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FLUORESCENCE IMAGING TO GUIDE DEBRIDEMENT OF FRACTURE AND INFECTION

A Phase I open label, single-center clinical trial of fluorescence imaging for predicting complications in orthopaedic surgical patients

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Study Product: ICG fluorescence imaging in trauma patients

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List of Abbreviations

AE：	Adverse Event/Adverse Experience
AUC:	Area under the curve
CIS:	Center of Innovation Surgery
CT:	Computed tomography
CTSA:	Clinical and Translational Science Institute
CCRC:	Clinical Cancer Review Committee CFR： Code of Federal Regulations
CMS:	Centers for Medicare and Medicaid Services
CRF:	Case Report Forms
CPHS:	Committee for the Protection of Human Subjects
DCC：	Data Coordinating Center
DCE：	Dynamic contrast-enhanced
DCE-MRI:	Dynamic contrast-enhanced magnetic resonance imaging
DHMC:	Dartmouth Hitchcock Medical Center
DSMC:	Data and safety monitoring committee
FDA:	Food and Drug Administration
FI	Fluorescence imaging
GCP:	Good Clinical Practice
Gd-DTPA:	gadolinium-diethylenetriamine penta-acetic acid
GFR:	Glomerular filtration rate
HVHC:	High Value Health Care
ICG:	Indocyanine green
HIPAA:	Health Insurance Portability and Accountability Act
IACUC:	Animal Care and Use Committee
ICF：	Informed Consent Form
iFI:	Intraoperative Fluorescence Imaging
IRB:	Institutional Review Board
I_{max} :	Maximum ICG fluorescence intensity
$I_{max\text{-}post}$:	ICG fluorescence intensity at post-debridement
ISM:	Independent Safety Monitor
LAR:	A legally authorized representative
MRI:	Magnetic resonance imaging
NIASM:	National Institute of Arthritis and Musculoskeletal and Skin Diseases
NIH:	National Institutes of Health
NIST:	National Institute of Standards and Technology
NCCC:	Norris Cotton Cancer Center
NSF:	nephrogenic sclerosing fibrosis
OCTOM:	Office of Clinical Trials Operations and Management, NIDCR, NIH
OHRP:	Office for Human Research Protections
OHSR:	Office of Human Subjects Research
OTA:	Orthopaedic Trauma Association
PI:	Principle investigator
PROs:	Patient reported outcomes
QA:	Quality assurance
QC:	Quality Control
RCTs:	Randomized controlled trials
REB:	Research Ethics Board
ROC:	Receiver operating characteristic
SAE:	Serious Adverse Event/Serious Adverse Experience
SOP:	Standard Operating Procedure
SSI :	Surgical site infection

Study Summary

Title	***Fluorescence imaging to Guide Debridement of Fracture and Infection***
Short Title	ICG fluorescence imaging in trauma patients
Protocol Number	N/A
Phase	Phase I
Methodology	This will be a prospective observational trial to better understand the range and variation associated with bone/soft tissue perfusion in fracture patients and examine the relationship between perfusion, measured using quantitative Indocyanine green (ICG) fluorescence and complications such as surgical site infection (SSI), persistent SSI, and fracture nonunion. Primary outcome measure is complication (either infection, recurrent infection or nonunion). Eligible consenting patients will receive standard of care treatment for their fracture or infection including irrigation and debridement of their operative site and/or fracture fixation. Participation in this study has no specific benefit to the subject. This treatment is not expected to alleviate the need for surgical intervention. After exposure, 0.1 mg/Kg ICG will be injected intravenously and video rate ICG fluorescence images will be acquired 20 seconds before and 4 minutes after the injection, each before and after debridement. A subset of 38 post-fracture complication patients will undergo surgical treatment for their infection in Center of Innovation Surgery (CIS) and have either an intraoperative DCE-MRI after exposure but before the ICG injection or a preoperative DCE-MRI. Patients receiving surgery in the CIS will be required to sign an additional CIS-specific consent document, Informed consent with MRI. The need for repeat surgical procedure will be left up to the treating surgeon. If repeat procedure is needed, pre- and post-debridement quantitative ICG fluorescence images will be obtained at each procedure. Study participants will be followed at 2 weeks, 6 weeks, 3 months and 6 months from their index study surgery. Complication including index infection, recurrent infection or delaying union/nonunion will be identified at the time of diagnosis and/or during each participant's assessment that occurs during routine outpatient clinic visit. Detailed information on the infection including date of diagnosis, participant signs and symptoms, culture test results, method of treatment(s), and date of resolution will be documented.
Study Duration	Five years
Study Center(s)	Single-center, Dartmouth-Hitchcock Medical Center
Objectives	The primary study objectives are to (1) establish the range and variation associated with bone/soft tissue perfusion in fracture patients and (2) examine the relationship between perfusion, measured using quantitative ICG fluorescence and complications such as SSI, persistent SSI, and fracture nonunion.
Number of Subjects	(1) 176 patients with open fracture patients; (2) 82 patients with a complications (infection or nonunion) following surgical treatment of fracture; (3) 100 patients with closed fracture.

Diagnosis and Main Inclusion Criteria	**Open Fracture Cohort (Cohort 1)** a. Patients 18 years of age or older. b. Open extremity fracture. c. Planned definitive fracture management with external fixation, internal fixation, or joint fusion. d. Will have all planned fracture care surgeries performed by a participating surgeon or delegate. e. Provision of informed consent. **Complication Cohort (Cohort 2)** f. Patients 18 years of age or older. g. Extremity fracture. h. Prior definitive fracture management with external fixation, internal fixation, or joint fusion. i. Superficial, deep, or organ space SSI (as per CDC criteria) at the fracture site that requires operative management. j. Will have all fracture care surgeries performed by a participating surgeon or delegate. k. Provision of informed consent. **Closed Fracture Cohort (Cohort 3)** l. Patients 18 years of age or older m. Closed extremity fracture n. Planned definitive fracture management with external fixation, internal fixation, or joint fusion. p. Provision of informed consent.
Study Product, Dose, Route, Regimen	*This protocol is an imaging study which does not specify any change in treatment protocol or and will not alter the surgical course for the patient enrolled, therefore should not be considered an experimental treatment..* ICG is an FDA approved fluorescence probe. After skin exposure, 0.1 mg/Kg ICG will be injected intravenously, each before and after debridement. Video rate ICG fluorescence images will be acquired 20 seconds before and 4 minutes after ICG injection, by an FDA approved fluorescence imaging system, (Spy Elite (Novodaq/Stryker US). The imaging is non-contact and the imaging head is at least 50 cm away from the bone.
Duration of administration	Patients are enrolled in the study from time of consent prior to surgery to the end of six months of the follow up period.
Reference methods	N/A
Statistical Considerations	Descriptive statistics will be present (mean and standard deviation) with 95% confidence intervals. We are planning to collect ICG data before and after the debridement. Pearson correlation between ICG parameters (pre-debridement, or/and post-debridement, or/and changes between pre- and post-debridement) and index infection/recurrent will be calculated for each of three patient cohorts). We will also include the corresponding coefficient of determination indicating how much variation in index infection/recurrent can be explained in ICG parameters. Finally, we will evaluate the significance of the correlation coefficient using two sided t-test. P-value less than 0.05 will indicate that the correlation is significant. Then, we will form receiver operating characteristic (ROC) curves to illustrate graphically the performance and to obtain the threshold for differentiating the recurrent infection from non-infection group, of each chosen model. Area under the ROC curve will be used to determine the optimal property for differentiating the recurrent infection patients from the non-infection patients.

1 Introduction

This document is a clinical research protocol and describes a human research study. This study will 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. All personnel involved in the conduct of this study have completed human subject protection training and primary oncologic surgeons performing study procedures will be trained on this protocol

1.1 BackgroundInformation

The focus of this Phase 1 clinical study is to (1) establish the range and variation associated with bone/soft tissue perfusion in fracture patients, using ICG fluorescence imaging; (2) examine the relationship between perfusion and complications such as surgical site infection (SSI), persistent SSI, and fracture nonunion; (3) to determine whether the quantitative ICG fluorescence can be used to guide bony debridement in the setting of open fracture or infected fracture to minimize complications. This research has been funded by the SYNERGY Pilot Translational Research Grant and by the Department of Orthopaedics.

Infection following trauma is one of the most prevalent and challenging complications faced by orthopedic surgeons in both military and civilian populations, occurring after up to 60% of open bone fractures[1-7]. Several factors specific to this trauma place patients at high risk for infectious complications, including: traumatized tissues, open contaminated fracture, soft tissue coverage issues, catabolic state due to polytrauma, prolonged hospitalization with exposure to nosocomial bacteria, and presence of metallic implants[8]. Infection requires one or more unplanned surgical procedures and leads to prolonged morbidity, loss of function, and potential loss of limb[1, 9-11]. Failed treatment for bone infection results in recurrent infection, requiring repeat surgical procedures in approximately 30% of patients.[12-15]

Deficient perfusion prevents delivery of antibiotics and endogenous immune cells to traumatized tissues. In the setting of open fracture and established infection, poorly perfused bone is a nidus for biofilm formation creating resistance to antibiotics. Because of this, initial management of open fractures and the management of SSIs at the fracture site are based on aggressive, thorough debridement in an effort to remove all poorly perfused bone. However, there is a critical lack of data to objectively inform bone perfusion and the corresponding amount of tissue to debride. This extensive gap in our understanding leads to substantial variation in extent of debridement and places patients at unnecessarily high risk for initial SSI and recurrent SSI.

While, to date, there is no gold standard technique established to measure bone perfusion in human patients, dynamic contrast-enhanced MRI (DCE-MRI) is the imaging modality that has been used most frequently and effectively to measure bone perfusion in patients without metal implants implants[16-21]. Because of this, developing a better understanding of the relationship between DCE-MRI, which assesses the bone and surrounding soft tissues in a three-dimensional format and ICG-based DCE-FI, which is surface-weighted, is an important next step. Data acquired from intraoperative DCE-MRI and DCE-FI will be used to create a fluorescence-imaging specific model which will correct for the surface-weighted nature of fluorescence imaging and more accurately reflect the fluorescence intensity and kinetics throughout the entire depth of the bone.

There are currently no accepted intraoperative tools that can be used to make objective decisions about bone perfusion. Methods currently used to guide debridement are quite rudimentary. Clinical judgement is based on the gross appearance of soft tissue and bone, including color, turgor, and extent of soft tissue stripping. A burr may be used to look for bleeding bone. More extensive debridement is thought to minimize risk of index infection or reduce the rate of persistent infection; however, this comes at the cost of increasingly complex reconstructive procedures to fill bony defects[22 23, 24]. Clearly what is needed is a functional imaging system which can assess vascular perfusion of surgical field tissue to guide surgeons in the amount of tissue to debride. In turn, this will lead to fewer infections and a more effective treatment of SSIs at the fracture site. Both scenarios will allow patients to return to duty or work sooner.

1.2 Investigational imaging method

ICG fluorescence imaging has been used to assess tissue perfusion in vivo in real-time intra-operative arterial and lymphatic perfusion imaging, [25, 26] osseous flap perfusion imaging[27]. Compared to the conventional medical imaging modalities, such as computed tomography (CT) or magnetic resonance imaging (MRI), ICG fluorescence imaging is nonionizing, low-cost, and portable. ICG is a dye that absorbs near-infrared light in the wavelength range between 790 to 805 nm and has an emission at the peak of 835 nm. ICG is almost 98% plasma protein bound so that once it takes up into the microcirculation, it remains within the lymphatic and circulatory vasculature. ICG has been approved for clinical use by FDA since 1959.[28] Since then, ICG has been used for human in angiography for ophthalmology and cardiology applications and is given routinely to patients in these clinical settings. In addition, as the clinical trials, ICG fluorescence imaging has been used to guide laparoscopic surgery, [29] robotic adrenalectomy,[30] assess tissue perfusion in vivo in real-time intra-operative arterial and lymphatic perfusion imaging,[25, 31, 32] and tissue flap perfusion imaging.[33, 34] ICG is ideal to measure perfusion/blood flow because it binds efficiently to blood lipoproteins and thus does not leak from circulation. In addition, the ICG half-life is short making repeated measures possible, and it is indirectly activated, so that the dynamic fluorescence due to bone and tissue perfusion can be captured by a video rate imaging system.

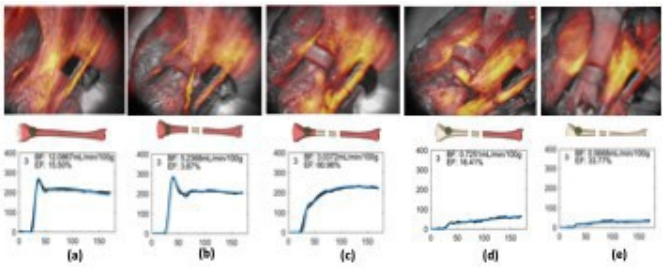

Figure 1: ICG fluorescence map overlay on the white light bone images as well as dynamic curves of green ROI. (a) baseline, (b) after double diaphyseal osteotomies, (c) half circumferential soft tissue stripping from the proximal bone close to the previous cut, (d) full circumferential soft tissue stripping from the proximal end, and (e) all circumferential soft tissue stripping to disrupt both periosteal and endosteal blood supply from entire tibia.

The dynamic fluorescence imaging systems used in this study will be SPY Elite (Novodaq/Stryker). Both of them are the publicly available imaging systems that can be used to assess tissue perfusion in real time in the operating room. The systems have a multi-directional imaging arm which contains a near-infrared light source that illuminates the fluorescent agent within the tissues, an HD video camera that captures the intensity of fluorescent marker in real-time, and software that allows the user to capture relative and absolute perfusion values within the surgical field. This product has been FDA approved for use to monitor blood flow, plastic surgery, microsurgery, reconstructive surgery, gastrointestinal imaging and coronary bypass surgery.

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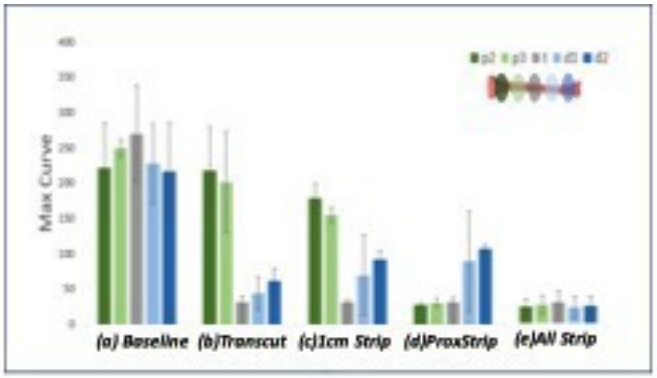

Figure 2: Summary of the maximum ICG fluorescence intensity in each ROI and condition.

We have conducted work utilizing a porcine model to demonstrate measurable, reproducible and predictable differences in bone perfusion as measured by quantitative ICG fluorescence in five conditions: (a) baseline, (b) two diaphyseal osteotomies, separating 1cm of tibia with circumferential soft tissue stripping (disrupting both periosteal and endosteal blood supply), (c) ~2 cm circumferential soft tissue stripping (disrupting periosteal blood supply) from the proximal bone close to osteotomy, (d) circumferential soft tissue stripping of the entire proximal bone segment (disrupting periosteal blood supply), and (e) circumferential soft tissue stripping of the entire bone (extensively disrupting periosteal blood supply). This study protocol has been approved by Dartmouth College's Institutional Animal Care and Use Committee (IACUC). Before imaging each condition, 0.1 mg/kg ICG was injected intravenously and the dynamic fluorescence change was captured by a fluorescence surgical microscope (Pentero, Zeiss) for 4 minutes. Between imaging each condition 20 minutes was allowed for elimination of residual ICG. Figure

1 shows the ICG fluorescence map overlay on the white light bone images as well as dynamic curves at the proximal bones (green ROIs in middle bone models). As shown in Figure 1 the ICG intensity and dynamic changes are substantially different in different conditions and ROIs. Baseline conditions, without disruption of either periosteal or endosteal blood supplies demonstrated the highest ICG intensity. Similarly, the next highest intensity was noted in ROIs proximal and distal to the osteotomy, prior to any removal of periosteum (again, leaving endosteal and periosteal blood supply intact, however, bone disrupted by an osteotomy). As sequential soft tissue stripping was performed, sequentially removing periosteal blood supply, the ICG intensity decreased. The osteotomized fragment of bone, with both periosteal and endosteal blood supplies disrupted, demonstrated the lowest ICG intensity and a flat dynamic curve. This experiment demonstrated that ICG intensity decreased in relationship to progressive disruption in bony blood supply. To date, we have collected fluorescence data on five limbs (three pigs) for this preliminary study. Figure 2 shows the summary data of the maximum intensity of each ROIs at each condition. The data of the first leg has been excluded from this summary due to the ICG concentration and experimental condition have been changed/optimized after the first pig experiment. From this summary graph, it is clear that ICG fluorescence is highest in bone with endosteal and periosteal blood supply intact compared both with bone with periosteal blood supply disrupted/endosteal blood supply intact and bone with both periosteal and endosteal blood supply disrupted. These studies validate the effectiveness of quantitative ICG fluorescence as a method of measuring bone perfusion in a quantitative manner.*1.3 Clinical Data to Date*

ICG is FDA approved for human use in angiography for ophthalmology and cardiology applications and is given routinely to patients in these clinical settings. In addition, as the clinical trials, ICG has been used to guide laparoscopic surgery, [29] robotic adrenalectomy,[30] assess tissue perfusion in vivo in real-time intra-operative arterial and lymphatic perfusion imaging,[25, 31, 32] and tissue flap perfusion imaging[33, 34]. The examples of these trials are summarized below.

(1) Guide laparoscopic surgery[29] ((0.1–0.5 mg/ml/kg): This study has been carried out in Minimally Invasive Surgery Research Center, at University of Insubria, Varese, Italy. 108 ICG-enhanced fluorescence guided laparoscopic procedures were performed. ICG imaging provided high-resolution real-time images of blood flow in vessels and organs as well as highlighted biliary excretion. As the result, “no intra-operative or injection-related adverse effects were reported, and the biliary/vascular anatomy was always clearly identified.” The real-time imaging provided sufficient information to conduct a safe cholecystectomy and ensure adequate vascular supply for colectomy, nephrectomy, or find lymph nodes.
(2) Guide robotic adrenalectomy[30]: (3.8–20 mg/subject) This study investigates the use of ICG florescence to guide an accurate dissection during robotic adrenalectomy (RA). A total of 40 patients had been involved and the borders of adrenal tumors delineated by ICG imaging was superior, equivalent, or inferior to conventional robotic view in 46.5%, 25.6%, and 27.9% of the procedures, respectively.
(3) Real-time intra-operative arterial and lymphatic perfusion imaging: [32] (2-6.6 mg/subject) 301 patients were involved in the trial, and 589 nodes were removed. 583 nodes (99%) were identified with ICG and the identify rate is higher than that the conventional 99mTc (452 nodes). No any significant acute side effects were found related to ICG in this study.
(4) Tissue flap perfusion imaging[33, 34]: (5 mg/subject) 77 single-pedicle breast reconstruction patients were involved. Perfusion was measured intraoperatively using ICG angiography following flap harvest and before transfer. Significant perfusion difference has been detected in various flaps and between zones. The results shown that the lower abdominal free flaps based on the inferior epigastric system have better perfusion when compared with pedicled transverse rectus abdominis muscle flaps.

1.4 Dose Rationale

We plan a sample size of 300 patients in this open label, single center, clinical trial of ICG fluorescence imaging for predicting recurrent infection in musculoskeletal trauma patients. For each subject, during the surgery, prior and after to the debridement, each of 0.1 mg/Kg ICG will be injected intravenously and video rate ICG fluorescence images will be acquired for each of 4 minutes. The ICG will

be supplied directly by Novodaq/Stryker, which is FDA approved. As it shown in Section 1.4, total does of 0.2 mg/kg is within the range of previous studies. Indeed, this is 10 times less than the maximum recommended dose by FDA of 2mg/kg.

The subset patients who will undergo surgical treatment in CIS may undergo gadolinium (Gd-DTPA) contrast-enhanced MRI either intraoperatively or preoperatively. Gd-enhanced MRI is FDA-approved clinical imaging. The Gd-DTPA injection procedures will follow FDA-approved guidelines which are accepted as part of standard care in this setting.

1.5 Potential Risks and Benefits

The risks to subjects are minimal in relation to anticipated benefits and/or knowledge that might reasonably be expected from the results of this clinical trial.

1.5.1 Potential Risks

Risk of ICG injection: ICG (Indocyanine Green) will be administered intravenously twice: once before and once after the debridement. ICG is FDA approved for human use in angiography for ophthalmology and cardiology applications and is given routinely to patients in these clinical settings. The risks are considered minimal and consist of nausea, vomiting, hives, increased heart rate in subjects with particular sensitivity to the dye. ICG does contain sodium iodide and patients with a history of allergy to iodides will be excluded. Anaphylactic or urticardial reactions are rare but have been reported in patients both with and without a history of allergy to iodides. Anaphylactic deaths have been reported following IGG administration during cardiac catheterization. Reported rates of mild, moderate and severe adverse reactions to ICG are 0.15%, 0.2% and 0.05%. Every effort will be made to minimize this risk as much as possible. It is the standard of care at this institution to obtain information related to allergies, sensitivities and past medical histories upon patient arrival. Patients will also be monitored throughout their hospitalization, as is the standard of care, by everyone involved in their medical care for evidence of new or previously unknown reactions or sensitivities. Additionally, at all times ICG fluorescence imaging assessments are taking place, research staff and both surgical and anesthesia staff will monitor patients closely for any adverse reaction to ICG. Research staff and surgical and anesthesia staff will also monitor patients for at least 30 minutes after all ICG fluorescence assessment procedures have been completed. In the event of an unexpected allergic reaction, Dartmouth-Hitchcock Medical Center and all affiliated groups within this institution have procedures in place for managing patients with unknown or unexpected allergic reactions. If such a reaction were to occur, all standard of care treatments will be provided including but not limited to treatment with appropriate agents such as epinephrine, antihistamines and corticosteroids. Risk of infection from IV injection is also extremely rare but can occur. Pregnant women are excluded from the study. A pregnancy test is administered to women of child-bearing age as part of standard of care pre-operative testing.

Risk of dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI): The risks associated with Gd contrast-enhanced MR are minimal. Gd-DTPA is an FDA-approved contrast agent and is widely used with few complications. The contrast injection procedures follow FDA-approved guidelines which are accepted as part of standard care in this setting. Consenting participants will have an intravenous catheter placed in an arm vein prior to their MRI examination by a trained MRI technologist conforming to the standard clinical methods used at DHMC.

Serious reactions to the gadolinium (Gd) are extremely rare and are primarily limited to nausea and vomiting. In response to the FDA notification regarding the potential association of nephrogenic sclerosing fibrosis (NSF) and intravenous gadolinium administration (primarily gadodiamide (Omniscan)), the Department of Radiology has developed a protocol which we will follow:

1. Identify the patients at higher risk of developing NSF (at the time of scheduling or before). Patients at risk include:

- Patients > 50y
- Patients already on dialysis
- Patients with known renal insufficiency but not currently on dialysis
- Diabetic patients

- Patients with known cardiovascular disease
- Patients who will be receiving high dose gadolinium (e.g. MR angiograms)
- Hypertensive patients

2. Obtain estimated Glomerular filtration rate (GFR) on this higher risk group of patients (unless already on dialysis).

- If a GFR value has been checked and is available in the patient's medical record within 1 month of contrast-enhanced MR, that value will be acceptable.
- If the participant is not a patient at DHMC, a "stat" GFR will be performed at DHMC one (1) hour prior to contrast-enhanced MR scan.

Subjects with a GFR <30 ml/min. will be considered ineligible for the contrast enhanced MRI portion of the study.

GFR values will not be sought at the time of scheduling on low risk patients, but the scheduler will check medical records at the time of the scan to see if an incidental GFR value is available.

Risk of infection from IV injection is also extremely rare but can occur. MR examination is used daily for many conditions and is considered safe. There are no known risks with MR imaging. Some individuals may feel claustrophobic inside the MR unit. Metal implants, such as surgical clips, and electronic devices, such as pacemakers, can be contra-indications for MR exams. Participants are questioned about these conditions and not enrolled accordingly.

Prolonged operating room time: Because the ICG fluorescence assessments will take place in the operating room, participation in this study may increase the amount of time spent under surgical anesthesia in the operating room. For the average case, an additional 10 minutes at most will be incurred. However, the research staff will make every effort to minimize this risk by performing the assessment test while other required operative procedures are being performed. For the subset patients who will have DCE-MR, the imaging sequence and time for DCE-MRI (~10-15 minutes) is not expected to change significantly from standard care of surgical treatment.

Risk of confidentiality breach: Subjects enrolled in research are always exposed to additional risks of a breach in confidentiality, for example, of some elements of their personal health information that is made available to study investigators as part of their participation. The risk is 100%, but the occurrence rate of an actual breach is < 1% (to date, we are not aware of any subject participating in our studies who has experienced a detrimental breach of confidentiality or confidential information).

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Risk of breach of confidentiality of the medical records and status of participants will be minimized. Databases which are used to store subject-sensitive information are password-protected and encrypted during file/data transfers from viewing terminals. Access will be limited to research team members who have undergone CPHS training at Dartmouth. Whenever possible and practical standard-of-care clinical data used in the research will be de-identified when under analysis.

1.5.2 Potential Benefits

Patients enrolling in this study will not benefit directly because no diagnostic or therapeutic decisions will occur based on the study, and thus, administration of the study is not intended to alter the surgical procedure. However, future patients may benefit from the knowledge gained from the study since this study may provide an objective, intraoperative, and real-time methodology to assess bone perfusion and thoroughness of debridement. This method may save the patients from unnecessarily high risk for index infection or nonunion—in the setting of open fracture—or recurrent infection in the setting of established infection, due to the substantial practice variation.

2Study Objectives

The primary study objective is to establish the range and variation associated with bone/soft tissue perfusion in fracture patients, using ICG fluorescence imaging and examine the relationship between perfusion and complications such as SSI, persistent SSI, and fracture nonunion.

The secondary study objective is to develop intraoperative hardware and software tools that will be used to guide debridement to optimize the quantity of bone/tissue resected to minimize complications.

3 3Study Design

3.1 General Design

This study is a Phase I open label, single-center clinical trial of fluorescence imaging for predicting recurrent infection in musculoskeletal trauma patients. Patients who is undergo fracture fixation or joint fusion or requiring open surgical debridement/saucerization for osteomyelitis, may recruited into the study. Although the SPY Elite imaging system and the use of ICG have not been used in this type of study previously both have been established in other clinical settings to assess tissue perfusion. The expected duration for enrolling study participants is five years, and up to 300 subjects may be enrolled. Eligible consenting patients will receive standard of care treatment for their fracture or complication (infection or nonunion) including irrigation and debridement of their operative site. Detail study procedures will be described in the following Section 6.

3.2Primary Study Endpoints

The primary study endpoint for the open fracture cohort (cohort 1) is SSI using the CDC National Healthcare Safety Network reporting criteria (https://www.cdc.gov/nhsn/pdfs/pscmanual/9pscssicurrent.pdf) within 12 months of fracture.

The primary study endpoint for the cohort of patients with complications, (cohort 2) is treatment failure (either persistent SSI using the CDC National Healthcare Safety Network reporting criteria or persistent nonunion requiring repeat surgical procedure).

The primary study endpoint for the closed fracture cohort (cohort 3) is SSI using the CDC National Healthcare Safety Network reporting criteria.

3.3 Secondary Study Endpoints

The secondary endpoint for all cohorts is unplanned fracture-related reoperation within 12 months of the initial open fracture. This endpoint has been used in previous fracture trials and is defined as any unplanned surgery that occurred from the time of index surgery to 12 months post-fracture that is associated with a wound healing problem, an infection at the operative site or contiguous to it, or fracture delayed union/nonunion.

4 4 Subject Selection and Withdrawal

4.1PatientSelection

Consecutive patients 18 years of age or older who present to DHMC with an open fracture will be considered for the open fracture cohort (Cohort 1). Fracture patients who present with a complication (either SSI or nonunion) following fracture fixation or joint fusion will be screened for the complication cohort (Cohort 2). Patients who present to DHMC with a closed fracture will be screened for the closed fracture cohort (Cohort 3).

4.2Inclusion Criteria

The following criteria must be met by study subjects to be eligible for study enrollment:

Open Fracture Cohort (Cohort 1)

(1) Patients 18 years of age or older.
(2) Open extremity fracture.
(3) Planned definitive fracture management with external fixation, internal fixation, or joint fusion.
(4) Will have all planned fracture care surgeries performed by a participating surgeon or delegate.
(5) Provision of informed consent.

Established SSI Fracture Cohort (Cohort 2)

a. Patients 18 years of age or older.
b. Extremity fracture.
c. Prior definitive fracture management with external fixation, internal fixation, or joint fusion.
d. Superficial, deep, or organ space SSI (as per CDC criteria) at the fracture site that requires operative management.
e. Will have all fracture care surgeries performed by a participating surgeon or delegate.
f. Provision of informed consent.

Closed Fracture Cohort (Cohort 3)

a. Patients 18 years of age or older.
b. Closed extremity fracture.
c. Planned definitive fracture management with external fixation, internal fixation, or joint fusion.
d. Will have all planned fracture care surgeries performed by a participating surgeon or delegate.
e. Provision of informed consent.

4.3 Exclusion Criteria

The following criteria would exclude a subject from study enrollment:

Open Fracture Cohort (Cohort 1)

(7) Fracture of the hand.
(8) Iodine allergy.
(9) Received previous surgical debridement or management of their fracture at a non-participating hospital or clinic.
(10) Open fracture managed outside of the participating orthopaedic service.
(11) Chronic or acute infection at or near the fracture site at the time of initial fracture surgery.
(12) Burns at the fracture site.
(13) Incarceration.
(14) Expected survival of less than 90 days.
(15) Problems, in the judgment of study personnel, with maintaining follow-up with the patient.

Established SSI Fracture Cohort (Cohort 2)

a. Fracture of the hand.
b. Iodine allergy.
c. Received previous surgical debridement to manage the SSI.
d. Incarceration.
e. Problems, in the judgment of study personnel, with maintaining follow-up with the patient.

Subset: DCE-MRI (Cohort 2-1)

f. the presence of an electronic implant, such as a pacemaker
g. the presence of a metal implant, such as an aneurysm clip
h. the presence of other contraindication(s), as determined by the MRI technologists and radiologists.
i. A history of allergy to iodides

j. A GFR < 30 ml/min as determined by blood test on the day of NIR/MR imaging, or from lab results within 3 months of DCE-MRI for this study

Closed Fracture Cohort (Cohort 3)

a. Fracture of the hand.
b. Iodine allergy.
c. Chronic or acute infection at or near the fracture site at the time of initial fracture surgery.
d. Burns at the fracture site.
e. Incarceration.
f. Expected survival of less than 90 days.
g. Problems, in the judgment of study personnel, with maintaining follow-up with the patient.

4.4 Subject Recruitment and Retention

Participation in this research requires informed consent according to Institutional Review Board (IRB) guidelines and a signed IRB-approved Consent Form as the means of documenting this understanding. Patients receiving surgery in the CIS will be required to sign an additional CIS-specific surgical consent document.

Potential recruits are instructed that their participation is completely voluntary and that their medical care will not be altered in any way should they elect not to participate at any time prior to surgery. Subjects are recruited from patients presenting or referred to Orthopedic Department at Dartmouth-Hitchcock Medical Center for treatment and meeting protocol inclusion criteria. This study requests a partial waiver of HIPAA for recruitment purposes. The partial waiver would allow for the surgeon to share with the research coordinator the patient name, sex, MRN number, age, underlying conditions as well as type and location of injury. There is an adequate plan to protect identifiers from improper use and disclosure as information will only be used by study team members to determine whether a patient may be eligible. Information will only be communicated and stored using D-HH approved secure platforms. Information is only being used to determine if a patient is eligible for the study, for recruitment purposes. Once approached, if a patient is not interested, information will be destroyed. Protected health information will not be re-used or disclosed for another purpose. It will only be used for recruitment. This research could not practically be done without a partial waiver of HIPAA. This information is needed for recruitment. Patients will sign a full HIPAA Authorization when consenting to the study.

Potential subjects may be contacted by Dr. Gitajn, Dr. Henderson or another surgeon in the Orthopaedic department to learn about the study and participation. Subjects will be invited to participate in this study by a member of the Orthopaedic Department, which will occur either at the time of consultation with the surgeon about the candidate's standard-of-care procedures or at another time agreed to by the potential participant, the candidate's surgeon and/or Dr. Gitajn and/or Dr. Henderson or their designee. No advertisements or other promotional material will be used. No finder fees or recruitment incentives will be offered. Women of child bearing potential are eligible for enrollment into this study because ICG administration is not considered to present any additional risk for these women. The study will exclude women who are pregnant or breast-feeding as indicated in the exclusion criteria. Women of child-bearing potential, if asked to participate, will be given a pregnancy test as part of their standard of care pre-operative testing to confirm pregnancy status before administration of ICG.

Subjects enrolled into this study prior to 01Nov2020 will be re-consented in order to allow for the use of their data in the following applicable studies:

Fluorescence Based Measurement of Bone Perfusion in Infection (STUDY 02000374)
ICG Fluorescence Imaging in Open Trauma (STUDY 02000365)

All follow-up visits are within standard practice for these injuries/diagnoses.

Several additional strategies may be used to maximize follow-up including: 1) at the time of enrollment each participant will provide their own telephone number, as well as the name and address of a primary care physician, and the names and phone numbers of three people at different addresses with home the participant does not live and who are likely to be aware of the patient's whereabouts; 2) participants will receive a reminder card upon discharge for their next follow-up visit by the clinical site study personnel; 3)

participants will receive text message reminders; 4) follow-up will coincide with normal surgical fracture clinic visits; and 5) if a participant refuses or is unable to return for the follow-up assessment, study personnel will determine his/her status with regards to major study outcomes by telephone, text, mail or email contact with the participant or the provided alternate contacts.

4.5Early Withdrawal of Subjects

4.5.1 When and How to Withdraw Subjects

Participants will be removed from the protocol if: Study imaging is not completed for any reason.

1) The subject withdraws consent.

The subject has an occurrence of a significant clinical event that precludes imaging.

2) The subject becomes pregnant.

If a participant is withdrawn from the protocol, the PI will mark the data of this subject as "withdrawn" and will add a detailed explanation about the cause of withdrawal in the database.

4.5.2 Data Collection and Follow-up for Withdrawn Subjects

For subjects who withdraw after the intraoperative imaging, we will follow-up to see if they still complete their clinical follow up in six months. For subjects who eventually finish their all clinical follow up visits, we will acquire their follow up results to include these subjects in the statistical analysis portion that only involves the intraoperative image data and infection status.

5 Study Drug and device

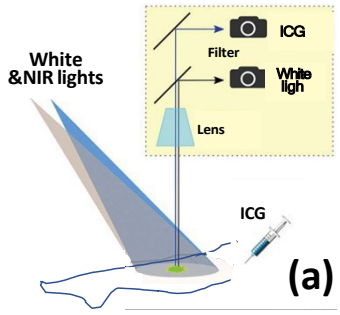

Figure (a): Schematic sketch of ICG fluorescence imaging.

5.1 Description

This study is ***neither a drug nor a device trial.*** Patients will be administered FDA approved ICG through intravenous injection and imaged by a FDA approved surgical microscope (Spy Elite) which is 0.5 meter away from the subject. Both ICG fluorescence and the two imaging systems have been used for routine clinical practice for many years. Figure (a) shows the Schematic sketch of the imaging systems. ICG fluorescence imaging utilizes intravenously injected ICG, which is a fluorescent dye that is FDA-approved for clinical use, illuminated with near-infrared light. The ICG dye is indirectly activated and the dynamic fluorescence due to bone perfusion can be captured by a video rate imaging system.

5.2 Preparation and Administration of Study Drug

ICG used in this study is purchased from Novodaq/Stryker.

ICG Preparation and Administration Procedures:

1) ICG is prepared by the Surgical and/or Anesthesia teams in the operating room on the day of surgery.
2) From the vial containing ICG, the required dose is withdrawn into a syringe, labeled, and administered through a peripheral intravenous line followed by a saline flush.

Prior to ICG administration, patients will undergo standard preoperative monitoring by the Same Day nursing staff that includes continuous pulse oximetry, blood pressure monitoring, and heart rate, and respiratory rate. These results will be available prior to surgery and prior to administration of ICG. ICG will be administered by an anesthetist in the operating room. Patients are under the care and observation of the anesthesia and surgical teams during this time. Postoperatively, patients are transferred to the Post-Anesthesia Care Unit (PACU) where they are monitored continuously by nursing staff supervised by anesthesiologists. During this entire time vital signs (including temperature, pulse, respiratory rate and blood pressure) are collected as standard of care. All clinical data will be reviewed for adverse events for a period of 30 minutes post-injection,

Day of surgery Procedures	Pre-Infusion	0-30 minutes post-infusion
Pulse	X	X
Blood Pressure	X	X
Temperature	X	X
Respiratory Rate	X	X
Adverse Events		X

A Pulse dye densometer (Pulsion Medical Systems), similar to a pulse oximetry probe, will be placed on the patient's finger to acquire an arterial blood input function during ICG injection. After exposure but prior to debridement, 0.1 mg/kg ICG will be injected intravenously. Video rate ICG fluorescence images will be acquired 20 seconds before and 4 minutes after ICG injection. Debridement will then proceed per standard of care. The wound will be irrigated with a minimum of 3 liters irrigation with normal saline. After irrigation and debridement is completed, 0.1 mg/kg ICG will be injected intravenously again and video rate ICG fluorescence images will be acquired 20 seconds before and 4 minutes after ICG injection. There will be a minimum of 5 minutes between ICG infusions to allow ICG to wash out of tissues.

At investigator discretion, only one ICG injection may be performed and only one series of ICG fluorescence imaging will be obtained. There will be no increased risk to the subject if the investigator opts to only obtain one series of images.

5.3 Subject Compliance Monitoring

Subjects participate in a "one-time" event (i.e., surgery) as part of this study and are monitored through the in-patient surgical service during the time of their participation.

5.4 Prior and Concomitant Therapy

N/A.

5.5 Packaging

N/A

5.6 Blinding of Study Drug

N/A.

5.7 Receiving, Storage, Dispensing and Return

5.7.1 Receipt of Drug Supplies

ICG will be shipped to the Pharmacy at Dartmouth-Hitchcock Medical Center by standard UAB. Pharmacy staff will count and verify that the shipment contains all the items noted in the shipment inventory, providing temperature monitoring data to UAB if necessary. Pharmacy staff will document receipt into their electronic investigational drug management database that keeps a perpetual inventory. Any damaged or unusable study drug in a given shipment will be documented in the study files, and destroyed on site following standard procedures.

5.7.2 Storage

ICG will be stored as the original packages that shipped from the maker (Stryker).

5.7.3 Dispensing of Study Drug

Once agent assignment is performed, research staff will transport ICG in its original package to the operating room. The surgeon or anesthesiologist will prepare the solution for injection per instructions from the Stryker. 0.1mg/kg ICG will then be administered per study protocol.

5.7.4 Drug Accountability

A member of the study team will document the amount of ICG administered and any amount remaining in the institution's electronic medical record.

5.7.5 Return or Destruction of Study Drug

ICG is FDA approved for routine clinical practice. No return or destruction of study drug is needed for this study.

6 Study Procedures

Consent Process

Eligible patients regardless of ethnicity or health status, will be identified and recruited subject to inclusion and exclusion criteria above. Patients who meet eligibility criteria will be asked to participate in the trial. If they agree written informed consent will be obtained from the patient or their healthcare proxy. Patients receiving surgery in the CIS will be required to sign an additional CIS-specific surgical consent document. To obtain informed consent, study personnel (surgeon or research coordinator) will adhere to the following procedures: (1) present study information in a manner that is understandable to the patient; (2) discuss the study with the patient and answer any questions; (3) allow the patient an opportunity to discuss participation with their family; (4) confirm that the patient understands the risks and benefits of participating in the study nd that their participation is voluntary; (5) complete the consent process and obtain signatures from the patient and research team. The process of obtaining and documenting informed consent forms will be completed in accordance with local Good Clinical Practice (GCP) recommendations. If the research team member obtaining consent is at all unsure about the patient's ability to consent, s/he will consult with the study PI.

Recognizing that consent is an ongoing process, the study team will encourage the participants to ask

additional questions that may arise during the course of their participation in the study.

This study will not involve the subjects who is under age 18.

The study team will comply with consent procedures outlined in SOP HRP-090.

This study does not involve the assessment of an experimental treatment to the subjects enrolled. The subjects will undergo a method for collecting imaging data that has been fully vetted and utilized in other clinical settings. The purpose of this research it to evaluate the utility of this imaging technique in orthopaedic trauma patients who have a closed orthopaedic trauma.

Cognitively Impaired Adults
Patients with impaired decision-making capacity will be included in this study. If the patient is unable to provide informed consent (e.g. due to their injury) at the time they are identified, informed consent may be obtained from their legally authorized representative (LAR). Allowing informed consent from a patient's LAR will reduce the risk of recruitment bias against the most severely infected patients.

For patient with impaired decision-making capacity, a LAR with knowledge of the potential participant will be approached to consent on the patient's behalf. If the patient cannot adequately answer at least two questions and it is determined that the patient's level of cognition is not likely to change before surgery, their LAR will be approached. The choice of LAR will follow standard procedures. The following will be approached in this order of priority:

- legal guardian
- proxy (health care agent) named in an advanced directive or durable power of attorney for health-care
- family member or other surrogate identified by state law on health care decisions.

The LAR will be advised to base the decision on the participant's expressed wishes, or, if these are not known, what they believe the participant would have desired under the circumstances of the injury, their beliefs and values. Recognizing that consent is an ongoing process, the study team will encourage the participants to ask additional questions that may arise during the course of their participation in the study.

Subjects participation in this study completely voluntary. If at any time a patient finds participation to be unduly stressful the patient or their legal authorized representative may withdraw the patient from the study with no repercussions.

Assent will not be requested from subjects unable to consent for themselves.

The study team will comply with consent procedures for cognitively impaired adults outlined in SOP HRP-013.

Surgical Procedures

In the surgery day, the patient will be prepared and transported to surgery as per routine at Dartmouth-Hitchcock. In the OR, patient positioning, preparation of the surgical field, and draping will follow standard practice, as fracture fixation or joint fusion or requiring open surgical debridement/saucerization for osteomyelitis.

A Pulse dye densometer (Pulsion Medical Systems), similar to a pulse oximetry probe, will be placed on the patient's finger to acquire an arterial blood input function during ICG injection. After exposure but prior to debridement, 0.1 mg/kg ICG will be injected intravenously. Video rate ICG fluorescence images will be acquired 20 seconds before and 4 minutes after ICG injection. Debridement will then proceed per standard of care. The wound will be irrigated with a minimum of 3 liters irrigation with normal saline. After irrigation and debridement is completed, 0.1 mg/kg ICG will be injected intravenously again and video rate ICG fluorescence images will be acquired 20 seconds before and 4 minutes after ICG injection. There will be a minimum of 5 minutes between ICG infusions to allow ICG to wash out of tissues.

A subset of 30 eligible consenting patients with post-fracture complications will undergo surgical treatment for their infection in CIS at DHMC. Patients receiving surgery in the CIS will be required to sign an additional CIS-specific consent document. For the patients who didn't receive contrast MRI in 48 hours prior to the

operation, after exposure and removal of hardware but prior to pre-debridement ICG injection, four MRI fiducials will be placed on the four corners of the damaged bone area and the MRI sequences will be acquired. Sequences will include a localizer, coronal T1 fast spin echo (FSE), axial short-tau inversion recovery (STIR), a T1 mapping sequence consisting of a T1-weighted 3D FLASH sequence with at least 3 different flip angles, and DCE acquisition, consisting of 3D FLASH sequences before and after the intravenous administration of 0.2 mL/kg Gadoterate meglumine (Dotarem®). The T1 mapping and DCE sequences will be acquired in the same plane (either the coronal or axial) for all patients. The protocol for the DCE acquisition has already been optimized for current clinical applications in the imaging of both bone and soft tissue tumors. For the patients who received MRI within 48 hours prior to the operation, we may use their pre-operation MRI, instead of intra-operative MRI.

The need for repeat debridement or tissue cultures will be left up to the treating surgeon. If repeat debridement is needed, pre- and post-debridement quantitative ICG fluorescence images will be obtained at each procedure. If the skin wound is unable to be closed, it will be left open with a sterile dressing until delayed coverage in collaboration with plastic surgery.

Upon admission and throughout the hospital course, patients will be treated with antibiotics per standard of care by the orthopaedic service or in collaboration with infectious disease recommendations. Management of study patients including clinical and radiographic assessments will not differ from standard of care.

Study participants will be followed at 6 weeks, 3 months, 6 months, 9 months and 12 months from their fracture (open and closed fracture cohorts) and from the date of consent (complication cohort), respectively. Final follow-up will be at 6 months to capture all infectious complications (Figure c.2)[35]. If a participant does not return to clinic, follow-up may be conducted by telephone. SSI and unplanned fracture-related reoperations will be identified at the time of diagnosis/occurrence and/or during each participant clinical assessment and medical record review that will occur during their routine outpatient clinic visits.

7 Statistical Plan

7.1 Sample Size Determination

The participating Statisticians on the study are members of the Norris Cotton Cancer Center and/or Department of Orthopaedics at Dartmouth-Hitchcock Medical Center. Sample size determination is based on the primary objective of the study as a pilot assessment of ICG fluorescence intensity of bone perfusion.

For patient cohort 1, we anticipate that approximately 10% of patients with open fracture will have an SSI within 12 months of their fracture[1, 2, 32]. A total sample size of 160 patients will result in an estimated 16 patients with an SSI and 144 patients without an SSI. We will have 86% statistical power to detect a mean maximum fluorescence intensity (I_{max}) difference of 24 (a.u.) between the SSI group and the non-SSI group with an estimated standard deviation of 30 (a.u.) and 15 (a.u.), respectively. All parameters were obtained from our previous porcine study. To be conservative, we increased the standard deviation by 50% for this power calculation. The type I error is set to be 5%. If we assume a drop-out rate of 10%, then a total of 176 patients will be enrolled.

For patient cohort 2, we anticipate approximately 30% of patients with an SSI will go on to develop a persistent SSI/treatment failure within 12 months of diagnosis of their SSI[12]. Based on this estimate, a sample size of 22 patients with SSIs and 52 patients without, will have 93% statistical power to detect a mean I_{max} difference of 24 (a. u.) between the persistent SSI/treatment failure group and the non-persistent SSI/non-treatment failure group with I_{max} estimated standard deviation of 30 (a. u.) and 15(a. u.). All parameters are obtained from our previous porcine study, which are consistent with a pilot study conducted at STC in human participants using ICG fluorescence for a different indication. To be conservative, we increased the standard deviation by 50% for this study. The type I error is set to be 5%. If we assume the dropout rate is10%, then a total of 82 patients will be recruited.

For patient cohort 2-1, the strength of the relationship between DCE-MR and DCE-FI measurements will

be assessed by determining the zero-order correlation and resulting R2 metric. A sample of 21 was estimated utilizing formula presented by Bonett & Wright [36] with a Type I error rate of 0.05 and Type II error

rate of 0.20 (1-Power) to estimate the magnitude of zero-order correlation and interval estimation. Formula inputs included expected zero-order relationship of r = 0.85 (95% CI 0.55 to 1.00). To optimize the imaging parameters of DCE-MRI, extra seven patients has been added into this cohort. Thus, total of 30 patients will be involved in this cohort.

Patient cohort 3 will serve as the control group for cohorts 1 and 2.

7.2 Data and Statistical Analysis

Patient cohort 1:

For the primary outcome, SSI is the dependent variable and bone and tissue perfusion, as measured by ICG fluorescence, is the independent variable. Unplanned fracture-related reoperation is the secondary dependent variable and bone and tissue perfusion as measured by ICG fluorescence, is the independent variable. Descriptive statistics, including means and standard deviations, will be presented with 95% confidence intervals. To select the most important ICG fluorescence kinetic curve-related variables, we will perform a bivariate analysis evaluating whether each variable predicts infection and we will select the variable with the highest individual area under the receiver operating curve (AUC ROC).

Our primary null hypothesis is that the post-debridement maximum ICG intensity ($I_{max\text{-}post}$) among patients with SSI is equal to or greater than that in the non-SSI patient group. The primary alternative hypothesis is that $I_{max\text{-}post}$ in the SSI group is smaller than that in the non-SSI group. We will then perform Students t-tests and chi-square analyses (depending on continuous or categorical variable status) to determine unadjusted associations between the most important ICG fluorescence variable and SSI. A p-value of 0.05 or less will be considered significant for differences between SSI and non-SSI groups. We will then apply multiple logistic model including ICG fluorescence and previously identified predictors for SSI[37-39]. Wald test will be used to determine if ICG fluorescence or any other predictors are significant in the model. We set the type I error at 5%. Table 7.1 shows a summary of the study outcomes, corresponding hypotheses, and method of analysis to be performed. We anticipate that we will demonstrate a statistically significant difference between the maximum post-debridement ICG fluorescence in patients with and without SSI

Table 7.1 Summary of Outcome Analysis Plan for patient cohort 1

Objective	Outcome		Hypothesis	Method of analysis
	Name	Type		
Determine the association between post-debridement bone perfusion as measured by quantitative ICG fluorescence and surgical site infection (SSI) in patients with open fracture	SSI	Binary	Mean perfusion will be lower among patients who develop SSI	Students t-test Multivariable logistic regression
	Unplanned fracture-related reoperation	Binary	Mean perfusion will be lower among patients who require unplanned fracture-related reoperation	Students t-test Multivariable logistic regression

Patient cohort 2:

For the primary outcome, treatment failure will be the dependent variable and bone and tissue perfusion, as measured by ICG fluorescence will be the independent variable. Descriptive statistics, including means and standard deviations, will be presented with 95% confidence intervals. To select the most important ICG fluorescence kinetic curve-related variables, we will perform a bivariate analysis evaluating whether each variable predicts infection and we will select the variable with the highest area under the curve (AUC).

Our primary null hypothesis is that the post-debridement maximum ICG intensity ($I_{max\text{-}post}$) among patients with persistent SSI/treatment failure is equal to or greater than that in the non-persistent SSI/non-treatment failure patient group. The primary alternative hypothesis is that $I_{max\text{-}post}$ in the persistent SSI/treatment failure

group is smaller than that in the non-persistent SSI/non-treatment failure group. We will then perform a Students t-test to determine the association between the most important ICG fluorescence variable and persistent SSI/treatment failure. A p-value of 0.05 or less will be considered significant for

differences between persistent SSI/treatment failure and non-persistent SSI/non-treatment failure groups.

We will then apply multiple logistic model including ICG fluorescence and previously identified predictors for persistent SSI/treatment failure[37-39]. Wald test will be used to determine if ICG fluorescence or any other predictors are significant in the model. We set the type I error at 5%. Table 7.2 shows a summary of the study outcome, corresponding hypotheses and method of analysis to be performed. We anticipate that we will demonstrate a statistically significant difference between the maximum post-debridement ICG fluorescence in patients who fail treatment for their SSI resulting in persistent SSI/treatment failure and those who do not.

For subset cohort 2-1, for each DCE-MR and DCE-FI parameter, statistics of median and percentiles will be calculated for each of ROIs. Due to the small cohort size, the correlations between DCE-MRI and DCE-FI will be utilized to analyze the data of each corresponding ROI, assuming that all ROIs in each subject were independent [40].

Table 7.2: Summary of Outcome Analysis Plan of patient cohort 2.

Objective	Outcome		Hypothesis	Method of analysis
	Name	Type		
Determine the association between post-debridement bone perfusion as measured by quantitative ICG fluorescence and persistent SSI/treatment failure in patientswith established SSI	Persistent SSI/Treatment failure	Binary	Mean perfusion will be lower among fracture patients with persistent SSI/treatment failure	Students t-test Logistic regression

Patient cohort 3: Classification into cohort categories (closed fracture, open fracture, complication) is the independent variable and ICG fluorescence magnitude is the dependent variable. Descriptive statistics, including means and standard deviations, will be presented with 95% confidence intervals.

7.3Subject Population(s) for Analysis

This study will involve consecutive patients 18 years of age or older who present to DHMC with an open fracture will be considered for the open fracture cohort (Cohort 1). Similarly fracture patients who present with an SSI following fracture fixation or joint fusion will be screened for the fracture SSI cohort (Cohort 2). Patients who present to DHMC with a closed fracture will be considered for the closed fracture cohort (Cohort 3). The image and clinical outcome data of all patients involved in this study will be analyzed.

8 Safety and Adverse Events

8.1 IRB Reporting Requirements for Adverse Events & Unanticipated Problems

Adverse events that are serious, unexpected, and possibly, probably or definitely related to participation in the research study will be reported to the Dartmouth Committee for the Protection of Human Subjects (CPHS) and to the sponsor. These events will be reported to the CPHS within 1 week. The sponsor will report these events to the FDA within 7 calendar days. All lethal events will be reported to the CPHS, Sponsor, and FDA.

Adverse events to be reported to the Sponsor are: any adverse experience, defined as any untoward or unfavorable medical occurrence in a human subject, including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's

participation in research, whether or not considered related to the subject's participation in the research), that is considered:

- Serious: Death; a life-threatening adverse drug experience; inpatient hospitalization or prolongation of existing hospitalization; a persistent or significant disability or incapacity; or a congenital anomaly or birth defect; and

- Unexpected: Any adverse experience, the specificity or severity of which is not consistent with the current investigator brochure or consent form; and
- Possibly related: There is a reasonable possibility that the incident, experience, or outcome may have been associated with the procedures involved in the research; and
- Is experienced by a participant in a trial open at a site subject to review by the CPHS.

An unanticipated problem involving risks to subjects or others is defined as any incident, experience, or outcome that meets each of the following criteria:

- Unanticipated in terms of nature, severity, or frequency given: (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and consent document; and (b) the characteristics of the subject population being studied; and
- Possibly related to participation in the research means there is a reasonable possibility that the incident, experience, or outcome may have been associated with research participation; and
- The problem suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, emotional, economic, legal, or social harms) than was previously known or recognized.

Copies of each report and documentation of IRB notification and receipt will be kept in the Clinical Investigator's study file.

Any unexpected or serious adverse events, or drug reaction beyond those expected from open sarcoma surgery that are determined likely to be related to the study procedures, will be reported to the FDA as required.

8.2 Institutional Safety and Data Monitoring

This study will be monitored by the Data Safety Monitoring and Accrual Committee (DSMAC). The Committee meets quarterly to review accrual rates and information for studies that have accrued participants. The Clinical Cancer Review Committee (CCRC) determines the frequency of DSMAC review. 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 CPHS (Dartmouth IRB) office.

9 Data Handling and Record Keeping

9.1 Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Risk of breach of confidentiality of the medical records and status of participants will be minimized. Databases which are used to store subject-sensitive information are password-protected and encrypted during file/data transfers from viewing terminals. Access will be limited to research team members who have undergone CPHS training at Dartmouth. Whenever possible and practical standard-of-care clinical data used in the research will be de-identified when under analysis.

9.2 Case Report Forms

Study case report forms (CRFs) will be the primary data collection instruments for the study. All data requested on CRFs will be recorded. Any missing data will be explained. If a space on the CRF is left blank because the procedure was not performed or the question was not asked, a written notation will be made. If an item is not applicable to an individual case, written notation will be made. Changes to the CRFs will be initialed and dated.

9.3 Record Retention

Following closure of the study, the investigator will maintain all site study records in a safe and secure location. The records are maintained to allow easy and timely retrieval when needed (e.g., audit or inspection) and, whenever feasible, to allow any subsequent review of data in conjunction with assessment

of the facility, supporting systems, and staff. Upon completion of study analysis, research information is stored in Dartmouth College Records Management off-site storage located at 6218 Etna Road, Hanover,

NH 03755. Documents are shredded on site after 50 years of storage.

10 Clinical Site Monitoring

10.1 On-Site Monitoring

Clinical research monitoring for regulatory compliance and data integrity will be conducted. Internal monitoring is conducted by appropriately trained staff of the 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, 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.

10.2 Auditing and Inspecting

The study investigators will permit study-related monitoring, audits, and inspections by the IRB, the sponsor, government regulatory bodies, and university compliance and quality assurance groups of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data, etc.). The study investigators will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.).

Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable university 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.

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. See Attachment for a copy of the Subject Informed Consent Form. This consent form will be submitted with the protocol for review and approval by the IRB. The formal consent of a subject, using the IRB-approved consent form, will be obtained before that subject undergoes any study procedure. The consent form will be signed by the subject or legally acceptable surrogate, and the investigator-designated research professional obtaining the consent.

12 Study Finances

12.1 Funding Source

This study is being funded by the Orthopaedics Department at Dartmouth Hitchcock Medical Center.

12.2 Conflict of Interest

All Dartmouth investigators will follow the Dartmouth conflict of interest policy, and will have disclosed potential conflicts-of-interest related to the study

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