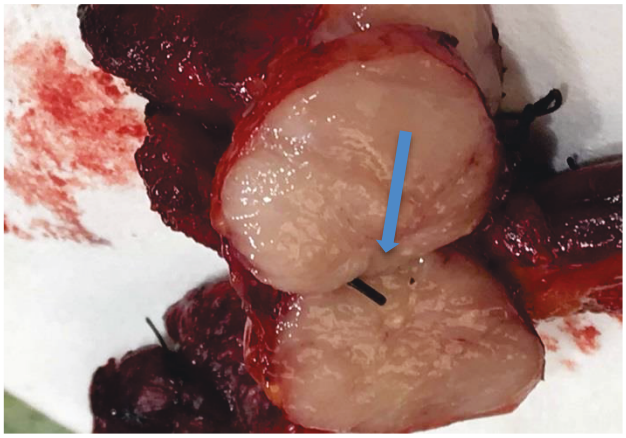


Figure 6. Removal of an OxyChip from a resected tumor (submandibular lymph node squamous cell carcinoma) from patient (#1785). The OxyChip (blue arrow) is within the transected tumor specimen.

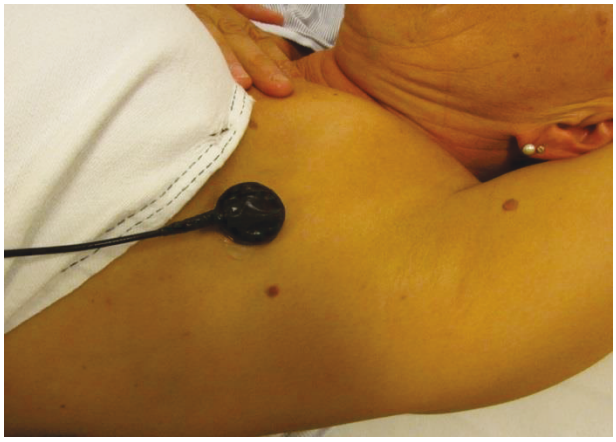


Figure 7. EPR measurement of an OxyChip in the breast malignancy of a patient (#1782) using a flexible resonator placed over the tumor.

1.5.2 Safety

The primary endpoint of this study is safety. Per protocol all patients have had physician graded toxicity using CTCAEv4.0 as well as evaluation of tissue pathology associated with the OxyChip. These findings are summarized in Table 1 for the first 23 patients.

DH Study ID	Diagnosis	Location of OxyChip Implantation	Treatment Prior to OxChip Removal	Max AE	Description AE	UADE	Location of OxyChip on microscopic evaluation	Pathologic Findings Associated with OxyChip	Pathologic Expectations in Context of Implantation Event
1735	Lipoma	Upper left back, subcutaneous	None	0	NA	No	Not within tumor; within superficial fascia of subcutaneous mass	Mild macrophage predominant chronic inflammatory reaction at needle site	Anticipated
1746	Melanoma	Left anterior tibia, skin	None	1	Minor bleeding from implantation needle	No	Within tumor	Minor hemorrhage at site of injection	Anticipated
1747	SCC Skin	Left nasal dorsum, skin	None	0	NA	No	Within tumor	Mild macrophage and foreign body type giant cell reaction at OxyChip site	Anticipated
1748	Melanoma	Scalp, skin	None	1	Pruritis, scalp	No	Within tumor	Tumor necrosis and mild hemorrhage immediately	Anticipated

								adjacent to injection site	
1749	BCC	Left temporal scalp, skin	None	1	Pruritis	No	Within tumor	Minor focal hemorrhage seen adjacent to the deep margin. Very focal collection of macrophages.	Anticipated
1750	SCC Skin	Scalp, posterior superior, skin	None	0	NA	No	Not found, presumed lost prior to surgery due to rapidly progressive tumor necrosis	NA	NA
1752	SCC Skin	Right posterior triangle neck, subcutaneous mass	None	1	Discomfort at surgical site	No	Outside of and adjacent to tumor within dermis	Focal disrupted tissue at edge of tumor with mild nonspecific chronic inflammation	Anticipated
1758	FTC	Thyroid	None	0	NA	No	Within tumor	No reaction	NA
1760	SCC Skin	Frontal Scalp, Left, skin	None	0	NA	No	Within tumor	No reaction	NA
1764	SCC Skin	Infraorbital cheek, Left, subcutaneous	None	1	Minor bleeding from implantation needle	No	Adjacent to tumor, but not within tumor; 0.4 cm from tumor margin	Focal organizing fat necrosis	Anticipated
1765	SCC Skin	Right temporal scalp. Skin	None	1	Minor bleeding from implantation needle	No	Within tumor	No reaction	NA
1768	SCC Skin	Right Neck, level II lymph node	None	1	Minor bleeding from implantation needle. Mild bruising.	No	Within tumor	Cystic degeneration of tumor	Anticipated
1770	IDC	Right Breast	None	1	Minimal bleeding associated with implantation. Mild bruising at needle insertion site.	No	Within tumor	No reaction	NA
1779	IDC	Left Breast	None	1	Minor bleeding from implantation needle.	No	Not within tumor, 1 mm from tumor edge	Minimal fat necrosis, macrophage infiltrate immediately	Anticipated

					Minor bruising			surrounding the OxyChip	
1761	IDC	Left Breast	Chemotherapy	1	Mild discomfort from implantation	No	Uncertain relationship to pretreatment tumor	No reaction	NA
1762	IDC	Left Breast	Chemotherapy	1	Mild discomfort and bleeding from implantation procedure. Bruising from implantation needle.	No	Uncertain relationship to pretreatment tumor	No reaction	NA
1771	IDC	Left Breast	Chemotherapy	1	Minor bleeding associated with implantation. Minor bruising near needle insertion site. Mild discomfort of left breast, not specifically associated with area of implantation.	No	Uncertain relationship to pretreatment tumor	No reaction	NA
1780	Sarcoma	Right Chest Wall	Radiotherapy	1	Minor bleeding from implantation procedure that resolved minutes after the completion of the procedure	No	Within collagenous soft tissue skeletal muscle fascia outside of viable tumor at least 6 mm	No reaction	NA
1781	IDC	Right Breast	Chemotherapy	1	Bleeding at needle insertion site	No	Uncertain relationship to pretreatment tumor	Focal fibrosis, few macrophages	
1782	IDC	Left Axillary Node	Chemotherapy	0	NA	No	No residual tumor	No reaction	NA
1783	IDC	Right Axillary Node	None	1	Bruising at implantation site	No	Within tumor	Focal fibrosis, macrophages, and apparent tumor capitation. Likely a function of tumor integrity and not abnormality	Anticipated

								in procedure. Tumor at the edge of the cystic cavity is viable and unaffected	
1784	SCC Skin	Above manubrium, skin	None	1	Bleeding from implantation	No	Unclear	Tumor necrosis within chip site	
1785	SCC Tonsil	Level II LN, neck	None	1	Pain and bleeding from implantation	No	Within tumor	No reaction	NA

Table 1: Summary of toxicity for first 23 patients. Maximum adverse events (AE) were graded using CTCAE v4.0. Abbreviations: SCC = squamous cell carcinoma; BCC = basal cell carcinoma; FTC = follicular thyroid cancer; IDC = invasive ductal carcinoma; AE = adverse event; UADE = unanticipated adverse event;

The resected tissues showed no signs of infection. There was evidence of an inflammatory response associated with expected trauma along the needle track from the implantation itself. In a few patients, a typical foreign body reaction was evident. As graded by the pathologist (J.P.) no findings indicative of significant, clinically concerning inflammation or reaction were found in any patient. In addition, via clinician assessment, no patients had any significant clinical toxicity associated with the implantation procedure or the presence of the OxyChip itself. The maximum CTCAE toxicity grade was 1.0.

Phase 1A and 1B still require removal of the OxyChip . However, it is important to note that based on the above safety data the FDA has now given permission to proceed with evaluation of permanent implantation of the OxyChip.

1.5.3 pO_2 measurements

To date, we have made a total of >60 pO_2 measurements. The pO_2 values successfully measured (**Figure 7**) from the patients, on multiple visits, are shown in **Table 1**. In each session, where an EPR signal from the OxyChip could be detected, pO_2 measurements were made continuously for 5-10 min while the patient breathed room air, followed by 10 min of breathing 100% oxygen, and then for 10 min after the patient had returned to room-air breathing. The tumor pO_2 values showed varying degrees of hypoxia and response to hyperoxygenation (**Figure 8**). The EPR signal could not be detected in some patients. Ultrasound imaging revealed that the OxyChips in these tumors were deeper (10-16 mm) than the usual depth in other tumors, which may explain our inability to obtain an EPR signal.

1.5.4 Integrity and calibration of OxyChip

All the explanted OxyChips were morphologically intact, except in 2 cases in which the chip was cut during removal from the excised tumor in the pathology lab. The oxygen-sensing calibration of the explanted chips was unchanged from the pre-implant calibration, suggesting that the oxygen-sensing ability of the chip was unaffected during its residency in the tissue for more than 4 months.

1.5.5 Compliance with FDA and Dartmouth IRB regulation

We have obtained approval from the FDA (Investigational Device Exemption) and the Dartmouth IRB (CPHS) to increase the total number of enrollments from the initial cohort of 12 to 60 patients, and to extend

the length of OxyChip residence in the tumor for up to 1 year. Currently, we have completed the Phase 1 safety and efficacy study requirement set by the FDA.

1.5.6 Summary

Safety: For the patients enrolled to date, we did not observe any reportable toxicity (grade 3 or higher according to the Common Terminology Criteria for Adverse Effects (CTCAE)) caused by the implanted OxyChips. We did not observe any unanticipated adverse device effects (UADE). The EPR measurements demonstrated that the OxyChip is robust and capable of making direct and repeated measurements of pO_2 for weeks and months without toxicity or change in oxygen sensitivity. Specifically, the measurements demonstrated the ability of the chip to measure tumor pO_2 values during room-air breathing and hyperoxygen intervention. The tumors showed variable responses to 100% O_2 breathing, with most tumors showing a positive (increase of pO_2) response to hyperoxygen intervention, while some tumors showed minimal response. **Figure 8** shows a complete listing of pO_2 data obtained from 39 measurements. Overall, the results indicated that the pO_2 values are both tumor-type and time dependent suggesting the importance of personalized and real-time measurement.

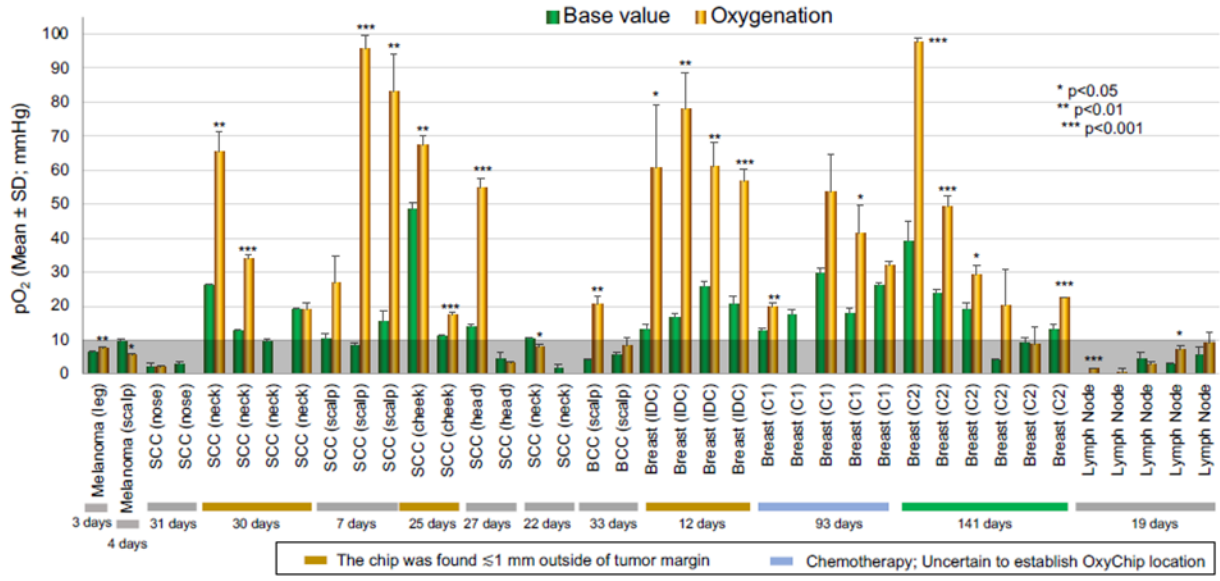


Figure 8, Tumor pO_2 values in patients breathing room air (Base value) and 100% oxygen (Oxygenation). The pO_2 values, measured from a total of 13 patients, are included in the plot. Multiple measurements from the same patient are indicated by each bar on the bottom. Below this bar is the total amount of days that the OxyChip was implanted. The data ranging from 0 to 10 mmHg is shaded to indicate the generally accepted hypoxic range. All data represent Mean±SEM. Significance compared to corresponding base value: * p<0.05; ** p<0.01; *** p<0.001.

Overall, the initial clinical measurements suggest that the OxyChip is robust and capable of making direct and repeated measurements of pO_2 for weeks without toxicity or change in oxygen sensitivity. Specifically, measurements in the first cohort of surgical cancer patients demonstrated the ability of the chip to measure tumor pO_2, whose values ranged from severely hypoxic (~1 mmHg) in an SCC tumor to well oxygenated (~24 mmHg) in a lipoma, under baseline conditions. The tumors showed variable responses to 100% O_2 breathing (via face mask). Histopathological evaluation of the excised tissues revealed no unexpected inflammation or hemorrhage at the site of the OxyChip implantation.

1.6 Justification of cohort design and revisions of the protocol

1.6.1 Rationale for expanding Phase 1A and Phase 1B Cohorts

Pilot studies for medical devices are intended to test the initial design of the device and procedures. It is expected that device modifications or procedure modifications will be made based on the experience of various providers in real clinical scenarios during the testing phase. The following are some reasons for conducting a pilot study for medical devices and how a larger study (*i.e.*, a more typical pilot study size of up to 30 patients) will be important for studying the safety and performance of the OxyChip.

We will initiate Phase 1A (oxygen measurements without therapy planned prior to surgical resection) with two cohorts of 3 patients. The table below provides justification to expand the Phase 1A study from these two cohorts of 3 patients to then enroll an additional 24 patients if no unacceptable toxicities including unanticipated adverse device effects (UADE) occur in the first 6 patients.

Goal	**Phase 1A - 6 patient study**	**Benefit to expanding the study**
Help address specific safety concerns	The initial 6 patients suggest safety of the device and study procedures in pre-surgical patients, but the study is small.	Increasing the Phase 1A to 30 patients will provide additional safety information for pre-surgical patients prior to proceeding with either a “Pivotal” study or a study combining oxygen measurements with an oxygen treatment for a therapeutic stud.
Permit initial assessment of device design	Initial device design suggests that the device and procedures are well tolerated.	Additional designs of the EPR measuring device (not in the OxyChip itself) are planned to help identify the OxyChip more efficiently in patients and in tumor specimens.
Better define/refine the clinical endpoints and intended patient population		Additional patients will allow more measurements of specific tumor types (*e.g.,* skin SCC, melanomas and SCC of the head and neck region) to collect baseline pO_2 and oxygen responsiveness in larger cohorts of these tumor types.
Provide investigators with initial device experience and identify special training needs	All injections were performed by only two clinicians, Dr. Chen and Dr. Jarvis.	Expanding the trial will allow more investigators to be trained in injection procedures. Specifically, Dr. Schaner will be trained to perform injections.
Estimate the required patient population size		Larger cohort of similar patients will allow for more accurate power calculation for larger “Pivotal” or therapeutic trial.

In a similar design strategy, we will initiate Phase 1B (oxygen measurements during either standard chemotherapy or radiotherapy prior to surgical resection) with one cohort of 3 patients receiving radiotherapy and one cohort of 3 patients receiving chemotherapy. Only after a full evaluation of 3 patients

receiving chemotherapy will additional patients receiving chemotherapy be enrolled. Similarly, only after a full evaluation of 3 patients receiving radiotherapy will additional patients receiving radiotherapy be enrolled. The two cohorts in Phase 1B are independent of each other, so either of these cohorts may continue accrual after the initial 3 patients while the other is still accruing the initial 3 patients. A total of 30 patients will be enrolled in Phase 1B.

1.6.2 Rationale for increasing the duration of residency of the OxyChip in a tumor

Patients in the first 2 cohorts of 3 patients in Phase 1A were implanted with the OxyChip for up to 32 days and monitored post explantation. No unacceptable toxicities or unanticipated adverse device effects were observed during or after study procedures. This indicated that the OxyChip is safe and does not elicit any reaction or undesirable effect on the host tissue. Based on these initial results and the need for monitoring patients for longer periods, especially those receiving radiation or chemotherapy treatments, it is desirable to keep the implant for longer periods until the treatment is completed. This is also important if the schedule for surgical resection of the tumor changes after implantation of the chip. We have completed safety and efficacy studies of OxyChips implanted in rat muscle and measured *in vivo* for 52 weeks. The results showed stable and repeated EPR oximetry measurements over the 52-week period without any toxicity or undesirable effects to the host tissue. The chip was intact with stable EPR and oxygen sensitivity. Hence, we requested and received approval for the OxyChip to be implanted in the patients' tumors for up to 52 weeks before removal during routine surgical resection.

1.6.3 Rationale for the option of implanting fiducials with the OxyChip

In order to assist with locating the OxyChip during treatment, during EPR measurements, and during/after resection, the protocol was revised to allow for fiducial(s) (either one or two) to be implanted along with the OxyChip during the procedure. The decision to implant fiducials is at the discretion of the investigator.

Because tumors size and shape can change during the course of radiation, the location of the OxyChip in relationship to the tumor may also change. By implanting fiducials with the OxyChip, the location of the OxyChip will be able to seen on CT scans that patients intermittently receive during radiation treatment as part of clinical care. This will allow for better interpretation of the pO_2 measurements that are collected throughout the treatment, as the location of the OxyChip in relation to tumor tissue will be evaluable.

Adding fiducials will also assist in locating the OxyChip for EPR measurements. Although OxyChips can be located in vivo using ultrasound guidance, it can be challenging to do so; fiducials can be located quicker than the OxyChip implanted alone and with higher sensitivity. This allows for faster placement of the resonator, which facilitates faster oxygen measurements. This is of great importance in the clinical translation of EPR oximetry, where time is limited.

Fiducials will also assist in procedures during and after resection. In order to assure that the OxyChip has been explanted, the pathology specimen will be scanned prior to surgical closure in the pathology lab. If the implanted fiducial(s) are located in the resected specimen, then there is high confidence that the OxyChip has been successfully explanted (See section 5.2.4 for more information on this procedure). Fiducials can also assist with assessing the pathology specimen after resection to ensure that the OxyChip is not cut during the dissection of the specimen, and to rapidly identify the location of the OxyChip in the specimen. If two fiducials are implanted, the orientation of the OxyChip will be known, so the pathologist will cut parallel to the two fiducials in order to not cut the OxyChip in the process.

A study has been completed where an OxyChip was successfully implanted into a phantom with two fiducials on each side of it in a number of different substrates without difficulty. EPR oximetry data in a known oxygen concentration was also collected from the OxyChip with and without fiducials present. These results showed that the presence of the fiducials had no significant effect on the pO_2 measurements (Figure 9).

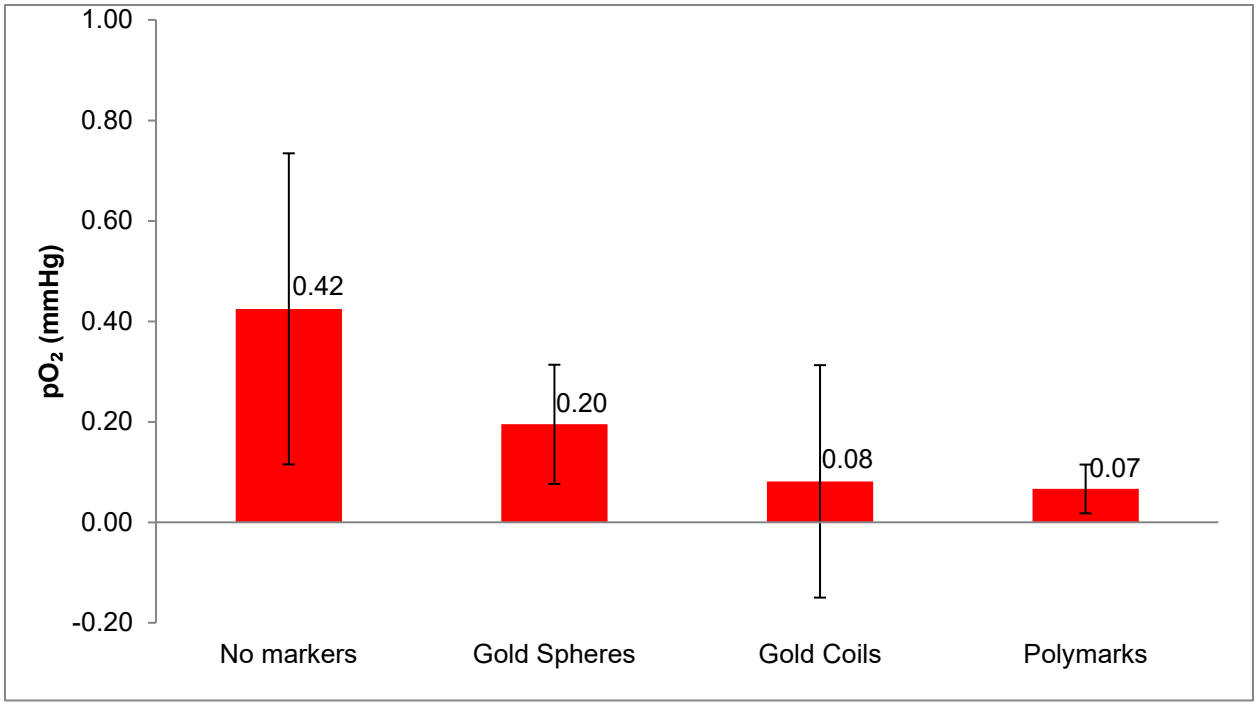


Figure 9: Effect of fiducials on OxyChip pO_2 measurements. An OxyChip was inserted into a nitrogen flow tube and EPR oximetry was performed ("no markers"). The same experiment was repeated with two fiducials flanking the OxyChip. No significant difference in pO_2 was noted, indicating no effect of the present of the fiducials on OxyChip derived pO_2 measurements.

The use of fiducials in the radiation oncology clinic is standard of care in a number of malignancies. For example, in order to facilitate image guidance daily during radiotherapy for prostate cancer the radiation oncologist places two to three fiducials into the prostate prior to treatment under ultrasound guidance in the clinic. These fiducials remain in place permanently after treatment. In the OxyChip protocol, the fiducials will be removed.

1.7 Risk/Benefits

In this Phase 1 Safety clinical trial, there are no expected benefits to the patients. The benefit is for those patients who have a desire to advance this technology and/or to improve radiotherapy for future patients. Furthermore, the knowledge gained by repeated measurement of tumor oxygen levels, especially during radiotherapy is critical for developing and implementing novel hypoxia-targeting therapies.

The risks are expected to be minimal. As with any implantation, there is a risk of pain, bleeding or infection. We expect this risk to be minimal and not more than the risk of a core needle biopsy, since the implantation procedure is minimally invasive, and performed with a needle insertion. Risks associated with placement beyond the risks associated with placement of the OxyChip itself are expected to be minimal. There is a very low risk of fiducial migration, estimated at 0.35% in one study of prostate cancer and fiducial placement [211]. Over 1000 fiducials have been implanted into patients in the Radiation Oncology clinic at DHMC as part of routine clinical care for prostate cancer with no significant complications (Alan Hartford, personal communication). Patients are consented for placement of fiducials in addition to the OxyChip. There is an unknown risk from LiNc-BuO in humans; however, no toxicity is expected based on studies using animal models and because the LiNc-BuO crystals are embedded in biocompatible PDMS to further eliminate any potential risk. Furthermore, the biocompatibility of OxyChips has been established in pre-clinical studies for

up to 52 weeks and, to date, in humans for up to 141 days with approval to be implanted for up to 52 weeks. In addition, the OxyChips will be placed in superficial locations and will be removed in this protocol when the tumor is resected. There is a possibility that the patient might be in an uncomfortable position during oxygen measurements, but re-positioning can be performed at any time if needed.

The study population will consist of patients who are ≥ 18 years of age, both genders, and must be competent to consent to participate in the trial. Risks of the device will be minimized by placement of the device under sterile conditions using a minimally-invasive procedure. The implantation site will be monitored closely.

2 Study Objectives

2.1 Primary Objective

This is a safety study to demonstrate that the implantation procedure, the OxyChip, and any subsequent oxygen measurements will be well-tolerated by the patient with minimal risk for complications. Specific adverse effects that will be monitored include:

- Allergic reactions or signs of inflammation at the site of implantation or systemically
- Infection at the site of implantation
- Bleeding at the site of implantation
- All tumors will be excised with the OxyChip in place, and histology will be analyzed for signs of tissue reaction and inflammation adjacent to the OxyChip
- Device (PDMS-embedded) breakage, loss of EPR or oxygen sensitivity, or change of calibration
- Erosion of skin over the implantation site
- Any unanticipated adverse device effects (UADE) will be assessed and reported. UADE will be defined as, “any serious adverse effect on health or safety or any life-threatening problem or death caused by, or associated with, a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the investigational plan or application, or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects” (21 CFR 812.3(s)).

2.2 Secondary Objective

This study will also determine the feasibility of repeated measurements of oxygen in tumors using the OxyChip and EPR oximetry. This objective is unrelated to the primary safety objective.

- To assess the feasibility of making repeated measurements of tumor oxygen levels in patients with easily-accessible, superficial tumors.
- To determine the tumor oxygen levels in patients without therapy prior to surgical excision (Phase 1A), or during a course of radiotherapy or neoadjuvant chemotherapy prior to surgical excision (Phase 1B).
- To determine the workflow and time required for each daily oxygen measurement.

2.3 Exploratory Objective

Collection and future use of tumor tissue surrounding the implanted device will be done to analyze molecular biomarkers related to hypoxia, angiogenesis, and tumor growth. The tissue analysis will explore the potential for any identifying biomarkers of the tumor that predict or otherwise are associated with variation in tumor growth or responsiveness to therapy.

3 Study Design

3.1 General Design

This is a Phase 1 clinical trial. Total enrollment will be 60 evaluable patients. Evaluable patients will be defined as patients who have the OxyChip implantation for the minimum of 3 days.

The study is split into Phase 1A (shorter duration of implantation with no other cancer therapy planned prior to excision) and Phase 1B (longer duration of implantation that is typically up to 12 weeks while receiving radiation therapy or longer if receiving neoadjuvant chemotherapy prior to surgical excision), as described below. OxyChip implant duration for both phases can be extended to 52 weeks (excluding the initial 6 evaluable patients in Phase 1A which may have the OxyChip implanted up to 4 weeks).

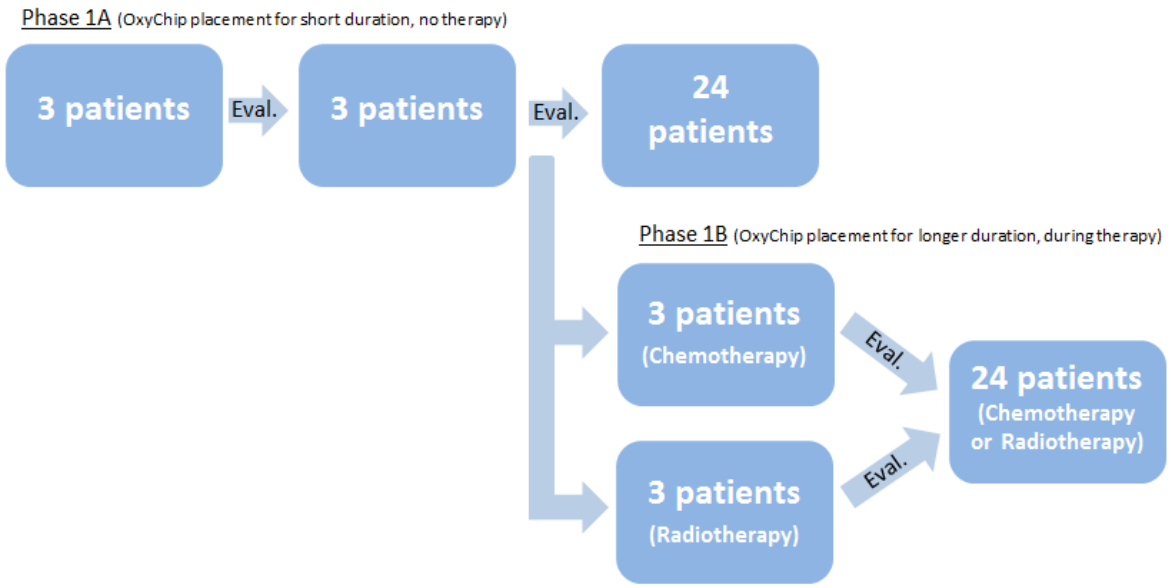
The initial 6 evaluable patients in Phase 1A will have the OxyChip placed for a short duration (up to 4 weeks) after which the OxyChip will be removed when the tumor mass is resected, prior to delivery of any further therapies. After successful implantation and removal of the OxyChip without any unacceptable toxicity in the first 6 evaluable patients, the Phase 1A cohort will be expanded to a total of 30 patients. In order to complete the safety and toxicity assessment of the short-term implantations, we will require that at least 3 patients have OxyChip implantations for a duration of at least 2 weeks, prior to proceeding to Phase 1B (long term implantation). This may require enrollment of additional patients in Phase 1A in order to meet this criterion. Phase 1B will consist of one cohort of 3 evaluable patients receiving chemotherapy treatment and one cohort of 3 evaluable patients receiving radiotherapy. Only after a full evaluation of the first 3 patients that underwent chemotherapy will additional patients receiving chemotherapy be enrolled. Similarly, only after a full evaluation of the first 3 patients that underwent radiotherapy will additional patients receiving radiotherapy be enrolled. The two cohorts of 3 patients in Phase 1B are independent of each other, so either of these cohorts may continue accrual after the initial 3 patients while the other is still accruing for the initial 3 patients. A total of 30 patients will be enrolled in Phase 1B. .

This larger cohort will allow further assessment of safety, as well as optimization of procedures for the OxyChip placement and subsequent oxygen measurements for different tumor types in different anatomical locations. This expanded Phase 1 study will also achieve a secondary goal of increasing patient numbers for specific tumor types (*i.e.*, melanomas, squamous cell carcinomas and sarcomas) to assess the levels of tumor oxygenation and their response to supplemental oxygen breathing.

The phase 1B patients will have up to five oxygen measurements per week during the course of radiation or chemotherapy. Patients will be evaluated weekly during radiotherapy or prior to each chemotherapy administration or follow-up appointment for assessment of any adverse effects, as per the primary objective. Oximetry measurements will be taken periodically, from at least one day after implantation up to the OxyChip's removal. The OxyChip will be removed at a planned surgery as part of standard-of-care therapy. Phase 1A patients will be evaluated at least monthly prior to OxyChip removal for assessment of adverse effects, again for the primary objective.

If a patient cannot or does not return for an evaluation, the study team will contact the patient by phone or email to check for development of new adverse events or changes in health status. Assessments by non-study physicians or physician extenders as part of standard care are also an acceptable replacement, if the patient is not accessible for an in-person evaluation. Following resection, the tissue surrounding the OxyChip will be examined for any adverse effects, as per the primary objective. For the exploratory objectives, the tissue will also be examined for biomarkers associated with hypoxia or growth.

Schema:



PHASE 1A	
Purpose	Primary: Safety of the OxyChip placement procedure and OxyChip removal after short duration (typically less than 4 weeks, but up to 52 weeks). Secondary: Feasibility of taking repeated oxygen measurements using the OxyChip.
Patient population	There will be a total of 30 patients enrolled in Phase 1A. Initially, 3 patients will be enrolled; the OxyChip will be placed; physical examination of the site will be made periodically (at least in conjunction with visits where oxygen measurements will be made) starting from one day following implantation for up to 52 weeks – at which point the tumor will be resected with concurrent removal of the OxyChip. If no adverse events are noted in the physical exams or tissue pathology, an additional 3 patients will be enrolled in this phase of the study. The decision to start the second cohort of 3 will be made by the study PI after review of surgical pathology reports and after the first 3 evaluable patients are at a time point more than 2 weeks following surgical removal of the OxyChip. Next, if no unacceptable toxicity is noted after completion of the first 6 patients, the enrollment will be open to 24 more, for a total of 30 patients. (Note: the enrollment totals will include patients enrolled at DHMC or Emory University, once the addition of Emory as a participating site is approved.) In addition the following eligibility and exclusion criteria will apply: • Superficial tumors located ≤ 3 cm from skin or mucosal surface. • Surgical resection of the tumor is planned at least 3 days following implantation. • Eligible tumors for this phase of the protocol are any tumors that (a) are identified by imaging or physical exam to be superficial enough for OxyChip placement and (b) will receive surgical resection with intent to remove the entire tumor.

	• Previous radiation to the site is not allowed. • Planned use of chemotherapy or radiation therapy or use of angiogenesis inhibitor prior to resection is not allowed. • Patients do not have to agree to an oxygen measurement in order to be eligible. Although oxygen measurements are desirable, the primary endpoint is safety of the OxyChip placement and removal over a short time frame. • Tumors must be $\geq$ 2.5 cm in minimum diameter to be eligible.
OxyChip placement	Placement of OxyChip will be through a minimally invasive procedure (*see methods for implantation protocol*).
OxyChip removal	The OxyChip will be removed at the time of surgical resection, which will occur within 52 weeks from the time of implantation. Surgery date must be at least three days after placement of the OxyChip. Tumor tissue surrounding the implanted device will be collected and examined for adverse events (primary) and stored to allow for future evaluation of molecular biomarkers (exploratory).
Oxygen measurements	The goal is for at least 2 oxygen measurements to be taken in the period between the day of OxyChip placement and removal (secondary objective). Note, however, that oxygen measurements are not necessary to determine the presence or absence of any unexpected adverse device events of implanting the OxyChip, for the study's primary goal.

PHASE 1B	
Purpose	Primary: Safety of the OxyChip during neoadjuvant radiotherapy or neoadjuvant chemotherapy with planned surgical resection as part of standard oncologic therapy. Secondary: Feasibility of oxygen measurements during neoadjuvant radiotherapy or neoadjuvant chemotherapy using the OxyChip.
Patient population	There will be a total of 30 patients enrolled in Phase 1B. Phase 1B will consist of one cohort of 3 evaluable patients receiving chemotherapy treatment and one cohort of 3 evaluable patients receiving radiotherapy. Only after a full evaluation of the first 3 patients that underwent chemotherapy will additional patients receiving chemotherapy be enrolled. Similarly, only after a full evaluation of the first 3 patients that underwent radiotherapy will additional patients receiving radiotherapy be enrolled. The decision to enroll additional patients in each cohort will be made by the study PI after review of surgical pathology reports and after the 3 evaluable patients are greater than 2 weeks after surgical removal of the OxyChip. The two cohorts of 3 patients in Phase 1B are independent of each other, so either of these cohorts may continue accrual after the initial 3 patients while the other is still accruing for the initial 3 patients. In addition, the following eligibility and exclusion criteria will apply: • If a patient is to receive neoadjuvant chemotherapy or radiotherapy at a hospital other than DH prior to resection of the cancer at DH, patients

	will still be eligible for enrollment as long as a PI or Co-PI on the study can evaluate the implanted site at least every two weeks during treatment. Of note, patients must receive their surgery at DH to be eligible. • Superficial tumors located ≤ 3 cm from skin or mucosal surface, where neoadjuvant radiotherapy or neoadjuvant chemotherapy followed by surgical resection is planned. • Eligible tumor histology for this protocol is any biopsy proven malignancy expected to undergo neoadjuvant chemotherapy or radiotherapy prior to resection • Previous radiation to the site is not allowed. • Use of angiogenesis inhibitors is not allowed. • Planned use of concurrent neoadjuvant chemoradiotherapy, or sequential neoadjuvant radiation therapy and chemotherapy, prior to resection, is not allowed. • Patients do not have to agree to an oxygen measurement in order to be eligible. Although oxygen measurements are desirable, the primary endpoint is safety of OxyChip during neoadjuvant radiation or chemotherapy. • Tumors must be ≥ 2.5 cm in minimum diameter to be eligible.
OxyChip placement	Placement of OxyChip will be through a minimally invasive procedure (*see methods for implantation protocol*).
OxyChip removal	The OxyChip will be removed with the tumor mass at the time of surgical resection and examined for adverse events (primary goal). Tumor tissue surrounding the implanted device will be collected and stored to allow for future evaluation of molecular biomarkers (exploratory goal).
Oxygen measurements	The goal is at least two measurements prior to the start of radiation therapy and then up to five times per week during radiotherapy or chemotherapy for the secondary objective. Note, however, that oxygen measurements are not necessary for the study's primary goal to determine the presence or absence of any adverse events after implantation of the OxyChip.

3.2 Primary Study Endpoint

For the primary objective (safety of the implantation procedure: the OxyChip itself and removal of the OxyChip is expected to be well-tolerated with minimal risk for complications). The primary endpoints will be

all grade 3 or higher toxicities according to the Common Terminology Criteria for Adverse Effects (CTCAE) that are attributed to the device or procedure.

3.3 Secondary Study Endpoint

For the secondary objective (feasibility of repeated oxygen measurements in tumors using the OxyChip and EPR oximetry) the secondary endpoint is completion of planned oxygen measurements.

4 Subject Selection and Withdrawal

4.1 Inclusion Criteria

1. Phase 1A: Any tumor identified by imaging or physical exam to be accessable to OxyChip implantation and measurements and that is going to receive surgical resection with intent to remove the entire tumor. The tumor must be sufficiently large to accommodate the OxyChip.
2. Phase 1B: Any biopsy-proven malignancy expected to undergo neoadjuvant chemotherapy or radiotherapy prior to resection. The tumor must be sufficiently large to accommodate the OxyChip.
3. The tumor must be within 3 cm of the surface of the skin or mucosa.
4. Age ≥18 years old.
5. Subject must be capable of giving informed consent
6. Anticipated time between implantation and planned surgical excision of at least three days.
7. Tumors must be ≥ 2.5 cm in minimum diameter to be eligible.

4.2 Exclusion Criteria

1. Pregnant women or women of childbearing potential without adequate contraception. Contraception, which can include abstinence, is required from the first day of the last menstrual period until the removal of the OxyChip.
2. Receipt of concurrent chemotherapy and radiotherapy, or planned sequential chemotherapy and radiotherapy, prior to resection (Phase 1B),
3. Receipt of Avastin, or other angiogenesis inhibitors, during the study.
4. Prior radiotherapy to the site of implantation.
5. Having other implanted (not removable) devices that generate electrical artifacts or that could be altered by the EPR magnetic field, such as cardiac pacemakers or defibrillators.
6. Concurrent enrollment in any clinical research study, in the absence of cancer recurrence, in which the other study can reasonably be anticipated to have the potential for causing adverse events that would affect our primary endpoint of assessing the safety of the OxyChip device. If a study is not felt to impact the evaluation of adverse events in this trial then the patient will be eligible for concurrent enrollment. In the presence of confirmed clinical recurrence after initial cancer therapy (and after removal of OxyChip) during the year-long follow up stipulated in the protocol, patients will be eligible for all clinical trials as deemed appropriate by the treating oncologist.
7. Patient platelet blood count < 50,000/μl of blood, and absolute neutrophil count < 1,000/μl of blood. Laboratory values must be obtained at least 3 months prior to implantation of the OxyChip.

4.3 Subject Recruitment and Screening

Patients will present to one of the DHMC surgical, medical or radiation oncologists who will review the case. If the patient meets criteria for enrollment and the surgeon is planning tumor resection, the case will be reviewed with the PI or a co-investigator to determine suitability for enrollment. The patient will then be recruited and consented by the PI or one of the co-investigators in collaboration with the treating surgical, medical or radiation oncologists.

Patient enrollment will be entered into the Velos eResearch Database.

The study will not be advertised.

Screening requirements for the study:

1. Any imaging of the tumor (including ultrasound, MRI or CT scans) must be available for review to guide the OxyChip implantation. A radiation-therapy-planning CT scan may be used to meet this requirement in Phase 1B. For patients with tumors involving the skin, which are easily visualized and palpated on clinical exam, imaging is not required. This requirement will be determined by PI and surgical oncologist, but in general, will be consistent with standard practice for surgical planning for each tumor.
2. Physical examination.
3. Pregnancy test, if applicable, within 14 days of OxyChip implantation.

4.4 Early Withdrawal of Subjects

4.4.1 When and how to withdraw subjects

A subject may be withdrawn from the study at any time at the discretion of the treating physicians or if the patient withdraws consent. If the OxyChip was placed before withdrawal, there will be two options for withdrawing from the study, as follows:

1. Discontinuing the oxygen measurements: Repeated oxygen measurements will be stopped at the patient's request for any reason. The reason will be recorded and tracked as part of the study feasibility outcome. Under these circumstances, the OxyChip will not be immediately removed. Preferably, the OxyChip will be removed at the time originally planned when the tumor is resected.
2. Removal of the OxyChip: The OxyChip may be removed at any time at the patient's request. If the patient wishes to withdraw completely from the study, then the OxyChip will be removed. The OxyChip will also be removed early if there is an adverse event associated with the implant or if it is having (or may have) an adverse effect on the patient. This will require either surgical excision of the OxyChip or an en bloc surgical resection of the tumor with inclusion of the OxyChip. The type of surgery that is performed will be at the discretion of the surgical oncologist.

4.4.2 Data collection and follow-up for withdrawn subjects

Survival data will be collected on all patients for up to a year following implantation, including subjects withdrawn from the study following implantation. Survival data will be collected from the social security death index as well as the DHMC medical record. In addition, the reasons for early withdrawal will be collected to determine if the device or measurement procedures contributed to the early withdrawal.

5 Study Device

5.1 Description

The device, OxyChip, used in this study is made in-house using a two-step process. In the first step, LiNc-BuO crystals are synthesized using commercially available starting materials. In the second step, the LiNc-

BuO crystals are embedded in PDMS, a biocompatible polymer. The product is made in the form of a cylindrical pellet (0.6-mm diameter and 5-mm length) for implantation.

5.1.1 Materials used for fabrication

LiNc-BuO crystals are synthesized using the published procedure[99]. The method uses lithium granules, 5,9,14,18,23,27,32,36-octa-*n*-butoxy-2,3-naphthalocyanine (Nc-BuO), *n*-pentanol, *n*-hexane, and *tert*-butyl methyl ether, all purchased from Sigma-Aldrich Chemical Co (St. Louis, MO, USA). PDMS is a medical grade silastic polymer (A-103) from Factor II, Incorporated (Lakeside, AZ).

5.1.2 Synthesis of LiNc-BuO crystals

Lithium granules (0.0053 g, 0.774 mmol) are added to *n*-pentanol (15 ml) and refluxed for 30 min under nitrogen atmosphere. The mixture is cooled to room temperature and Nc-BuO (0.1 g, 0.0774 mmol) is added and refluxed gently for 2.5 hours under nitrogen atmosphere. After cooling down to room temperature, 300 ml of *tert*-butyl methyl ether is added and filtered through a small silica gel plug. The solvent is then evaporated under reduced pressure to 3 ml of solution. The concentrate is dissolved in 100 ml of *n*-hexane. The greenish solution is slowly evaporated under reduced pressure to yield shiny crystals of lithium 5,9,14,18,23,27,32,36-octa-*n*-butoxy-2,3-naphthalocyanine (LiNc-BuO). The crystals are washed with methanol and dried under a vacuum. Please see **Attachment 2** for details of LiNc-BuO synthesis.

5.1.3 Method for fabrication of OxyChip

OxyChips are fabricated by cast-molding and polymerization, as reported[102]. The silastic polymer is mixed at the recommended base-to-crosslinker ratio (10:1), and crystals of LiNc-BuO are thoroughly dispersed into this mixture using a spatula. The base-crosslinker/LiNc-BuO microcrystal mixture is poured into a polystyrene weighing boat and degassed under vacuum. The degassed mixture is cured in an oven at 70°C for 3.5 hours. Please see **Attachment 2** for details of SOP for fabrication of OxyChips.

5.1.4 Sterilization

As a “critical device” *(i.e.,* a device that is introduced into a sterile area of the body), each OxyChip is sterilized with a SAL of 10-6 and validated as per the published standard (Sterilization of health care products – Moist Heat – Part 2: Guidance on the application of ANSI/AAMI/ISO 17665-1; TIR17665-2:2009). The sterilization procedure is performed by trained staff, using the clinical autoclaves and standard procedures. Sterilizations are recorded. Individual OxyChips devices are placed in sealable autoclave bags with process indicators and are labeled with product identification number. The steam sterilization is done in the autoclave at gravity cycle (at 121^{o}C (249.8^{o}F)) and 15 PSIG for 30 minutes. The sterilization cycle is validated using biological indicators (BI) and chemical indicators (CI). If the indicator results are appropriate, the implant is released for use. The implant will remain sterile in storage as long as the integrity of the package is maintained. Importantly the device chemistry (PDMS and LiNc-BuO) is stable under high temperature conditions. (See Standard Method 67.0 for further details.)

5.1.5 Calibration of oxygen sensitivity

Prior to autoclaving, each OxyChip is calibrated. Each chip is placed individually in a 5-mm diameter NMR tube (Norell) modified for gas delivery. A calibration line is generated using the measured linewidths collected from an L-band EPR spectrometer under known conditions. EPR Linewidths are plotted relative to the % oxygen exposure to obtain the calibration curve, which is used to calculate pO_2 from the measured linewidths using the corresponding samples implanted within the tumors. In addition, after each chip is implanted and subsequently excised along with the tumor, the OxyChip is re-calibrated and the pre- and post-implants are compared.

5.2 Treatment Regimen

5.2.1 OxyChip and fiducial placement

Fiducial(s) (one or two) may be implanted along with the OxyChip during the procedure at the discretion of the investigator. It is anticipated that fiducials will be used in most tumors, excepting those where superficiality and expected ease of OxyChip assessment make their use sub-optimal (for example for a, fungating skin tumor where the OxyChip is placed < 1 cm from the surface). Placement of the OxyChip and fiducials (if implanted) will occur via a **collaborative effort between the surgical oncologist and the PI or one of the co-investigators** with the following goals and protocol:

(i) The OxyChip and fiducials will need to be removed in their entirety when the tumor is resected.

(ii) The OxyChip will be positioned within the tumor and within 3 cm of the skin or mucosal surface.

(iii) The selection of the specific site for insertion is guided (by available pre-operative MRI, CT, or PET imaging, or physical exam) to be inside the viable tumor and not in a necrotic core. All implantations will be performed with sterile technique with the skin prepped with alcohol or betadine and draped in a sterile fashion. The OxyChip and fiducials will be implanted by a minimally invasive, needle-based approach. The needle will be loaded by the provider injecting the OxyChip in the following order depending on what is determined to be implanted. Loading occurs immediately prior to implantation, in the sterile field:

a. OxyChip only: The needle is loaded with a single OxyChip.

b. One fiducial and OxyChip: The needle is loaded with both the fiducial and single OxyChip. Order within the needle for implantation is not significant; therefore, the fiducial may be loaded first into the needle and then the OxyChip or vice versa.

c. Two fiducials and OxyChip: The needle is loaded in the following order: one fiducial, the single OxyChip, then one fiducial.

(iv) A local anesthetic will be offered to all patients prior to the implantation. After numbing, the loaded needle will be placed in the tumor guided by palpation (if sufficiently superficial and large), ultrasound, or a CT-guided procedure, and the needle contents will be deployed into the tumor using a stylet (Figure 6). The placement of the OxyChip may be performed by protocol trained Radiology staff or by protocol trained study personnel.

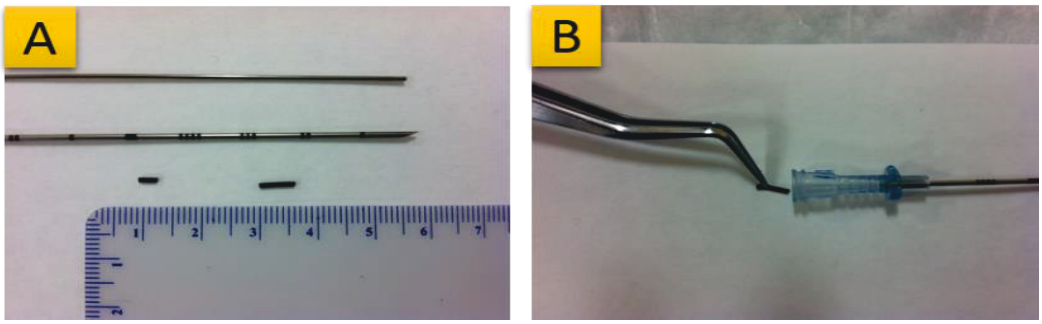


Figure 6. OxyChip for implantation. (**A**) The OxyChip, a cylindrical pellet that resembles a pencil lead, is prepared for loading into the needle for implantation. (**B**) The needle is loaded with a single OxyChip. When the needle tip is in the proper position in the tumor, the OxyChip is pushed into the tumor using the needle's stylet.

5.2.2 Oxygen measurements

Patients will be positioned in the whole-body clinical EPR scanner located on the second floor of DHMC in Radiation Oncology (**Figure 7A**). The scanner is equipped with an electromagnet, and a translating bed, or chair, that facilitates rapid repositioning of measurement subjects. This magnet has a pole separation of 50 cm, suitable for measurement in humans.

An external loop resonator will be positioned on the skin surface (**Figure 7B**) above the OxyChip. Portable ultrasound may be used to assist in finding the OxyChip in order to facilitate the placement of this. Ultrasound imaging may be performed by trained Radiology staff or one of the trained study team members.

EPR measurements will be performed using standard data collection procedures, collecting multiple EPR spectra and converting the observed absorption linewidths to pO_2 using pre-established calibration curves. If the patient agrees to breathing 100% oxygen, the measurement will be divided into three, continuous, approximately 10-minute time periods (Baseline- patient breathing room air, Hyperoxygenation- patient breathing pure (100%) oxygen, Recovery- patient return to breathing room air). If the patient is unwilling to breathe 100% oxygen, data associated with baseline tumor oxygen will be collected. Any measurement may be concluded prior to completion at any time at the discretion of the operator/physician/patient.

See Standard Method 68.x for further details regarding oxygen measurements.

The time from patient positioning to completion of the last measurement will be recorded for each reading.

Dartmouth's IRB, in reviewing two other protocols using the same clinical EPR scanner to make oxygen measurements (CPHS 12459 and CPHS16578), has previously determined that the EPR scanner presents a non-significant risk and therefore does not require an IDE process for its use for investigational purposes. See Standard Method 68.x for further details.)

5.2.3 Safety of EPR measurements

Our clinical EPR scanner[1] shown in **Figure 7** operates at ~430 G (0.043 T) magnetic field strength and ~1.2 GHz microwave energy as the RF source. The field is very low compared to whole-body clinical MRI scanners that operate typically at 1.5T or 3T magnetic field strength. The 1.2 GHz RF source in our clinical EPR scanner will use <20-mW power continuously when turned on for a few minutes (2-5 minutes). The maximum specific absorption rate (SAR) in the tissue at the position that should have the greatest density of RF, directly under the loop of the surface-loop resonator, has been estimated to be 3.7±1.2 W/kg at 100 -mW incident 1.2 GHz RF power using a surface-loop resonator with an efficiency of about 0.1 $mT/W^{1/2}$ [210]. This estimated value is substantially below the recommended limit (12 W/kg) in the relevant regulation for

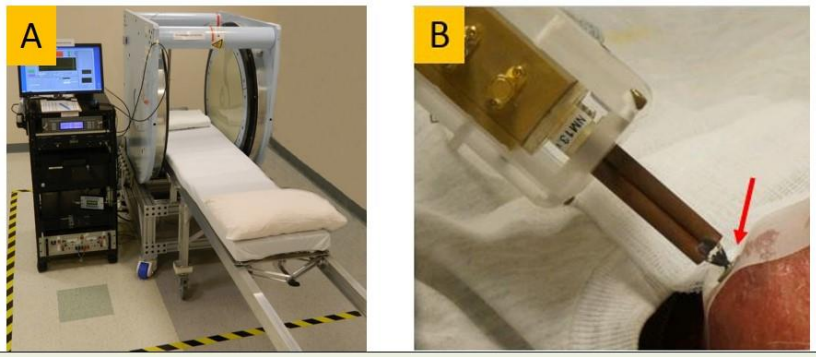


Figure 7. Setup for EPR measurement of pO_2 in human. (**A**) A custom-designed and custom-built 400-Gauss whole-body magnet and coil assembly with a 50-cm gap to facilitate whole-body measurements of tissue oxygenation. (**B**) A head and neck cancer patient undergoing EPR measurement using a surface-loop resonator (shown by arrow) placed just above the tumor.

[1] Note that this is the same EPR scanner as used in the protocol CCRC D0626/CPHS #12459 previously approved by Full Committee Review of CPHS.

extremities. Thus, the SAR for our measurements, even at 100-mW power, is well within the acceptable range for human applications. The protocol for our clinical EPR scanner will not use rapidly changing magnetic field gradients such as those used in MRI. However, the EPR measurements use three types of magnetic fields that change in time during the measurement:

1. Main magnetic field sweep: The sweeping of the main magnetic field is done at slow rates, typically a linear sweep of 8 G over a 30-sec period, which translates to a rate of ~$3x10^{-5}$ T/sec.
2. Modulation field: We typically use a modulation field of 1 G or less at 20 kHz, which will amount to 2T/sec.
3. Multisite oximetry: We apply a static gradient magnetic field which will be applied during the entire 3-sec duration of data acquisition.

In all three scenarios, the rate of change of the magnetic field is well below the limit of 20 T/sec set by the 1988 or 1995 Magnetic Resonance Diagnostic Devices (MRDD) Guidance. Hence the time-rate of change in the magnetic field is not expected to have any significant effect on the subject.

5.2.4 OxyChip and Fiducial removal

The OxyChip and fiducial(s) will be excised with the surgical specimen. The surgeon determines the timing of surgery, but it will typically be planned to be within 4 weeks for Phase 1A, or after completion of radiotherapy or chemotherapy for Phase 1B, although the OxyChip may remain implanted for up to 52 weeks for both Phases (excluding the first 6 patients enrolled into Phase 1A). The minimum time from implantation to surgical excision required for inclusion is 3 days. Note, patients in Phase 1B are expected to have the OxyChip in place for a minimum of 5 weeks (the expected duration of radiation therapy). The protocol and goals for removal will be as follows:

(i) All attempts will be made to maintain the OxyChip in the tissue specimen, without disrupting the shape or position within the tumor mass.

(ii) In collaboration with the surgeon, after the patient has been anesthetized, imaging of the fiducial(s) will occur prior to surgery in order to identify the putative location of the OxyChip and facilitate surgical removal.

a. We anticipate the fluoroscopy will be sufficient for identification of the fiducial prior to surgical removal (and after). However, due to concerns that more advanced imaging may be necessary, the first three patients with fiducial implantation will have their surgery performed at DHMC in the Center for Surgical Innovation (CSI). If fluoroscopy is sufficient for identification, then subsequent surgeries can be performed in standard operating rooms as desired if fluoroscopy is available for identification of fiducials before and after surgery.

    i. In the first three patients in the CSI, if the fiducial is not found prior to surgery either CT or MRI imaging will be performed to identify the fiducials. If the fiducials are found within the expected surgical field, or reasonably adjacent, fiducials will be removed during surgery. If the fiducials are found in an unanticipated area, consultation between the PI and the surgeon will occur as to whether surgical resection is acceptable. All decisions regarding surgery will be at the discretion of the surgeon. If no fiducials are found after advanced imaging prior to surgery, then surgery will proceed as planned as all reasonable measures will have been taken to identify the location of the OxyChip at that time.

b. After surgical resection, fluoroscopy will be performed on the specimen prior to surgical closure in order to ensure that the fiducials have been removed. If the fiducials are present in the specimen, it is highly likely that the OxyChip has been removed and surgical closure will occur. If the fiducials are not found then advanced imaging via CT or MRI will occur to attempt to identify the fiducials in the patient. If the fiducials are then identified, in consultation with the surgeon, that area will be removed if it is deemed acceptably close to the surgical field. If the fiducial is not visualized, a discussion will be had with the surgeon regarding further excisions of margins, as well as a search of the surgical field for an

OxyChip/fiducial that may have fallen out at the time of surgery. In this circumstance, all attempts will be reasonably made to ensure removal of the OxyChip, but the surgeon will have discretion about what is clinically appropriate.

If a definitive surgery is deemed unnecessary after chemotherapy therapy or radiotherapy (Phase 1B), or if a definitive surgery is delayed beyond the expected time point (> 52 weeks) due to unforeseen circumstances, the OxyChip may be removed in a minor procedure under local anesthesia. The OxyChip will be identified using the fiducials as surrogates via ultrasound or fluoroscopy, and that area will be excised using similar considerations noted above. This procedure will be performed at the first available time deemed safe for the patient as determined by the primary oncology team.

In the circumstance where a patient dies prior to surgery and the OxyChip is still in place, a discussion will be had with the next of kin regarding removal of the OxyChip prior to burial. If next of kin agrees to allow OxyChip removal, it may be removed via a small surgical procedure.

5.2.5 Tissue processing

For the primary goal, immediately after tumor resection, the OxyChip will be removed from the tissue and inspected visually to confirm that the device remained intact. The tissue at the site of the OxyChip will be submitted for processing with standard DHMC pathology procedures. Tissue sections spanning the OxyChip placement site will be stained with hematoxylin and eosin and evaluated for the presence of acute or chronic inflammation or other tissue reaction (scar, fibrosis, capsule formation, or other). After removal from the tissue, the OxyChip will be recalibrated to confirm that there was no change in the oxygen sensitivity during the course of the implantation. The pathologist, the PI, or a co-investigator who is present during specimen dissection will note if the OxyChip has been damaged during the extraction. Details in standard method 70.x.

If the OxyChip cannot be identified in the surgical specimen by dissection, the specimen will be subjected to MRI, ultrasound, or EPR analysis to visualize the OxyChip, which will facilitate the dissection.

For the exploratory goals, this tissue will be examined or stored for expression of biomarkers that may be associated with hypoxia or tumor growth.

5.3 Subject Compliance Monitoring

Patients in Phase 1A will typically have the OxyChip implanted for a short duration (≤ 4 weeks), but may remain for up to 52 weeks, and the patient will be seeing the surgeon for surgical planning and post-operative evaluations, making non-compliance unlikely. Phase 1A patients will be evaluated at least monthly prior to the OxyChip removal to check for any adverse events, as per the primary objective. Patients in Phase 1B will be receiving daily radiotherapy or a course of chemotherapy and therefore will be seen by a physician at least weekly or with each chemotherapy cycle for standard evaluation. At these appointments, the PI, co-investigators or staff protocol members perform safety monitoring by querying the patient about his or her local symptoms relating to the OxyChip implantation, and by inspection of the implantation site if possible, depending on the patient's willingness to allow inspection. Evaluation can also be performed at Norris Cotton Cancer Center North by one of the DH radiation or medical oncologists who are protocol staff, if the patients elects to receive radiation or chemotherapy at that facility prior to surgery (Phase 1B).

If the patient is unwilling to see the PI or co-investigators during a visit at which toxicity is to be assessed, or if the patient is unable to be seen due to logistical considerations, the patient by phone to evaluate potential toxicity. All efforts will be made to see the patient in clinic and perform an inspection of the implanted site. If the patient is receiving oxygen measurements, the patient will be seen by the EPR team at that time, ensuring compliance. It is possible that patients will decline oxygen measurements, but this will have a minor impact on the secondary objective (feasibility), and no impact on the primary objective (safety). Assessments by non-study physicians as part of standard of care are also an acceptable replacement if the patient is not accessible for an in-person evaluation.

5.4 Prior and Concomitant Therapy

- In Phase 1B, radiotherapy or chemotherapy or biologic or endocrine therapy will be delivered.
- All supportive medications are allowed on this protocol, including antibiotics, if needed.
- In Phase 1B, chemotherapy will not be given **with** radiotherapy (concurrently) or consecutively prior to surgical explantation of the tumor in this protocol.
- Angiogenesis inhibitors will not be given with radiotherapy in this protocol.
- Prior irradiation to the site where the OxyChip will be placed is not allowed.

5.5 Packaging

- There will be no comparative device for the OxyChip.
- The OxyChips will be sterilized in individual packaging, as described previously.
- Labels identifying the units as “Investigational Devices: Sterilized OxyChips” will be used on all storage containers.

5.6 Receiving, Storage, Dispensing and Return

5.6.1 Receipt of study device

The date of manufacture of the OxyChips by the EPR Center will be kept on an inventory, which will include the name of the technician manufacturing the device, and a device log filled out to track the devices. Any damaged or unusable study devices found at the time of the implantation procedure will be documented in the study files and on the inventory. Devices delivered to the operating room or procedure room will also be logged in the Sponsor’s inventory log.

5.6.2 Storage

The sterilized OxyChips will be stored in sterile packaging at room temperature (20°C to 23°C [68°F to 75°F]) and at a relative humidity range not to exceed 70%. All packaging materials will be examined regularly for defects and extraneous matter. If wet or damaged packages are observed, they will not be released. Access to the location where the OxyChips are stored will be limited (i.e. in a lock box or in a room with a lock).The OxyChips will be stored on a closed shelf in a room with a lock to limit access. The OxyChips will be stored away from any sprinkler heads, 18 inches away from the floor or ceiling and 2 inches away from the wall. The OxyChips will be positioned such that they do not get bent, crushed, punctured, or compressed.

5.6.3 Dispensing of study device

Regular device reconciliation checks will be performed. Information tracked will include which device was implanted in a given subject; which device containers were opened to select the specific device to be implanted in the subject; whether the subject actually received the assigned device; which devices were inadvertently damaged in handling/dispensing. This reconciliation will be logged on the device reconciliation form and signed and dated by the study team. After removal of the OxyChip from the pathology specimen, the OxyChip will be returned to the sponsor for recalibration and inspection.

5.6.4 Return or destruction of study device

At the completion of the study, there will be a final reconciliation of study devices manufactured, devices consumed, and devices remaining. This reconciliation will be logged on the device reconciliation form, signed and dated. Any discrepancies noted will be investigated, resolved, and documented prior to completion of the study. Devices destroyed on site will be documented in the study files.

6 Study Procedures

6.1 Pre-enrollment (Phase 1A, 1B)

1. Review of any prior imaging studies; this could include an MRI, CT scan or PET/CT scan.
2. Review of pathology to confirm tumor histology for Phase 1B
3. The study will be introduced to the patient and the IRB-approved consent form reviewed and signed if the patient agrees to participate. No study related procedures should be performed prior to informed consent being obtained.
4. Physical exam performed within 6 weeks of OxyChip implantation procedure. This may be performed by non-study physician or physician extender as standard of care or by study physician.
5. Pregnancy test or other standard proof of non-pregnancy for women of reproductive capability at the discretion of the physician.
6. All other eligibility criteria will be reviewed and recorded, *e.g.,* age, no pacemaker.
    a. If platelet blood count and/or absolute Neurophil count (ANC) have not been drawn for standard of care within the last months prior to the implantation of the OxyChip, the patient will be given the option to have a venipuncture for research purposes only in order to confirm eligibility.

6.2 Placement of the OxyChip (Phase 1A, 1B)

1. Placement of the OxyChip with a minimally invasive approach (see 5.2.1).

6.3 Post OxyChip implantation assessment (Phase 1A, 1B)

1. Phase 1A patients will be evaluated at least monthly prior to OxyChip removal for assessment* with respect to any adverse effects for the primary objective.
2. Phase 1B patients will be evaluated weekly during radiotherapy or prior to each chemotherapy administration or follow-up appointment for assessment* of any adverse effects for the primary objective.

    * Assessments by non-study physicians or physician extenders as part of standard of care are acceptable. If a patient cannot or does not return for an evaluation, the study team will contact the patient by phone or email to assess for development of new adverse effects or changes in health status.

6.4 Oxygen measurements (Phase 1A, 1B)

1. The area over the OxyChip is examined for signs of infection or inflammation at each session.
2. Non-invasive tumor oxygenation measurements are obtained.(see 5.2.2)
3.

6.5 Pre-operative assessment (Phase 1A, 1B)

1. The area over the OxyChip is examined for signs of infection or inflammation. This assessment can be performed within two weeks prior to surgery depending on logistical constraints, or by the treating physician prior to surgery.
2. Physical exam is performed within two weeks of surgery. This may be performed by non-study physician or physician extender as standard of care or by study physician.

6.6 Removal of OxyChip (Phase 1A, 1B)

1. Surgical resection of the tumor with OxyChip removal in the specimen followed by pathology evaluation of specimen. (see 5.2.4 and 5.2.5)

6.7 Visits after removal of the OxyChip (Phase 1A, 1B)

1. Patients will be evaluated 1-2 weeks after surgical resection with removal of the OxyChip for evaluation of healing. This may be performed by a non-study physician as standard of care or by a study physician. If the patient cannot or does not return for post-operative evaluation, the study team will contact the patient by phone or email to check for development of new adverse events or changes in health status.
2. While we do not expect any long-term effects following removal of the OxyChip, we will collect and examine results from laboratory tests and physical exams obtained *ad hoc* as a part of routine follow-up up to 12 months following implantation.

7 Statistical Plan

7.1 Statistical Methods

This is a first-in-humans pilot study[2]/Phase I trial designed to (1) test the safety and toxicity of the OxyChip and patient tolerance of its presence and to (2) help design a larger confirmatory study. As such, the study was sequentially designed into different cohorts, as previously described in section_3.1. The criteria for proceeding with each cohort is not based on statistical significance but on the presence and severity of any unexpected adverse events and whether they can be attributed to the OxyChip. The sample size was selected to be within the range recommended for early phase I trials[3,4].

For purposes of examining the secondary goal, the feasibility of using the OxyChip to assess PO_2, we will use a qualitative assessment about whether any attempted measurements were successful, and assess the time for making measurements. Oxygen measurements will be averaged across visit days (if available) per subject for all subjects in Phase 1A and Phase 1B. We will use a paired t-test to detect statistically significant acute changes in pO_2. To compare time period measurements per visit across patients, we will use an ANOVA model to account for individual variation of the baseline pO_2. Finally, if the oxygen level exhibits a nonlinear pattern we will use a nonlinear fitting technique. To account for substantial variations of pO_2 across patients, methods of repeated measurements and mixed modeling will be used.

7.2 Subject Population(s) for Analysis

The subject populations whose data will be subjected to analysis (both primary and secondary) include all evaluable participants in this Phase I trial.

---

[2] US Dept of Health and Human Services, Food and Drug Administration Center for Devices and Radiological Health. Center for Biologics Evaluation and Research. “Investigational Device Exemptions (IDEs) for Early Feasibility Medical Device Clinical Studies, including Certain First in Human (FIH) Studies: Guidance for Industry and Food and Drug Administration Staff”. Document issued October 1, 2013. http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/HowtoMarketYourDevice/InvestigationalDeviceExemptionIDE/ucm162453.htm.

[3]Browne RH: On the use of a pilot sample for sample size determination. Stat Med. 1995, 14: 1933-1940.

[4]Sim J, Lewis M: The size of a pilot study for a clinical trial should be calculated in relation to considerations of precision and efficiency. J Clin Epidemiol. 2012, 65: 301-308.

8 Safety and Adverse Events

8.1 Definitions

8.1.1 Adverse event definitions

Adverse effect. Any untoward medical occurrence in a clinical study of an investigational device; regardless of the causal relationship of the problem with the device or, if applicable, other study treatment or diagnostic product(s).

Associated with the investigational device or, if applicable, other study treatment or diagnostic product(s). There is a reasonable possibility that the adverse effect may have been caused by the investigational device or, if applicable, the other study treatment or diagnostic product(s).

Disability. A substantial disruption of a person's ability to conduct normal life functions.

Life-threatening adverse effect. Any adverse effect that places the subject, in the view of the investigator-sponsor, at immediate risk of death from the effect as it occurred (*i.e.,* does not include an adverse effect that, had it actually occurred in a more severe form, might have caused death).

Serious adverse effect. Any adverse effect that results in any of the following outcomes: death, a life-threatening adverse effect, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant disability/incapacity, or a congenital anomaly/birth defect.

- *Hospitalization* shall include any initial admission (even if less than 24 hours) to a healthcare facility as a result of a precipitating clinical adverse effect; to include transfer within the hospital to an intensive care unit. Hospitalization or prolongation of hospitalization in the absence of a precipitating, clinical adverse effect *(e.g.,* for a preexisting condition not associated with a new adverse effect or with a worsening of the preexisting condition; admission for a protocol-specified procedure) is not, in itself, a serious adverse effect.

Unexpected adverse effect. Any adverse effect, the frequency, specificity or severity of which is not consistent with the risk information described in the clinical study protocol(s) or elsewhere in the current IDE application, as amended.

8.1.2 Unanticipated adverse device effect (UADE)

Unanticipated adverse device effect (UADE). Any serious adverse effect on health or safety or any life-threatening problem or death caused by, or associated with, a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the investigational plan or IDE application (including a supplementary plan or application), or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects.

8.2 Recording of Adverse Events

Clinical study subjects will be questioned about adverse effects at study visits. The visits will be documented in the clinical records. Adverse effects with suspected causal relationship to study procedures, determined by the PI or co-I, will be recorded on study adverse effects forms and summarized in CRFs.

8.3 Reporting of Adverse Device Effects and Unanticipated Problems

8.3.1 Investigator reporting: Notifying the study sponsor

8.3.1.1 Sponsor contact information for reporting purposes

Report adverse device effects by phone and facsimile to: Periannan Kuppusamy, Phone: (603) 653-3577; Fax (603) 650-1940; Philip Schaner, Phone (603) 650-5000

All observed or volunteered adverse effects (serious or non-serious) and abnormal test findings, regardless of treatment group, if applicable, with suspected causal relationship to the investigational device or, if applicable, other study treatment or diagnostic product(s) will be recorded in the subjects' case histories. For all adverse effects, sufficient information will be pursued and/or obtained so as to permit (i) an adequate determination of the outcome of the effect *(i.e.,* whether the effect should be classified as a *serious adverse effect*) and; (ii) an assessment of the casual relationship between the adverse effect and the investigational device or, if applicable, the other study treatment or diagnostic product(s).

Adverse effects or abnormal test findings felt to be associated with the investigational device or, if applicable, other study treatment or diagnostic product(s) will be followed until the effect (or its sequelae) or the abnormal test finding resolves or stabilizes at a level acceptable to the investigator-sponsor.

8.3.1.2 Abnormal test findings

An abnormal test finding will be classified as an *adverse effect* if one or more of the following criteria are met:

- The test finding is accompanied by clinical symptoms.
- The test finding necessitates additional diagnostic evaluation(s) or medical/surgical intervention; including significant additional concomitant drug or other therapy. (Note: simply repeating a test finding, in the absence of any of the other listed criteria, does not constitute an adverse effect.)
- The test finding leads to a change in study dosing or exposure or discontinuation of subject participation in the clinical study.
- The test finding is considered an adverse effect by the investigator-sponsor.

8.3.1.3 Causality and severity assessment

The investigator-sponsor will promptly review documented adverse effects and abnormal test findings to determine (i) if the abnormal test finding should be classified as an adverse effect; (ii) if there is a reasonable possibility that the adverse effect was caused by the investigational device or, if applicable, other study treatment or diagnostic product(s); and (ii) if the adverse effect meets the criteria for a *serious adverse effect*.

If the investigator-sponsor's final determination of causality is "unknown and of questionable relationship to the investigational device or, if applicable, other study treatment or diagnostic product(s)", the adverse effect will be classified as *associated with the use of the investigational device or study treatment or diagnostic drug product(s)* for reporting purposes. If the investigator-sponsor's final determination of causality is "unknown but not related to the investigational device or, if applicable, other study treatment or diagnostic product(s)", this determination and the rationale for the determination will be documented in the respective subject's case history.

8.3.1.4 Reporting of adverse effects to the FDA.

The investigator-sponsor will submit a completed FDA Form 3500A to the FDA's Center for Devices and Radiological Health for any observed or volunteered adverse effect that is determined to be an *unanticipated adverse device effect. A* copy of this completed form will be provided to all participating sub-investigators.

The completed FDA Form 3500A will be submitted to the FDA as soon as possible and, in no event, later than 10 working days after the investigator-sponsor first receives notice of the adverse effect.

If the results of the sponsor-investigator's follow-up evaluation show that an adverse effect that was initially determined to not constitute an *unanticipated adverse device effect* does, in fact, meet the requirements for reporting; the investigator-sponsor will submit a completed FDA Form 3500A as soon as possible, but in no event later than 10 working days, after the determination was made.

For each submitted FDA Form 3500A, the sponsor-investigator will identify all previously submitted reports that that addressed a similar adverse effect experience and will provide an analysis of the significance of newly reported adverse effect in light of the previous, similar report(s).

Subsequent to the initial submission of a completed FDA Form 3500A, the investigator-sponsor will submit additional information concerning the reported adverse effect as requested by the FDA.

8.3.2 Reporting of adverse effects to the responsible IRB

In accordance with applicable policies of the Dartmouth College's Institutional Review Board (IRB)—the Committee for Protection of Human Subjects (CPHS)—the investigator-sponsor will report, to the IRB, any observed or volunteered adverse effect that is determined to meet all of the following criteria: (i) *associated with the investigational device or, if applicable, other study treatment or diagnostic product(s)*; (ii) *a serious adverse effect*; and (iii) an *unexpected adverse effect*. Adverse event reports will be submitted to the IRB in accordance with the respective IRB procedures.

Applicable adverse effects will be reported to the IRB as soon as possible and, in no event, later than 10 working days after an investigator learns of the effect.

Follow-up information to reported adverse effects will be submitted to the IRB as soon as the relevant information is available. If the results of the sponsor-investigator's follow-up investigation show that an adverse effect that was initially determined to not require reporting to the IRB does, in fact, meet the requirements for reporting; the investigator-sponsor will report the adverse effect to the IRB as soon as possible, but in no event later than 10 working days, after an investigator learns of the effect.

8.3.2.1 Withdrawal of IRB approval

The Sponsor shall notify the FDA, all participating IRBs and participating investigators of any withdrawal of approval of the study by a reviewing IRB within 5 working days after receipt of the withdrawal of approval.

8.3.2.2 FDA reporting process

Medical Device Reports, whether for anticipated or unanticipated device-related effects, are to be submitted on FDA Form 3500A (MEDWATCH Form). The contact information for submitting MDR reports is noted below:

Food and Drug Administration
Center for Devices and Radiological Health
Medical Device Reporting
PO Box 3002
Rockville, MD 20847-3003

8.4 Stopping Rules

The study will be stopped for any grade 3 or higher toxicity. The study will not re-open until the event is analyzed and deemed to be unrelated to the OxyChip or oxygen measurements.

8.5 Medical Monitoring

It is the responsibility of the PI to oversee the safety of the study at all sites. This safety monitoring will include careful assessment and appropriate reporting of adverse effects as noted above, as well as the construction and implementation of a site data and safety-monitoring plan (see section 9 Auditing, Monitoring and Inspecting). Medical monitoring will include a regular assessment of the number and type of serious adverse events. The sponsor and local PIs will meet monthly via conference call to discuss progress, safety issues and regulatory processes.

9 Data Handling and Record Keeping

9.1 Confidentiality

Information about all study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study?
- Who will have access to that information and why?
- Who will use or disclose that information?
- The rights of a research subject to revoke their authorization for use of their PHI.

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (i.e. that the subject is alive) at the end of their scheduled study period.

9.2 Source Documents

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial.

9.3 Case Report Forms

The study case report form (CRF) is the primary data collection instrument for the study. All data requested on the CRF must be recorded. All missing data must be explained. If a space on the CRF is left blank because the procedure was not done or the question was not asked, write "N/D". If the item is not applicable to the individual case, write "N/A". All entries should be printed legibly in black ink. If any entry error has been made, to correct such an error, draw a single straight line through the incorrect entry and enter the correct data above it. All such changes must be initialed and dated. DO NOT ERASE OR WHITE OUT ERRORS. For clarification of illegible or uncertain entries, print the clarification above the item, then initial and date it.

9.4 Records Retention

Locally, electronic information will be kept in the secure, password-protected research database. After the study is completed, documents containing research information are stored in Dartmouth College Records Management off-site storage. Documents are shredded off site after 50 years.

10 Study Monitoring, Auditing, and Inspecting

10.1 Study Monitoring Plan

This study will be monitored by the Data Safety Monitoring and Accrual Committee (DSMAC) of the Norris Cotton Cancer Center (NCCC) of Dartmouth-Hitchcock Medical Center. The Clinical Cancer Review Committee (CCRC) of NCCC determines the frequency of DSMAC review. The DSMAC meets quarterly to review accrual rates and information about studies that have accrued participants, including adverse device effects. The DSMAC has the authority to suspend or to recommend termination to the CCRC of all research

activities that fall within its jurisdiction. In the event that a study is suspended or terminated, that information will be forwarded to the responsible IRB office.

10.2 On-Site Monitoring

Clinical research monitoring for regulatory compliance and data integrity will be conducted according to the NCI-approved NCCC Data and Safety Monitoring Plan. Internal monitoring is conducted by appropriately trained staff of the NCCC Office of Clinical Research and Dartmouth-Hitchcock Medical Center Clinical Trials Office who are not involved in the study. This monitoring will include periodic assessment of the regulatory compliance, data quality, pharmacy records and study integrity. Study records will be reviewed and directly compared to source documents and the conduct of the study will be discussed with the investigator. Monitors may request access to all regulatory documents, source documents, CRFs, and other study documentation for on-site inspection. Direct access to these documents is guaranteed by the investigator, who must provide support at all times for these activities.

10.3 Auditing and Inspecting

The investigator will permit study-related monitoring, audits, and inspections by the CPHS, government regulatory bodies, and NCCC compliance and quality assurance groups of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data, etc.). The investigator will ensure the capability for inspections of applicable study-related facilities. Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable institutional compliance and quality assurance offices.

11 Ethical Considerations

This study is to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and Institutional research policies and procedures. This protocol and any amendments will be submitted to a properly constituted or Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study conduct. The decision of the IRB concerning the conduct of the study will be made in writing to the investigator. All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. The written consent of a subject, using the IRB-approved consent form, must be obtained before that subject undergoes any study procedure. The consent form must be signed by the subject and the investigator-designated research professional obtaining the consent.

11.1 Funding Source

This study is being funded as a part of a Program Project Grant (PPG) from the National Cancer Institute (P01 CA190193 – Project 2; Direct and Repeated Clinical Measurement of pO_2 using OxyChip for Enhancing Cancer Therapy (07/01/2015 – 06/30/2020) with Dr. Kuppusamy is the PI.

11.2 Conflict of Interest

Because the information to be disclosed differs for the FDA and Dartmouth, the relevant disclosures have been filed in a separate supporting document for the CPHS application as well as filed in annual and other disclosures required by Dartmouth. The disclosures for the FDA have been signed and filed in a regulatory binder by all investigators named in this protocol.

12 Publication Plan

As of now, there is no industry sponsor for this study. The OxyChip-based clinical EPR trial has been developed by the study investigators at Dartmouth. We plan to publish results from this study in peer-reviewed journals with all investigators who participate in the study as co-authors. The primary investigators, Drs. Kuppusamy and Schaner, will have the responsibility for publication.

Neither the complete nor any part of the results of the study carried out under this protocol, nor any of the information provided by the sponsor for the purposes of performing the study, will be published or passed on to any third party without the consent of the primary investigators. Any investigator involved with this study is obligated to provide the primary investigators with complete test results and all data derived from the study.

13 References

1. Brizel DM, Dodge RK, Clough RW, Dewhirst MW. Oxygenation of head and neck cancer: changes during radiotherapy and impact on treatment outcome. *Radiother Oncol*. 53: 113-117 (1999).

2. Vaupel P, Thews O, Hoeckel M. Treatment resistance of solid tumors: role of hypoxia and anemia. *Med Oncol*. 18: 243-259 (2001).

3. Dewhirst MW, Cao Y, Moeller B. Cycling hypoxia and free radicals regulate angiogenesis and radiotherapy response. *Nature reviews. Cancer*. 8: 425-437 (2008).

4. Matsumoto S, Batra S, Saito K, Yasui H, Choudhuri R, Gadisetti C, Subramanian S, Devasahayam N, Munasinghe JP, Mitchell JB, Krishna MC. Antiangiogenic agent sunitinib transiently increases tumor oxygenation and suppresses cycling hypoxia. *Cancer Res*. 71: 6350-6359 (2011).

5. Matsumoto S, Yasui H, Mitchell JB, Krishna MC. Imaging cycling tumor hypoxia. *Cancer Res*. 70: 10019-10023 (2010).

6. Swartz HM, Walczak T. Developing in vivo EPR oximetry for clinical use. *Advances in experimental medicine and biology*. 454: 243-252 (1998).

7. Doll CM, Milosevic M, Pintilie M, Hill RP, Fyles AW. Estimating hypoxic status in human tumors: a simulation using Eppendorf oxygen probe data in cervical cancer patients. *Int J Radiat Oncol Biol Phys*. 55: 1239-1246 (2003).

8. Evans SM, Judy KD, Dunphy I, Jenkins WT, Nelson PT, Collins R, Wileyto EP, Jenkins K, Hahn SM, Stevens CW, Judkins AR, Phillips P, Geoerger B, Koch CJ. Comparative measurements of hypoxia in human brain tumors using needle electrodes and EF5 binding. *Cancer Res*. 64: 1886-1892 (2004).

9. Gagel B, Piroth M, Pinkawa M, Reinartz P, Zimny M, Kaiser HJ, Stanzel S, Asadpour B, Demirel C, Hamacher K, Coenen HH, Scholbach T, Maneschi P, DiMartino E, Eble MJ. pO polarography, contrast enhanced color duplex sonography (CDS), [18F] fluoromisonidazole and [18F] fluorodeoxyglucose positron emission tomography: validated methods for the evaluation of therapy-relevant tumor oxygenation or only bricks in the puzzle of tumor hypoxia? *BMC Cancer*. 7: 113 (2007).

10. Hockel M, Knoop C, Schlenger K, Vorndran B, Baussmann E, Mitze M, Knapstein PG, Vaupel P. Intratumoral pO2 predicts survival in advanced cancer of the uterine cervix. *Radiother Oncol*. 26: 45-50 (1993).

11. Vaupel P, Frinak S, O'Hara M. Direct measurement of reoxygenation in malignant mammary tumors after a single large dose of irradiation. *Advances in experimental medicine and biology*. 180: 773-782 (1984).

12. Vaupel P, Mayer A. Hypoxia in cancer: significance and impact on clinical outcome. *Cancer Metastasis Rev*. 26: 225-239 (2007).

13. Cosse JP, Michiels C. Tumour hypoxia affects the responsiveness of cancer cells to chemotherapy and promotes cancer progression. *Anticancer Agents Med Chem*. 8: 790-797 (2008).

14. Shannon AM, Bouchier-Hayes DJ, Condron CM, Toomey D. Tumour hypoxia, chemotherapeutic resistance and hypoxia-related therapies. *Cancer Treat Rev*. 29: 297-307 (2003).

15. Nordsmark M, Bentzen SM, Rudat V, Brizel D, Lartigau E, Stadler P, Becker A, Adam M, Molls M, Dunst J, Terris DJ, Overgaard J. Prognostic value of tumor oxygenation in 397 head and neck tumors after primary radiation therapy. An international multi-center study. *Radiother Oncol*. 77: 18-24 (2005).

16. Nordsmark M, Overgaard J. Tumor hypoxia is independent of hemoglobin and prognostic for loco-regional tumor control after primary radiotherapy in advanced head and neck cancer. *Acta Oncol*. 43: 396-403 (2004).

17. Rudat V, Stadler P, Becker A, Vanselow B, Dietz A, Wannenmacher M, Molls M, Dunst J, Feldmann HJ. Predictive value of the tumor oxygenation by means of pO2 histography in patients with advanced head and neck cancer. *Strahlenther Onkol*. 177: 462-468 (2001).

18. Nordsmark M, Loncaster J, Aquino-Parsons C, Chou SC, Gebski V, West C, Lindegaard JC, Havsteen H, Davidson SE, Hunter R, Raleigh JA, Overgaard J. The prognostic value of pimonidazole and tumour pO2 in human cervix carcinomas after radiation therapy: a prospective international multi-center study. *Radiother Oncol*. 80: 123-131 (2006).

19. Rofstad EK. Microenvironment-induced cancer metastasis. *International journal of radiation biology*. 76: 589-605 (2000).

20. Tatum JL, Kelloff GJ, Gillies RJ, Arbeit JM, Brown JM, Chao KS, Chapman JD, Eckelman WC, Fyles AW, Giaccia AJ, Hill RP, Koch CJ, Krishna MC, Krohn KA, Lewis JS, Mason RP, Melillo G, Padhani AR, Powis G, Rajendran JG, Reba R, Robinson SP, Semenza GL, Swartz HM, Vaupel P, Yang D, Croft B, Hoffman J, Liu G, Stone H, Sullivan D. Hypoxia: importance in tumor biology, noninvasive measurement by imaging, and value of its measurement in the management of cancer therapy. *International journal of radiation biology*. 82: 699-757 (2006).

21. Vaupel P. Hypoxia and aggressive tumor phenotype: implications for therapy and prognosis. *The oncologist*. 13 Suppl 3: 21-26 (2008).

22. Brizel DM, Rosner GL, Harrelson J, Prosnitz LR, Dewhirst MW. Pretreatment oxygenation profiles of human soft tissue sarcomas. *Int J Radiat Oncol Biol Phys*. 30: 635-642 (1994).

23. Brizel DM, Rosner GL, Prosnitz LR, Dewhirst MW. Patterns and variability of tumor oxygenation in human soft tissue sarcomas, cervical carcinomas, and lymph node metastases. *Int J Radiat Oncol Biol Phys*. 32: 1121-1125 (1995).

24. Takeshita K, Kawaguchi K, Fujii-Aikawa K, Ueno M, Okazaki S, Ono M, Krishna MC, Kuppusamy P, Ozawa T, Ikota N. Heterogeneity of regional redox status and relation of the redox status to oxygenation in a tumor model, evaluated using electron paramagnetic resonance imaging. *Cancer Res*. 70: 4133-4140 (2010).

25. Baudelet C, Ansiaux R, Jordan BF, Havaux X, Macq B, Gallez B. Physiological noise in murine solid tumours using T2*-weighted gradient-echo imaging: a marker of tumour acute hypoxia? *Physics in medicine and biology*. 49: 3389-3411 (2004).

26. Brurberg KG, Graff BA, Rofstad EK. Temporal heterogeneity in oxygen tension in human melanoma xenografts. *Br J Cancer*. 89: 350-356 (2003).

27. Brurberg KG, Skogmo HK, Graff BA, Olsen DR, Rofstad EK. Fluctuations in pO2 in poorly and well-oxygenated spontaneous canine tumors before and during fractionated radiation therapy. *Radiother Oncol*. 77: 220-226 (2005).

28. Cardenas-Navia LI, Yu D, Braun RD, Brizel DM, Secomb TW, Dewhirst MW. Tumor-dependent kinetics of partial pressure of oxygen fluctuations during air and oxygen breathing. *Cancer Res*. 64: 6010-6017 (2004).

29. Cardenas-Navia LI, Mace D, Richardson RA, Wilson DF, Shan S, Dewhirst MW. The pervasive presence of fluctuating oxygenation in tumors. *Cancer Res*. 68: 5812-5819 (2008).

30. Pogue BW, Braun RD, Lanzen JL, Erickson C, Dewhirst MW. Analysis of the heterogeneity of pO2 dynamics during photodynamic therapy with verteporfin. *Photochem Photobiol*. 74: 700-706 (2001).

31. Pogue BW, O'Hara JA, Goodwin IA, Wilmot CJ, Fournier GP, Akay AR, Swartz H. Tumor PO(2) changes during photodynamic therapy depend upon photosensitizer type and time after injection. *Comp Biochem Physiol A Mol Integr Physiol*. 132: 177-184 (2002).

32. Yasui H, Matsumoto S, Devasahayam N, Munasinghe JP, Choudhuri R, Saito K, Subramanian S, Mitchell JB, Krishna MC. Low-field magnetic resonance imaging to visualize chronic and cycling hypoxia in tumor-bearing mice. *Cancer Res*. 70: 6427-6436 (2010).

33. Magat J, Jordan BF, Cron GO, Gallez B. Noninvasive mapping of spontaneous fluctuations in tumor oxygenation using 19F MRI. *Med Phys*. 37: 5434-5441 (2010).

34. Dewhirst MW, Kimura H, Rehmus SW, Braun RD, Papahadjopoulos D, Hong K, Secomb TW. Microvascular studies on the origins of perfusion-limited hypoxia. *Br J Cancer Suppl*. 27: S247-251 (1996).

35. Kimura H, Braun RD, Ong ET, Hsu R, Secomb TW, Papahadjopoulos D, Hong K, Dewhirst MW. Fluctuations in red cell flux in tumor microvessels can lead to transient hypoxia and reoxygenation in tumor parenchyma. *Cancer Res*. 56: 5522-5528 (1996).

36. O'Hara JA, Blumenthal RD, Grinberg OY, Demidenko E, Grinberg S, Wilmot CM, Taylor AM, Goldenberg DM, Swartz HM. Response to radioimmunotherapy correlates with tumor pO2 measured by EPR oximetry in human tumor xenografts. *Radiat Res*. 155: 466-473 (2001).

37. O'Hara JA, Goda F, Dunn JF, Swartz HM. Potential for EPR oximetry to guide treatment planning for tumors. *Advances in experimental medicine and biology*. 411: 233-242 (1997).

38. Goel S, Duda DG, Xu L, Munn LL, Boucher Y, Fukumura D, Jain RK. Normalization of the vasculature for treatment of cancer and other diseases. *Physiol Rev*. 91: 1071-1121 (2011).

39. Jain RK. Normalization of tumor vasculature: an emerging concept in antiangiogenic therapy. *Science*. 307: 58-62 (2005).

40. Buerk DG. Measuring tissue PO2 with microelectrodes. *Methods Enzymol*. 381: 665-690 (2004).

41. Griffiths JR, Robinson SP. The OxyLite: a fibre-optic oxygen sensor. *The British journal of radiology*. 72: 627-630 (1999).

42. Springett R, Swartz HM. Measurements of oxygen in vivo: overview and perspectives on methods to measure oxygen within cells and tissues. *Antioxid Redox Signal*. 9: 1295-1301 (2007).

43. Mason RP, Hunjan S, Le D, Constantinescu A, Barker BR, Wong PS, Peschke P, Hahn EW, Antich PP. Regional tumor oxygen tension: fluorine echo planar imaging of hexafluorobenzene reveals heterogeneity of dynamics. *Int J Radiat Oncol Biol Phys*. 42: 747-750 (1998).

44. Braun RD, Lanzen JL, Snyder SA, Dewhirst MW. Comparison of tumor and normal tissue oxygen tension measurements using OxyLite or microelectrodes in rodents. *Am J Physiol Heart Circ Physiol*. 280: H2533-2544 (2001).

45. Nozue M, Lee I, Yuan F, Teicher BA, Brizel DM, Dewhirst MW, Milross CG, Milas L, Song CW, Thomas CD, Guichard M, Evans SM, Koch CJ, Lord EM, Jain RK, Suit HD. Interlaboratory variation in oxygen tension measurement by Eppendorf "Histograph" and comparison with hypoxic marker. *Journal of surgical oncology*. 66: 30-38 (1997).

46. O'Hara JA, Hou H, Demidenko E, Springett RJ, Khan N, Swartz HM. Simultaneous measurement of rat brain cortex PtO2 using EPR oximetry and a fluorescence fiber-optic sensor during normoxia and hyperoxia. *Physiol Meas*. 26: 203-213 (2005).

47. O'Hara JA, Khan N, Hou H, Wilmo CM, Demidenko E, Dunn JF, Swartz HM. Comparison of EPR oximetry and Eppendorf polarographic electrode assessments of rat brain PtO2. *Physiol Meas*. 25: 1413-1423 (2004).

48. Vikram DS, Zweier JL, Kuppusamy P. Methods for noninvasive imaging of tissue hypoxia. *Antioxid Redox Signal*. 9: 1745-1756 (2007).

49. Baudelet C, Gallez B. How does blood oxygen level-dependent (BOLD) contrast correlate with oxygen partial pressure (pO2) inside tumors? *Magnetic resonance in medicine : official journal of the Society of Magnetic Resonance in Medicine / Society of Magnetic Resonance in Medicine*. 48: 980-986 (2002).

50. Jordan BF, Beghein N, Crokart N, Baudelet C, Gregoire V, Gallez B. Preclinical safety and antitumor efficacy of insulin combined with irradiation. *Radiother Oncol*. 81: 112-117 (2006).

51. Robinson SP, Howe FA, Rodrigues LM, Stubbs M, Griffiths JR. Magnetic resonance imaging techniques for monitoring changes in tumor oxygenation and blood flow. *Semin Radiat Oncol*. 8: 197-207 (1998).

52. Ahmad R, Kuppusamy P. Theory, instrumentation, and applications of electron paramagnetic resonance oximetry. *Chem Rev*. 110: 3212-3236 (2010).

53. Atsarkin VA, Demidov VV, Vasneva GA, Dzheparov FS, Ceroke PJ, Odintsov BM, Clarkson RB. Mechanism of oxygen response in carbon-based sensors. *Journal of magnetic resonance*. 149: 85-89 (2001).

54. Grinberg OY, Williams BB, Ruuge AE, Grinberg SA, Wilcox DE, Swartz HM, Freed JH. Oxygen effects on the EPR signals from wood charcoals: experimental results and the development of a model. *J Phys Chem B*. 111: 13316-13324 (2007).

55. Ilangovan G, Zweier JL, Kuppusamy P. Mechanism of oxygen-induced EPR line broadening in lithium phthalocyanine microcrystals. *Journal of magnetic resonance*. 170: 42-48 (2004).

56. Pandian RP, Dolgos M, Marginean C, Woodward PM, Hammel PC, Manoharan PT, Kuppusamy P. Molecular packing and magnetic properties of lithium naphthalocyanine crystals: hollow channels enabling permeability and paramagnetic sensitivity to molecular oxygen. *J Mater Chem*. 19: 4138-4147 (2009).

57. Elas M, Ahn KH, Parasca A, Barth ED, Lee D, Haney C, Halpern HJ. Electron paramagnetic resonance oxygen images correlate spatially and quantitatively with Oxylite oxygen measurements. *Clin Cancer Res*. 12: 4209-4217 (2006).

58. Froncisz W, Lai CS, Hyde JS. Spin-label oximetry: kinetic study of cell respiration using a rapid-passage T1-sensitive electron spin resonance display. *Proceedings of the National Academy of Sciences of the United States of America*. 82: 411-415 (1985).

59. Gallez B, Bacic G, Goda F, Jiang J, O'Hara JA, Dunn JF, Swartz HM. Use of nitroxides for assessing perfusion, oxygenation, and viability of tissues: in vivo EPR and MRI studies. *Magnetic resonance in medicine : official journal of the Society of Magnetic Resonance in Medicine / Society of Magnetic Resonance in Medicine*. 35: 97-106 (1996).

60. Grinberg OY, Friedman BJ, Swartz HM. Intramyocardial pO2 measured by EPR. *Advances in experimental medicine and biology*. 428: 261-268 (1997).

61. Halpern HJ, Yu C, Peric M, Barth ED, Karczmar GS, River JN, Grdina DJ, Teicher BA. Measurement of differences in pO2 in response to perfluorocarbon/carbogen in FSa and NFSa murine fibrosarcomas with low-frequency electron paramagnetic resonance oximetry. *Radiat Res*. 145: 610-618 (1996).

62. Hou H, Dong R, Li H, Williams B, Lariviere JP, Hekmatyar SK, Kauppinen RA, Khan N, Swartz H. Dynamic changes in oxygenation of intracranial tumor and contralateral brain during tumor growth and carbogen breathing: a multisite EPR oximetry with implantable resonators. *Journal of magnetic resonance*. 214: 22-28 (2012).

63. Ilangovan G, Li H, Zweier JL, Kuppusamy P. Effect of carbogen-breathing on redox status of the RIF-1 tumor. *Advances in experimental medicine and biology*. 510: 13-17 (2003).

64. Ilangovan G, Liebgott T, Kutala VK, Petryakov S, Zweier JL, Kuppusamy P. EPR oximetry in the beating heart: myocardial oxygen consumption rate as an index of postischemic recovery. *Magnetic resonance in medicine : official journal of the Society of Magnetic Resonance in Medicine / Society of Magnetic Resonance in Medicine*. 51: 835-842 (2004).

65. Khan M, Kutala VK, Wisel S, Chacko SM, Kuppusamy ML, Kwiatkowski P, Kuppusamy P. Measurement of oxygenation at the site of stem cell therapy in a murine model of myocardial infarction. *Advances in experimental medicine and biology*. 614: 45-52 (2008).

66. Khan M, Mohan IK, Kutala VK, Kotha SR, Parinandi NL, Hamlin RL, Kuppusamy P. Sulfaphenazole protects heart against ischemia-reperfusion injury and cardiac dysfunction by overexpression of iNOS,

leading to enhancement of nitric oxide bioavailability and tissue oxygenation. *Antioxid Redox Signal*. 11: 725-738 (2009).

67. Kuppusamy P, Afeworki M, Shankar RA, Coffin D, Krishna MC, Hahn SM, Mitchell JB, Zweier JL. In vivo electron paramagnetic resonance imaging of tumor heterogeneity and oxygenation in a murine model. *Cancer Res*. 58: 1562-1568 (1998).

68. Liu KJ, Gast P, Moussavi M, Norby SW, Vahidi N, Walczak T, Wu M, Swartz HM. Lithium phthalocyanine: a probe for electron paramagnetic resonance oximetry in viable biological systems. *Proceedings of the National Academy of Sciences of the United States of America*. 90: 5438-5442 (1993).

69. Krishna MC, English S, Yamada K, Yoo J, Murugesan R, Devasahayam N, Cook JA, Golman K, Ardenkjaer-Larsen JH, Subramanian S, Mitchell JB. Overhauser enhanced magnetic resonance imaging for tumor oximetry: coregistration of tumor anatomy and tissue oxygen concentration. *Proceedings of the National Academy of Sciences of the United States of America*. 99: 2216-2221 (2002).

70. Matsumoto A, Matsumoto K, Matsumoto S, Hyodo F, Sowers AL, Koscielniak JW, Devasahayam N, Subramanian S, Mitchell JB, Krishna MC. Intracellular hypoxia of tumor tissue estimated by noninvasive electron paramagnetic resonance oximetry technique using paramagnetic probes. *Biological & pharmaceutical bulletin*. 34: 142-145 (2011).

71. Rosen GM, Halpern HJ, Brunsting LA, Spencer DP, Strauss KE, Bowman MK, Wechsler AS. Direct measurement of nitroxide pharmacokinetics in isolated hearts situated in a low-frequency electron spin resonance spectrometer: implications for spin trapping and in vivo oxymetry. *Proceedings of the National Academy of Sciences of the United States of America*. 85: 7772-7776 (1988).

72. Zweier JL, Kuppusamy P. Electron paramagnetic resonance measurements of free radicals in the intact beating heart: a technique for detection and characterization of free radicals in whole biological tissues. *Proceedings of the National Academy of Sciences of the United States of America*. 85: 5703-5707 (1988).

73. Gallez B, Debuyst R, Dejehet F, Liu KJ, Walczak T, Goda F, Demeure R, Taper H, Swartz HM. Small particles of fusinite and carbohydrate chars coated with aqueous soluble polymers: preparation and applications for in vivo EPR oximetry. *Magnetic resonance in medicine : official journal of the Society of Magnetic Resonance in Medicine / Society of Magnetic Resonance in Medicine*. 40: 152-159 (1998).

74. Gallez B, Jordan BF, Baudelet C. Microencapsulation of paramagnetic particles by pyrroxylin to preserve their responsiveness to oxygen when used as sensors for in vivo EPR oximetry. *Magnetic resonance in medicine : official journal of the Society of Magnetic Resonance in Medicine / Society of Magnetic Resonance in Medicine*. 42: 193-196 (1999).

75. Glockner JF, Swartz HM. In vivo EPR oximetry using two novel probes: fusinite and lithium phthalocyanine. *Advances in experimental medicine and biology*. 317: 229-234 (1992).

76. He G, Shankar RA, Chzhan M, Samouilov A, Kuppusamy P, Zweier JL. Noninvasive measurement of anatomic structure and intraluminal oxygenation in the gastrointestinal tract of living mice with spatial and spectral EPR imaging. *Proceedings of the National Academy of Sciences of the United States of America*. 96: 4586-4591 (1999).

77. He J, Beghein N, Ceroke P, Clarkson RB, Swartz HM, Gallez B. Development of biocompatible oxygen-permeable films holding paramagnetic carbon particles: evaluation of their performance and stability in EPR oximetry. *Magnetic resonance in medicine : official journal of the Society of Magnetic Resonance in Medicine / Society of Magnetic Resonance in Medicine*. 46: 610-614 (2001).

78. Jordan BF, Baudelet C, Gallez B. Carbon-centered radicals as oxygen sensors for in vivo electron paramagnetic resonance: screening for an optimal probe among commercially available charcoals. *Magma*. 7: 121-129 (1998).

79. O'Hara JA, James PE, Wadghiri YZ, Panz T, Grinberg OY, Jain N, Dunn JF, Swartz HM. Determining the anatomic position and histological effects in murine tumors of gloxy, an oxygen-sensitive paramagnetic material. *Advances in experimental medicine and biology*. 428: 107-113 (1997).

80. Vahidi N, Clarkson RB, Liu KJ, Norby SW, Wu M, Swartz HM. In vivo and in vitro EPR oximetry with fusinite: a new coal-derived, particulate EPR probe. *Magnetic resonance in medicine : official journal of the*

Society of Magnetic Resonance in Medicine / Society of Magnetic Resonance in Medicine. 31: 139-146 (1994).

81. Swartz HM, Liu KJ, Goda F, Walczak T. India ink: a potential clinically applicable EPR oximetry probe. Magnetic resonance in medicine : official journal of the Society of Magnetic Resonance in Medicine / Society of Magnetic Resonance in Medicine. 31: 229-232 (1994).

82. Goda F, Liu KJ, Walczak T, O'Hara JA, Jiang J, Swartz HM. In vivo oximetry using EPR and India ink. Magnetic resonance in medicine : official journal of the Society of Magnetic Resonance in Medicine / Society of Magnetic Resonance in Medicine. 33: 237-245 (1995).

83. Nakashima T, Goda F, Jiang J, Shima T, Swartz HM. Use of EPR oximetry with India ink to measure the pO2 in the liver in vivo in mice. *Magnetic resonance in medicine : official journal of the Society of Magnetic Resonance in Medicine / Society of Magnetic Resonance in Medicine*. 34: 888-892 (1995).

84. Jiang J, Nakashima T, Liu KJ, Goda F, Shima T, Swartz HM. Measurement of PO2 in liver using EPR oximetry. *J Appl Physiol*. 80: 552-558 (1996).

85. Khan N, Hou H, Hein P, Comi RJ, Buckey JC, Grinberg O, Salikhov I, Lu SY, Wallach H, Swartz HM. Black magic and EPR oximetry: from lab to initial clinical trials. *Advances in experimental medicine and biology*. 566: 119-125 (2005).

86. Williams BB, Khan N, Zaki B, Hartford A, Ernstoff MS, Swartz HM. Clinical electron paramagnetic resonance (EPR) oximetry using India ink. *Advances in experimental medicine and biology*. 662: 149-156 (2010).

87. Afeworki M, Miller NR, Devasahayam N, Cook J, Mitchell JB, Subramanian S, Krishna MC. Preparation and EPR studies of lithium phthalocyanine radical as an oxymetric probe. *Free radical biology & medicine*. 25: 72-78 (1998).

88. Ilangovan G, Bratasz A, Kuppusamy P. Non-invasive measurement of tumor oxygenation using embedded microparticulate EPR spin probe. *Advances in experimental medicine and biology*. 566: 67-73 (2005).

89. Ilangovan G, Bratasz A, Li H, Schmalbrock P, Zweier JL, Kuppusamy P. In vivo measurement and imaging of tumor oxygenation using coembedded paramagnetic particulates. *Magnetic resonance in medicine : official journal of the Society of Magnetic Resonance in Medicine / Society of Magnetic Resonance in Medicine*. 52: 650-657 (2004).

90. Ilangovan G, Li H, Zweier JL, Krishna MC, Mitchell JB, Kuppusamy P. In vivo measurement of regional oxygenation and imaging of redox status in RIF-1 murine tumor: effect of carbogen-breathing. *Magnetic resonance in medicine : official journal of the Society of Magnetic Resonance in Medicine / Society of Magnetic Resonance in Medicine*. 48: 723-730 (2002).

91. Ilangovan G, Li H, Zweier JL, Kuppusamy P. Electrochemical preparation and EPR studies of lithium phthalocyanine. 3. Measurements of oxygen concentration in tissues and biochemical reactions. *J. Phys. Chem. B*. 105: 5323-5330 (2001).

92. Ilangovan G, Pal R, Zweier JL, Kuppusamy P. Electrochemical preparation and EPR studies of lithium phthalocyanine. 4. Effect of nitric oxide. *J. Phys. Chem. B*. 106: 11929-11935 (2002).

93. Ilangovan G, Zweier JL, Kuppusamy P. Electrochemical preparation and EPR studies of lithium phthalocyanine. Part 2: Particle-size-dependent line broadening by molecular oxygen and its implications as an oximetry probe. *J. Phys. Chem. B*. 104: 9404-9410 (2000).

94. Ilangovan G, Zweier JL, Kuppusamy P. Electrochemical preparation and EPR studies of lithium phthalocyanine: Evaluation of the nucleation and growth mechanism and evidence for potential-dependent phase formation. *J. Phys. Chem. B*. 104: 4047-4059 (2000).

95. Pandian RP, Chacko SM, Kuppusamy ML, Rivera BK, Kuppusamy P. Evaluation of lithium naphthalocyanine (LiNc) microcrystals for biological EPR oximetry. *Advances in experimental medicine and biology*. 701: 29-36 (2011).

96. Pandian RP, Dang V, Manoharan PT, Zweier JL, Kuppusamy P. Effect of nitrogen dioxide on the EPR property of lithium octa-n-butoxy 2,3-naphthalocyanine (LiNc-BuO) microcrystals. *J Magn Reson*. 181: 154-161 (2006).

97. Pandian RP, Dolgos M, Dang V, Sostaric JZ, Woodward PM, Kuppusamy P. Structure and Oxygen-Sensing Paramagnetic Properties of a New Lithium 1,8,15,22-Tetraphenoxyphthalocyanine Radical Probe for Biological Oximetry. *Chem. Mater.* 19: 3545-3552 (2007).

98. Pandian RP, Kim YI, Woodward PM, Zweier JL, Manoharan PT, Kuppusamy P. Open molecular framework in lithium octabutoxy-naphthalocyanine paramagnetic crystal: Implications for the detection of oxygen and nitric oxide by EPR spectroscopy. *J. Mater. Chem.* 16: 3609-3618 (2006).

99. Pandian RP, Parinandi NL, Ilangovan G, Zweier JL, Kuppusamy P. Novel particulate spin probe for targeted determination of oxygen in cells and tissues. *Free radical biology & medicine*. 35: 1138-1148 (2003).

100. Mahy P, De Bast M, Gallez B, Gueulette J, Koch CJ, Scalliet P, Gregoire V. In vivo colocalization of 2-nitroimidazole EF5 fluorescence intensity and electron paramagnetic resonance oximetry in mouse tumors. *Radiother Oncol*. 67: 53-61 (2003).

101. Ilangovan G, Manivannan A, Li H, Yanagi H, Zweier JL, Kuppusamy P. A naphthalocyanine-based EPR probe for localized measurements of tissue oxygenation. *Free radical biology & medicine*. 32: 139-147 (2002).

102. Meenakshisundaram G, Eteshola E, Pandian RP, Bratasz A, Lee SC, Kuppusamy P. Fabrication and physical evaluation of a polymer-encapsulated paramagnetic probe for biomedical oximetry. *Biomedical microdevices*. 11: 773-782 (2009).

103. Meenakshisundaram G, Eteshola E, Pandian RP, Bratasz A, Selvendiran K, Lee SC, Krishna MC, Swartz HM, Kuppusamy P. Oxygen sensitivity and biocompatibility of an implantable paramagnetic probe for repeated measurements of tissue oxygenation. *Biomedical microdevices*. 11: 817-826 (2009).

104. Meenakshisundaram G, Pandian RP, Eteshola E, Lee SC, Kuppusamy P. A paramagnetic implant containing lithium naphthalocyanine microcrystals for high-resolution biological oximetry. *Journal of magnetic resonance*. 203: 185-189 (2010).

105. Pandian RP, Meenakshisundaram G, Bratasz A, Eteshola E, Lee SC, Kuppusamy P. An implantable Teflon chip holding lithium naphthalocyanine microcrystals for secure, safe, and repeated measurements of pO2 in tissues. *Biomedical microdevices*. 12: 381-387 (2010).

106. Vaupel P, Schlenger K, Knoop C, Hockel M. Oxygenation of human tumors: evaluation of tissue oxygen distribution in breast cancers by computerized O2 tension measurements. *Cancer Res*. 51: 3316-3322 (1991).

107. Aboagye EO, Maxwell RJ, Horsman MR, Lewis AD, Workman P, Tracy M, Griffiths JR. The relationship between tumour oxygenation determined by oxygen electrode measurements and magnetic resonance spectroscopy of the fluorinated 2-nitroimidazole SR-4554. *Br J Cancer*. 77: 65-70 (1998).

108. Kim JG, Zhao D, Song Y, Constantinescu A, Mason RP, Liu H. Interplay of tumor vascular oxygenation and tumor pO2 observed using near-infrared spectroscopy, an oxygen needle electrode, and 19F MR pO2 mapping. *J Biomed Opt*. 8: 53-62 (2003).

109. Laukemper-Ostendorf S, Scholz A, Burger K, Heussel CP, Schmittner M, Weiler N, Markstaller K, Eberle B, Kauczor HU, Quintel M, Thelen M, Schreiber WG. 19F-MRI of perflubron for measurement of oxygen partial pressure in porcine lungs during partial liquid ventilation. *Magn Reson Med*. 47: 82-89 (2002).

110. Mason RP, Rodbumrung W, Antich PP. Hexafluorobenzene: a sensitive 19F NMR indicator of tumor oxygenation. *NMR in biomedicine*. 9: 125-134 (1996).

111. Prasad PV, Edelman RR, Epstein FH. Noninvasive evaluation of intrarenal oxygenation with BOLD MRI. *Circulation*. 94: 3271-3275 (1996).

112. Swartz HM, Khan N, Buckey J, Comi R, Gould L, Grinberg O, Hartford A, Hopf H, Hou H, Hug E, Iwasaki A, Lesniewski P, Salikhov I, Walczak T. Clinical applications of EPR: overview and perspectives. *NMR in biomedicine*. 17: 335-351 (2004).

113. Khan N, Williams BB, Hou H, Li H, Swartz HM. Repetitive tissue pO2 measurements by electron paramagnetic resonance oximetry: current status and future potential for experimental and clinical studies. *Antioxid Redox Signal*. 9: 1169-1182 (2007).

114. Khan N, B. WB, Swartz HM. Clinical applications of in vivo EPR: Rationale and initial results. *J Appl Magn Reson*. 30: 185-199 (2006).

115. Rink C, Roy S, Khan M, Ananth P, Kuppusamy P, Sen CK, Khanna S. Oxygen-sensitive outcomes and gene expression in acute ischemic stroke. *J Cereb Blood Flow Metab*. 30: 1275-1287 (2010).

116. Liu KJ, Bacic G, Hoopes PJ, Jiang J, Du H, Ou LC, Dunn JF, Swartz HM. Assessment of cerebral pO2 by EPR oximetry in rodents: effects of anesthesia, ischemia, and breathing gas. *Brain Res*. 685: 91-98 (1995).

117. Goda F, O'Hara JA, Liu KJ, Rhodes ES, Dunn JF, Swartz HM. Comparisons of measurements of pO2 in tissue in vivo by EPR oximetry and microelectrodes. *Advances in experimental medicine and biology*. 411: 543-549 (1997).

118. Hoopes PJ, Liu KJ, Bacic G, Rolett EL, Dunn JF, Swartz HM. Histological assessment of rodent CNS tissues to EPR oximetry probe material. *Advances in experimental medicine and biology*. 411: 13-21 (1997).

119. Liu KJ, Hoopes PJ, Rolett EL, Beerle BJ, Azzawi A, Goda F, Dunn JF, Swartz HM. Effect of anesthesia on cerebral tissue oxygen and cardiopulmonary parameters in rats. *Advances in experimental medicine and biology*. 411: 33-39 (1997).

120. Dunn JF, Grinberg O, Roche M, Nwaigwe CI, Hou HG, Swartz HM. Noninvasive assessment of cerebral oxygenation during acclimation to hypobaric hypoxia. *J Cereb Blood Flow Metab*. 20: 1632-1635 (2000).

121. Rolett EL, Azzawi A, Liu KJ, Yongbi MN, Swartz HM, Dunn JF. Critical oxygen tension in rat brain: a combined (31)P-NMR and EPR oximetry study. *Am J Physiol Regul Integr Comp Physiol*. 279: R9-R16 (2000).

122. Lei H, Grinberg O, Nwaigwe CI, Hou HG, Williams H, Swartz HM, Dunn JF. The effects of ketamine-xylazine anesthesia on cerebral blood flow and oxygenation observed using nuclear magnetic resonance perfusion imaging and electron paramagnetic resonance oximetry. *Brain Res*. 913: 174-179 (2001).

123. Dunn JF, O'Hara JA, Zaim-Wadghiri Y, Lei H, Meyerand ME, Grinberg OY, Hou H, Hoopes PJ, Demidenko E, Swartz HM. Changes in oxygenation of intracranial tumors with carbogen: a BOLD MRI and EPR oximetry study. *J Magn Reson Imaging*. 16: 511-521 (2002).

124. Hou H, Grinberg OY, Taie S, Leichtweis S, Miyake M, Grinberg S, Xie H, Csete M, Swartz HM. Electron paramagnetic resonance assessment of brain tissue oxygen tension in anesthetized rats. *Anesth Analg*. 96: 1467-1472, table of contents (2003).

125. Miyake M, Grinberg OY, Hou H, Steffen RP, Elkadi H, Swartz HM. The effect of RSR13, a synthetic allosteric modifier of hemoglobin, on brain tissue pO2 (measured by EPR oximetry) following severe hemorrhagic shock in rats. *Advances in experimental medicine and biology*. 530: 319-329 (2003).

126. Swartz HM, Taie S, Miyake M, Grinberg OY, Hou H, el-Kadi H, Dunn JF. The effects of anesthesia on cerebral tissue oxygen tension: use of EPR oximetry to make repeated measurements. *Advances in experimental medicine and biology*. 530: 569-575 (2003).

127. Swartz HM. Using EPR to measure a critical but often unmeasured component of oxidative damage: oxygen. *Antioxid Redox Signal*. 6: 677-686 (2004).

128. Hou H, Grinberg OY, Grinberg SA, Demidenko E, Swartz HM. Cerebral tissue oxygenation in reversible focal ischemia in rats: multi-site EPR oximetry measurements. *Physiol Meas*. 26: 131-141 (2005).

129. Hou H, Khan N, O'Hara JA, Grinberg OY, Dunn JF, Abajian MA, Wilmot CM, Demidenko E, Lu S, Steffen RP, Swartz HM. Increased oxygenation of intracranial tumors by efaproxyn (efaproxiral), an allosteric hemoglobin modifier: In vivo EPR oximetry study. *Int J Radiat Oncol Biol Phys*. 61: 1503-1509 (2005).

130. Sakata YS, Grinberg OY, Grinberg S, Springett R, Swartz HM. Simultaneous NIR-EPR spectroscopy of rat brain oxygenation. *Advances in experimental medicine and biology*. 566: 357-362 (2005).

131. Aron JH, Fink GW, Swartz MF, Ford B, Hauser MC, O'Leary CE, Puskas F. Cerebral oxygen desaturation after cardiopulmonary bypass in a patient with raynaud's phenomenon detected by near-infrared cerebral oximetry. *Anesth Analg*. 104: 1034-1036, tables of contents (2007).

132. Hou H, Grinberg O, Williams B, Grinberg S, Yu H, Alvarenga DL, Wallach H, Buckey J, Swartz HM. The effect of oxygen therapy on brain damage and cerebral pO(2) in transient focal cerebral ischemia in the rat. *Physiol Meas*. 28: 963-976 (2007).

133. Williams BB, Hou H, Grinberg OY, Demidenko E, Swartz HM. High spatial resolution multisite EPR oximetry of transient focal cerebral ischemia in the rat. *Antioxid Redox Signal*. 9: 1691-1698 (2007).

134. Khan N, Li H, Hou H, Lariviere JP, Gladstone DJ, Demidenko E, Swartz HM. Tissue pO2 of orthotopic 9L and C6 gliomas and tumor-specific response to radiotherapy and hyperoxygenation. *Int J Radiat Oncol Biol Phys*. 73: 878-885 (2009).

135. Khan N, Mupparaju S, Hekmatyar SK, Hou H, Lariviere JP, Demidenko E, Gladstone DJ, Kauppinen RA, Swartz HM. Effect of hyperoxygenation on tissue pO2 and its effect on radiotherapeutic efficacy of orthotopic F98 gliomas. *Int J Radiat Oncol Biol Phys*. 78: 1193-1200 (2010).

136. Li H, Hou H, Sucheta A, Williams BB, Lariviere JP, Khan MN, Lesniewski PN, Gallez B, Swartz HM. Implantable resonators--a technique for repeated measurement of oxygen at multiple deep sites with in vivo EPR. *Advances in experimental medicine and biology*. 662: 265-272 (2010).

137. Dunn JF, Khan MN, Hou HG, Merlis J, Abajian MA, Demidenko E, Grinberg OY, Swartz HM. Cerebral oxygenation in awake rats during acclimation and deacclimation to hypoxia: an in vivo electron paramagnetic resonance study. *High Alt Med Biol*. 12: 71-77 (2011).

138. Hou H, Li H, Dong R, Mupparaju S, Khan N, Swartz H. Cerebral oxygenation of the cortex and striatum following normobaric hyperoxia and mild hypoxia in rats by EPR oximetry using multi-probe implantable resonators. *Advances in experimental medicine and biology*. 701: 61-67 (2011).

139. Hou H, Dong R, Lariviere JP, Mupparaju SP, Swartz HM, Khan N. Synergistic combination of hyperoxygenation and radiotherapy by repeated assessments of tumor pO2 with EPR oximetry. *J Radiat Res*. 52: 568-574 (2011).

140. Mupparaju S, Hou H, Lariviere JP, Swartz H, Jounaidi Y, Khan N. Repeated tumor oximetry to identify therapeutic window during metronomic cyclophosphamide treatment of 9L gliomas. *Oncol Rep*. 26: 281-286 (2011).

141. Mupparaju S, Hou H, Lariviere JP, Swartz HM, Khan N. Tumor pO as a surrogate marker to identify therapeutic window during metronomic chemotherapy of 9L gliomas. *Advances in experimental medicine and biology*. 701: 107-113 (2011).

142. Mupparaju S, Hou H, Lariviere JP, Swartz HM, Khan N. Tumor pO(2) as a surrogate marker to identify therapeutic window during metronomic chemotherapy of 9L gliomas. *Advances in experimental medicine and biology*. 701: 107-113 (2011).

143. Khan N, Mupparaju S, Hou H, Williams BB, Swartz H. Repeated assessment of orthotopic glioma pO(2) by multi-site EPR oximetry: a technique with the potential to guide therapeutic optimization by repeated measurements of oxygen. *J Neurosci Methods*. 204: 111-117 (2012).

144. Hou H, Mupparaju SP, Lariviere JP, Hodge S, Gui J, Swartz HM, Khan N. Assessment of the changes in 9L and C6 glioma pO2 by EPR oximetry as a prognostic indicator of differential response to radiotherapy. *Radiat Res*. 179: 343-351 (2013).

145. Chacko SM, Khan M, Kuppusamy ML, Pandian RP, Varadharaj S, Selvendiran K, Bratasz A, Rivera BK, Kuppusamy P. Myocardial oxygenation and functional recovery in infarct rat hearts transplanted with mesenchymal stem cells. *Am J Physiol Heart Circ Physiol*. 296: H1263-1273 (2009).

146. Gogna R, Madan E, Kuppusamy P, Pati U. Chaperoning of mutant p53 protein by wild-type p53 protein causes hypoxic tumor regression. *J Biol Chem*. 287: 2907-2914 (2012).

147. Khan M, Kutala VK, Vikram DS, Wisel S, Chacko SM, Kuppusamy ML, Mohan IK, Zweier JL, Kwiatkowski P, Kuppusamy P. Skeletal myoblasts transplanted in the ischemic myocardium enhance in situ oxygenation and recovery of contractile function. *Am J Physiol Heart Circ Physiol*. 293: H2129-2139 (2007).

148. Khan M, Meduru S, Mohan IK, Kuppusamy ML, Wisel S, Kulkarni A, Rivera BK, Hamlin RL, Kuppusamy P. Hyperbaric oxygenation enhances transplanted cell graft and functional recovery in the infarct heart. *J Mol Cell Cardiol*. 47: 275-287 (2009).

149. Khan M, Meduru S, Pandian RP, Rivera BK, Kuppusamy P. Effect of oxygenation on stem-cell therapy for myocardial infarction. *Advances in experimental medicine and biology*. 701: 175-181 (2011).

150. Mohan IK, Khan M, Wisel S, Selvendiran K, Sridhar A, Carnes CA, Bognar B, Kalai T, Hideg K, Kuppusamy P. Cardioprotection by HO-4038, a novel verapamil derivative, targeted against ischemia and reperfusion-mediated acute myocardial infarction. *Am J Physiol Heart Circ Physiol*. 296: H140-151 (2009).

151. Selvendiran K, Bratasz A, Kuppusamy ML, Tazi MF, Rivera BK, Kuppusamy P. Hypoxia induces chemoresistance in ovarian cancer cells by activation of signal transducer and activator of transcription 3. *Int J Cancer*. 125: 2198-2204 (2009).

152. Selvendiran K, Kuppusamy ML, Ahmed S, Bratasz A, Meenakshisundaram G, Rivera BK, Khan M, Kuppusamy P. Oxygenation inhibits ovarian tumor growth by downregulating STAT3 and cyclin-D1 expressions. *Cancer Biol Ther*. 10: 386-390 (2010).

153. Vikram DS, Bratasz A, Ahmad R, Kuppusamy P. A comparative evaluation of EPR and OxyLite oximetry using a random sampling of pO(2) in a murine tumor. *Radiat Res*. 168: 308-315 (2007).

154. Grinberg OY, Grinberg SA, Friedman BJ, Swartz HM. Myocardial oxygen tension and capillary density in the isolated perfused rat heart during pharmacological intervention. *Advances in experimental medicine and biology*. 411: 171-181 (1997).

155. Abramovic Z, Hou H, Julijana K, Sentjurc M, Lariviere JP, Swartz HM, Khan N. Modulation of tumor hypoxia by topical formulations with vasodilators for enhancing therapy. *Advances in experimental medicine and biology*. 701: 75-82 (2011).

156. Ansiaux R, Baudelet C, Cron GO, Segers J, Dessy C, Martinive P, De Wever J, Verrax J, Wauthier V, Beghein N, Gregoire V, Buc Calderon P, Feron O, Gallez B. Botulinum toxin potentiates cancer radiotherapy and chemotherapy. *Clin Cancer Res*. 12: 1276-1283 (2006).

157. Ansiaux R, Baudelet C, Jordan BF, Beghein N, Sonveaux P, De Wever J, Martinive P, Gregoire V, Feron O, Gallez B. Thalidomide radiosensitizes tumors through early changes in the tumor microenvironment. *Clin Cancer Res*. 11: 743-750 (2005).

158. Bratasz A, Pandian RP, Ilangovan G, Kuppusamy P. Monitoring oxygenation during the growth of a transplanted tumor. *Advances in experimental medicine and biology*. 578: 375-380 (2006).

159. Crokart N, Danhier F, Daugimont L, Goncalves N, Jordan BF, Gregoire V, Feron O, Bouquet C, Gallez B, Preat V. Potentiation of radiotherapy by a localized antiangiogenic gene therapy. *Radiother Oncol*. 107: 252-258 (2013).

160. Crokart N, Jordan BF, Baudelet C, Ansiaux R, Sonveaux P, Gregoire V, Beghein N, DeWever J, Bouzin C, Feron O, Gallez B. Early reoxygenation in tumors after irradiation: determining factors and consequences for radiotherapy regimens using daily multiple fractions. *Int J Radiat Oncol Biol Phys*. 63: 901-910 (2005).

161. Danhier F, Danhier P, Magotteaux N, De Preter G, Ucakar B, Karroum O, Jordan B, Gallez B, Preat V. Electron paramagnetic resonance highlights that the oxygen effect contributes to the radiosensitizing effect of paclitaxel. *PLoS One*. 7: e40772 (2012).

162. Doloff JC, Khan N, Ma J, Demidenko E, Swartz HM, Jounaidi Y. Increased tumor oxygenation and drug uptake during anti-angiogenic weekly low dose cyclophosphamide enhances the anti-tumor effect of weekly tirapazamine. *Curr Cancer Drug Targets*. 9: 777-788 (2009).

163. Fujii H, Sakata K, Katsumata Y, Sato R, Kinouchi M, Someya M, Masunaga S, Hareyama M, Swartz HM, Hirata H. Tissue oxygenation in a murine SCC VII tumor after X-ray irradiation as determined by EPR spectroscopy. *Radiother Oncol*. 86: 354-360 (2008).

164. Gallez B, Jordan BF, Baudelet C, Misson PD. Pharmacological modifications of the partial pressure of oxygen in murine tumors: evaluation using in vivo EPR oximetry. *Magnetic resonance in medicine : official journal of the Society of Magnetic Resonance in Medicine / Society of Magnetic Resonance in Medicine*. 42: 627-630 (1999).

165. Goda F, O'Hara JA, Rhodes ES, Liu KJ, Dunn JF, Bacic G, Swartz HM. Changes of oxygen tension in experimental tumors after a single dose of X-ray irradiation. *Cancer Res*. 55: 2249-2252 (1995).

166. Hou H, Abramovic Z, Lariviere JP, Sentjurc M, Swartz H, Khan N. Effect of a topical vasodilator on tumor hypoxia and tumor oxygen guided radiotherapy using EPR oximetry. *Radiat Res*. 173: 651-658 (2010).

167. Hou H, Khan N, Grinberg OY, Yu H, Grinberg SA, Lu S, Demidenko E, Steffen RP, Swartz HM. The effects of Efaproxyn (efaproxiral) on subcutaneous RIF-1 tumor oxygenation and enhancement of radiotherapy-mediated inhibition of tumor growth in mice. *Radiat Res*. 168: 218-225 (2007).

168. Hou H, Khan N, O'Hara JA, Grinberg OY, Dunn JF, Abajian MA, Wilmot CM, Makki M, Demidenko E, Lu S, Steffen RP, Swartz HM. Effect of RSR13, an allosteric hemoglobin modifier, on oxygenation in murine tumors: an in vivo electron paramagnetic resonance oximetry and bold MRI study. *Int J Radiat Oncol Biol Phys*. 59: 834-843 (2004).

169. Hou H, Lariviere JP, Demidenko E, Gladstone D, Swartz H, Khan N. Repeated tumor pO(2) measurements by multi-site EPR oximetry as a prognostic marker for enhanced therapeutic efficacy of fractionated radiotherapy. *Radiother Oncol*. 91: 126-131 (2009).

170. Jordan BF, Beghein N, Aubry M, Gregoire V, Gallez B. Potentiation of radiation-induced regrowth delay by isosorbide dinitrate in FSaII murine tumors. *Int J Cancer*. 103: 138-141 (2003).

171. Jordan BF, Gregoire V, Demeure RJ, Sonveaux P, Feron O, O'Hara J, Vanhulle VP, Delzenne N, Gallez B. Insulin increases the sensitivity of tumors to irradiation: involvement of an increase in tumor oxygenation mediated by a nitric oxide-dependent decrease of the tumor cells oxygen consumption. *Cancer Res*. 62: 3555-3561 (2002).

172. Jordan BF, Misson P, Demeure R, Baudelet C, Beghein N, Gallez B. Changes in tumor oxygenation/perfusion induced by the no donor, isosorbide dinitrate, in comparison with carbogen: monitoring by EPR and MRI. *Int J Radiat Oncol Biol Phys*. 48: 565-570 (2000).

173. Jordan BF, Sonveaux P, Feron O, Gregoire V, Beghein N, Gallez B. Nitric oxide-mediated increase in tumor blood flow and oxygenation of tumors implanted in muscles stimulated by electric pulses. *Int J Radiat Oncol Biol Phys*. 55: 1066-1073 (2003).

174. Karroum O, Kengen J, Danhier P, Magat J, Mignion L, Bouzin C, Verrax J, Charette N, Starkel P, Calderon PB, Sonveaux P, Feron O, Gregoire V, Gallez B, Jordan BF. Tumor reoxygenation following administration of Mitogen-Activated Protein Kinase inhibitors: a rationale for combination with radiation therapy. *Radiother Oncol*. 105: 64-71 (2012).

175. Karroum O, Kengen J, Gregoire V, Gallez B, Jordan BF. Tumor Reoxygenation Following Administration of the EGFR Inhibitor, Gefitinib, in Experimental Tumors. *Advances in experimental medicine and biology*. 789: 265-271 (2013).

176. Khan N, Mupparaju SP, Hou H, Lariviere JP, Demidenko E, Swartz HM, Eastman A. Radiotherapy in conjunction with 7-hydroxystaurosporine: a multimodal approach with tumor pO2 as a potential marker of therapeutic response. *Radiat Res*. 172: 592-597 (2009).

177. O'Hara JA, Blumenthal RD, Grinberg OY, Grinberg S, Wilmot C, Goldenberg DM, Swartz HM. Tumor pO2 assessments in human xenograft tumors measured by EPR oximetry: location of paramagnetic materials. *Advances in experimental medicine and biology*. 530: 205-214 (2003).

178. O'Hara JA, Goda F, Demidenko E, Swartz HM. Effect on regrowth delay in a murine tumor of scheduling split-dose irradiation based on direct pO2 measurements by electron paramagnetic resonance oximetry. *Radiat Res*. 150: 549-556 (1998).

179. O'Hara JA, Goda F, Liu KJ, Bacic G, Hoopes PJ, Swartz HM. The pO2 in a murine tumor after irradiation: an in vivo electron paramagnetic resonance oximetry study. *Radiat Res*. 144: 222-229 (1995).

180. Sersa G, Krzic M, Sentjurc M, Ivanusa T, Beravs K, Cemazar M, Auersperg M, Swartz HM. Reduced tumor oxygenation by treatment with vinblastine. *Cancer Res*. 61: 4266-4271 (2001).

181. Fisher E, Khan M, Steiner R, Kuppusamy P. Challenges to intestinal pO measurement using EPR. *Advances in experimental medicine and biology*. 701: 37-44 (2011).

182. Matsumoto A, Matsumoto S, Sowers AL, Koscielniak JW, Trigg NJ, Kuppusamy P, Mitchell JB, Subramanian S, Krishna MC, Matsumoto K. Absolute oxygen tension (pO(2)) in murine fatty and muscle tissue as determined by EPR. *Magnetic resonance in medicine : official journal of the Society of Magnetic Resonance in Medicine / Society of Magnetic Resonance in Medicine*. 54: 1530-1535 (2005).

183. Jordan BF, Kimpalou JZ, Beghein N, Dessy C, Feron O, Gallez B. Contribution of oxygenation to BOLD contrast in exercising muscle. *Magnetic resonance in medicine : official journal of the Society of Magnetic Resonance in Medicine / Society of Magnetic Resonance in Medicine*. 52: 391-396 (2004).

184. Towner RA, Sturgeon SA, Khan N, Hou H, Swartz HM. In vivo assessment of nodularin-induced hepatotoxicity in the rat using magnetic resonance techniques (MRI, MRS and EPR oximetry). *Chem Biol Interact*. 139: 231-250 (2002).

185. James PE, Bacic G, Grinberg OY, Goda F, Dunn JF, Jackson SK, Swartz HM. Endotoxin-induced changes in intrarenal pO2, measured by in vivo electron paramagnetic resonance oximetry and magnetic resonance imaging. *Free radical biology & medicine*. 21: 25-34 (1996).

186. Krzic M, Sentjurc M, Kristl J. Improved skin oxygenation after benzyl nicotinate application in different carriers as measured by EPR oximetry in vivo. *J Control Release*. 70: 203-211 (2001).

187. Biswas S, Roy S, Banerjee J, Hussain SR, Khanna S, Meenakshisundaram G, Kuppusamy P, Friedman A, Sen CK. Hypoxia inducible microRNA 210 attenuates keratinocyte proliferation and impairs closure in a murine model of ischemic wounds. *Proceedings of the National Academy of Sciences of the United States of America*. 107: 6976-6981 (2010).

188. Fries RB, Wallace WA, Roy S, Kuppusamy P, Bergdall V, Gordillo GM, Melvin WS, Sen CK. Dermal excisional wound healing in pigs following treatment with topically applied pure oxygen. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*. 579: 172-181 (2005).

189. Lu C, Rollins M, Hou H, Swartz HM, Hopf H, Miclau T, Marcucio RS. Tibial fracture decreases oxygen levels at the site of injury. *Iowa Orthop J*. 28: 14-21 (2008).

190. Lu C, Saless N, Hu D, Wang X, Xing Z, Hou H, Williams B, Swartz HM, Colnot C, Miclau T, Marcucio RS. Mechanical stability affects angiogenesis during early fracture healing. *J Orthop Trauma*. 25: 494-499 (2011).

191. Eubank TD, Roberts RD, Khan M, Curry JM, Nuovo GJ, Kuppusamy P, Marsh CB. Granulocyte macrophage colony-stimulating factor inhibits breast cancer growth and metastasis by invoking an anti-angiogenic program in tumor-educated macrophages. *Cancer Res*. 69: 2133-2140 (2009).

192. Khan M, Meduru S, Gogna R, Madan E, Citro L, Kuppusamy ML, Sayyid M, Mostafa M, Hamlin RL, Kuppusamy P. Oxygen cycling in conjunction with stem cell transplantation induces NOS3 expression leading to attenuation of fibrosis and improved cardiac function. *Cardiovasc Res*. 93: 89-99 (2012).

193. Wisel S, Chacko SM, Kuppusamy ML, Pandian RP, Khan M, Kutala VK, Burry RW, Sun B, Kwiatkowski P, Kuppusamy P. Labeling of skeletal myoblasts with a novel oxygen-sensing spin probe for noninvasive monitoring of in situ oxygenation and cell therapy in heart. *Am J Physiol Heart Circ Physiol*. 292: H1254-1261 (2007).

194. Wisel S, Khan M, Kuppusamy ML, Mohan IK, Chacko SM, Rivera BK, Sun BC, Hideg K, Kuppusamy P. Pharmacological preconditioning of mesenchymal stem cells with trimetazidine (1-[2,3,4-trimethoxybenzyl]piperazine) protects hypoxic cells against oxidative stress and enhances recovery of myocardial function in infarcted heart through Bcl-2 expression. *J Pharmacol Exp Ther*. 329: 543-550 (2009).

195. Abbasi F, Mirzadeh H, Katbab AA. Modification of polysiloxane polymers for biomedical applications: a review. *Polymer International*. 50: 1279-1287 (2001).

196. Belanger MC, Marois Y. Hemocompatibility, biocompatibility, inflammatory and in vivo studies of primary reference materials low-density polyethylene and polydimethylsiloxane: a review. *J Biomed Mater Res*. 58: 467-477 (2001).

197. Eteshola E, Pandian RP, Lee SC, Kuppusamy P. Polymer coating of paramagnetic particulates for in vivo oxygen-sensing applications. *Biomedical microdevices*. (2008).

198. Mata A, Fleischman AJ, Roy S. Characterization of polydimethylsiloxane (PDMS) properties for biomedical micro/nanosystems. *Biomedical microdevices*. 7: 281-293 (2005).

199. Shanbhag PV, Sirkar KK. Ozone and oxygen permeation behavior of silicon capillary membranes employed in membrane ozonators. *Journal of Applied Polymer Science*. 69: 1263-1273 (1998).

200. Zhang ZY. Facilitated oxygen transport in a novel silicone polymer membrane containing carboxylic cobalt groups. *Journal of Applied Polymer Science*. 90: 1038-1044 (2003).

201. Kuncova-Kallio J, Kallio PJ. PDMS and its Suitability for Analytical Microfluidic Devices. 2006.

202. Bergamini G, Alicandri-Ciufelli M, Molteni G, Villari D, Luppi MP, Genovese E, Presutti L. Therapy of Unilateral Vocal Fold Paralysis With Polydimethylsiloxane Injection Laryngoplasty: Our Experience. *J Voice*. 24: 119-125 (2010).

203. Hagemann M, Seifert E. The use of polydimethylsiloxane for injection laryngoplasty. *World J Surg*. 32: 1940-1947 (2008).

204. Sittel C, Thumfart WF, Pototschnig C, Wittekindt C, Eckel HE. Textured polydimethylsiloxane elastomers in the human larynx: safety and efficiency of use. *J Biomed Mater Res*. 53: 646-650 (2000).

205. Zullo MA, Plotti F, Bellati F, Muzii L, Angioli R, Panici PB. Transurethral polydimethylsiloxane implantation: a valid option for the treatment of stress urinary incontinence due to intrinsic sphincter deficiency without urethral hypermobility. *J Urol*. 173: 898-902 (2005).

206. Guys JM, Breaud J, Hery G, Camerlo A, Le Hors H, De Lagausie P. Endoscopic injection with polydimethylsiloxane for the treatment of pediatric urinary incontinence in the neurogenic bladder: Long-term results. *J Urology*. 175: 1106-1110 (2006).

207. Eddings MA, Gale BK. A PDMS-based gas permeation pump for on-chip fluid handling in microfluidic devices. *Journal of Micromechanics and Microengineering*. 16: 2396-2402 (2006).

208. Mills CA, Martínez E, Errachid A, Gomila G, Samsó A, Samitier J. Small scale structures: the fabrication of polymeric nanostructures for biomedical applications using pattern replication techniques. *Contributions to Science*. 3: 47-56 (2005).

209. Quake SR, Scherer A. From micro- to nanofabrication with soft materials. *Science*. 290: 1536-1540 (2000).

210. Salikhov IK, Swartz HM. Measurement of specific absorption rate for clinical EPR at 1200 MHz. *Appl. Magn. Reson.* 29: 287-291 (2005).

211. Fawaz ZA, Yassa M, Nguyen DH, Vavassis P. Fiducial marker implantation in prostate cancer radiation therapy: complication rates and techniques. *Cancer Radiother.* 18 (8): 736-9 (2014).

Attachments (as submitted to the FDA in Aug 6, 2014)

14 This section should contain all pertinent documents associated with the management of the study.

IDE Checklist

1. Description of methods, facilities and controls used for the manufacture of the device
2. A sample Investigation Agreement Form
3. Certification of Investigator agreement
4. List of Institutional IRB chairpersons
5. Approval letter from CCRC (Pre-IRB review)
6. Participant informed consent form
7. Description of EPR device & measurement protocol
8. Publications related to EPR oximetry & OxyChip
9. Report of ISO 10993-12: 2012 Chemical characterization analysis