PROTOCOL TITLE: *ICG Fluorescence Imaging in Lower Extremity Amputation Patients*

D-HH IRB OVERSIGHT:

One of the following must be true in order to submit to the D-HH IRB. Please check all that apply:

☒ The Principal Investigator is employed by D-H
☒ The study will utilize any D-H data or specimens
☒ The study will enroll D-H patients or recruit from D-H sites
☒ The study will utilize any D-H resources, e.g. study procedures will occur at D-H locations and/or use of D-H equipment or shared resources

PROTOCOL TITLE:

ICG Fluorescence Imaging in Lower Extremity Amputation Patients

PRINCIPAL INVESTIGATOR:

Ida Leah Gitajn
Department of Orthopaedics
603-650-7484
Ida.Leah.Gitajn@hitchcock.org

VERSION NUMBER/DATE:

Version 5, August 18, 2022

REVISION HISTORY

Revision #	Version Date	Summary of Changes	Consent Change?
4	12/11/2020	Included consenting of Spanish Speaking Subject Changes make to recruitment, vulnerable populations Requesting a partial waiver of HIPAA for recruitment purposes	Yes
5	08/18/2022	Adding SPY PHI and drug receipt change	Yes

PROTOCOL TITLE: *ICG Fluorescence Imaging in Lower Extremity Amputation Patients*

Table of Contents

1.0 Study Summary

Study Title	ICG fluoresce imaging in lower extremity amputation patients
Study Design	Prospective nonrandomized trial
Primary Objective	Evaluate changes to bone perfusion in association with osteotomy and soft tissue stripping in patients undergoing lower extremity amputation
Secondary Objective(s)	None
Research Intervention(s)/ Investigational Agent(s)	ICG contrast infusion and ICG-based fluorescence imaging
IND/IDE #	NA
Study Population	Patients undergoing lower extremity amputation
Sample Size	30 patients
Study Duration for individual participants	During surgical procedure only
Study Specific Abbreviations/ Definitions	AE: adverse event/adverse experience BWH: Brigham and Women's Hospital CT: computed tomography CRF: case report forms DCE-FI: dynamic contrast enhanced fluorescence imaging DHMC: Dartmouth-Hitchcock Medical Center GDP: Good Clinical Practice ICG: indocyanine green Imx: maximum ICG fluorescence intensity LAR: legally authorized representative SAE: serious adverse event/serious adverse experience SOP: standard operating procedure

2.0 Objectives*

Specific Aim 1: Identify bone perfusion kinetics at baseline and following osteotomy and subsequent soft tissue stripping in patients undergoing scheduled lower extremity amputation.

Hypothesis 1—Human bone perfusion kinetics will demonstrate predictable and reproducible changes when undergoing vascular compromise, osteotomy, and soft tissue stripping.

Specific Aim 2: Optimize image data processing and identify ICG-based DCE-FI kinetic curve properties most closely associated with low bone perfusion.

Hypothesis 2—Low post-debridement bone perfusion is associated with higher rates of infection.

3.0 Background*

Infection following high-energy trauma or blast injury is one of the most challenging complications facing musculoskeletal trauma patients, with an estimated annual cost of $35 billion in the US (https://stacks.cdc.gov/view/cdc/11550). Infection can be catastrophic, potentially causing prolonged morbidity, reduced function, permanent disability and amputation. Failed treatment results in chronic bone infection requiring repeat surgical procedures in approximately 30% of patients. [1-4]

Blood supply plays a critical role in the bone health for trauma patients by delivering necessary oxygen, nutrients, antibiotics, and immune cells necessary to treat infection successfully. [5-9]. The management of trauma-related infection is therefore based on **aggressive, thorough debridement** of all poorly perfused bone and connective tissue, followed by fracture stabilization to prevent further injury. More extensive debridement is believed to improve treatment success; however, additional tissue loss requires increasingly complex reconstructive procedures to fill bone and connective tissue voids. **At present there are no objective, intraoperative, and real-time techniques to assess bone perfusion and thoroughness of debridement.** This unmet clinical need results in substantial practice variation and places patients at unnecessarily high risk for chronic infection and potentially avoidable surgical procedures.

Our multidisciplinary research team has demonstrated that quantitative indocyanine green (ICG)-based Dynamic Contrast Enhanced Fluorescence Imaging (DCE-FI) can assess bone perfusion in a measurable, reproducible and predictable way using a porcine model.[10] Furthermore, we have initiated an Institutional Review Board (IRB)-approved protocol to perform similar imaging in human trauma patients with good success. To this end, the primary objective of this project is to gather critical pilot data that will accelerate translation of ICG-based DCE-FI to clinical practice for human trauma patients. During this translational work we will systematically develop and evaluate an ICG-based DCE-FI methodology and analytic tools for intraoperative assessment of bone perfusion. Our long-term goal is to apply these sophisticated methods to guide surgical debridement that will enhance trauma patients' short- and long-term recovery.

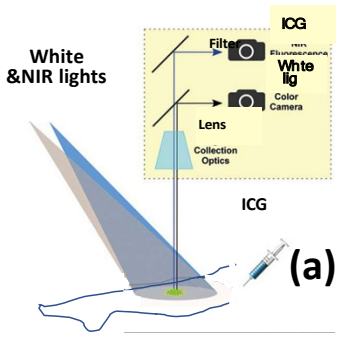
4.0 Study Endpoints*

Primary outcome measure is ICG-based DCE-FI changes that occur as a result of osteotomy and soft tissue stripping associated with the amputation.

5.0 Study Intervention/Investigational Agent

5.1 Description:

ICG fluorescence imaging has been used to assess tissue perfusion in vivo in real-time in arterial and lymphatic perfusion imaging[25, 26], osseous flap perfusion imaging[27]. ICG has been approved for clinical use by the FDA since 1959. The dynamic fluorescence imaging systems used in this study will be the Pentero surgical microscope (Zeiss, Germany), SPY Elite (Novodaq/Stryker), or SPY PHI (Novodaq/Stryker). Both are 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.



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 (Pentero, SPY Elite, or SPY PHI) 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 fluorecent dy 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.

This study is similar to STUDY00031575 in terms of methodology with a nearly identical intervention. That study is approved and active, but in a different patient population, both of which are highly clinically relevant.

5.2 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.

5.3 Receipt of Drug Supplies

Indocyanine green (ICG)/drape kits used in this study are purchased from Stryker. Each kit includes 25mg ICG, 10cc saline and a sterile drape. Kits will be delivered to DHMC Investigational Drug Services and to each relying site when needed who will count and verify that the shipment contains all of the items noted in the shipment inventory. Any damaged kits will be documented in study files and destroyed on site. This is standard of care for ICG/drape kits used clinically in non-research indications.

5.4 Storage

ICG will be stored in a locked temperature controlled room, as is standard of care for ICG/drape kits used clinically in non-research indications.

5.5 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.

5.6 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 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.

6.0 Procedures Involved*

This is a prospective non-randomized clinical trial which will assess bone perfusion using

ICG fluorescence imaging at several step in the surgical procedure to assess the effect of these steps on bone perfusion. This is neither a drug nor a device trial. Patients will be administered FDA approved ICG through intravenous injection and imaged using an FDA approved surgical microscope (Pentero,SPY Elite, or SPY PHI).

6.1 Patient selection

Consecutive patients 18 years of age or older who present to DHMC and are scheduled to undergo a lower extremity amputation (either below knee, through knee or above knee) will be considered for study enrollment.

6.2 Inclusion Criteria

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

a. Patients 18 years of age or older
b. Scheduled for a lower extremity amputation (either below knee, through knee or above knee)
c. Provision of informed consent

6.3 Exclusion Criteria

The following criteria would exclude a subject from study enrollment

a. Iodine allergy
b. Pregnant or breastfeeding women

6.4 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. 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. Potential subjects will be approached by a member of their care team to gauge interest about participation in research prior to being approached by a member of the study team. 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 at no cost to confirm pregnancy status before administration of ICG.

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. 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 and 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.

A legally authorized representative (LAR) with reasonable knowledge of the potential participant will be approached to consent on the patient's behalf if the patient cannot adequately answer at least 2 questions and it is determined that the patient's level of cognition is not likely to change before surgery.

The choice of LAR will follow standard procedures. The following with be approached in this order of priority:

- Legal guardian
- Proxy (health care agent) named in an advance directive or durable power of attorney for health care
- Family member or other surrogate identified by the state law on health care decisions. Guidance will be provided to assist the LAR in making the consent decision. They will 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. If the LAR does not know what the participant would have wanted, the LAR will advised to base the decision with the participant's best interest in mind. They will be asked to carefully consider how much leeway the participant would likely give the LAR in making the choice about participation in the study.

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.

6.5 Early Withdrawal of Subjects

Participants will be removed from the protocol if:

1) Study imaging is not completed for any reason. The subject withdraws consent.
2) The subject has an occurrence of a significant clinical event that precludes imaging.
3) 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.

6.6 Study drug and device

6.6a 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 (Pentero,SPY Elite, or SPY PHI) 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.

6.6.b Preparation and Administration of Study Drug

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

6.6.c 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.

6.7 Study Procedures

On 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.

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 of the tibia or femur 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. Osteotomy or bone cut will be performed and then 0.1 mg/kg ICG will be injected intravenously and, again, video rate ICG fluorescence images will be acquired 20 seconds before and 4 minutes after ICG injection. Soft tissue stripping from tibia or femur will then be performed, per standard surgical procedure. Then a third dose of 0.1mg/kg ICG will be injected 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.

Surgical plan will not be affected as a result of the intraoperative fluorescence imaging. Surgical care team will be blinded to the imaging results in the operating room. The

imaging screen will be either be covered or hidden from view of the surgical team. The operator of the imagine device may visualize the imaging screen to ensure integrity of imaging data collection but this person will not communicate the results to the surgical team.

There is no follow-up associated with this study.

7.0 Data and Specimen Banking*

Not applicable

8.0 Sharing of Results with Subjects*

This study is not intended to provide any results to the subjects because this is the investigational study and the subjects will be under standard practice for their condition and no diagnostic or therapeutic decisions will occur based on the study.

9.0 Study Timelines*

This study is a one-time imaging during the subject's surgery. The duration of each subject enrolled in the study is from the date the subject signs the consent to the day that the surgery is carried out.

The duration to enroll all study subjects is Jan.2, 2020 – Dec.31, 2021.

The estimated date for the investigators to complete this study is Jan. 31, 2022

10.0 Inclusion and Exclusion Criteria*

Inclusion Criteria

a. Patients 18 years of age or older
b. Scheduled for a lower extremity amputation (either below knee, through knee or above knee)
c. Provision of informed consent

Exclusion Criteria

a. Iodine allergy
b. Pregnant women
c. Patients under the age of 18.
d. Prisoners
e. Pregnant of nursing mothers

Vulnerable Populations*

After the consent has been reviewed with a potential subject, the subject will be asked to tell the research team member two things about the research they recall. If they are not able to do so the subject will be considered incompetent to consent to the research trial. Patients found to be in capable of providing informed consent will be deemed by the research staff to have impaired decision making and therefore will only be consented if a LAR agrees to their participation and signs the consent form.

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

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 be obtained from all patients that are able to sign their name. If patients are injured so severely that they are not able to sign their name verbal assent will be obtained. Assent will not be obtained from patients whom are unconscious due to the nature of their injury.

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

11.0 Local Number of Subjects

The primary outcome measure is total bone blood flow (TBBF). Informed by data collected from the porcine study, a total sample size of 30 trans-tibial amputation patients is required to determine the difference in TBBF baseline bone perfusion kinetics and change in the bone perfusion kinetics associated with osteotomy and subsequent soft tissue stripping. Considering that the controlled environment associate with the porcine model may not be replicated to the same degree in the amputation sample, a more conservative difference of 0.84 standard deviations between baseline and osteotomy and soft tissue stripping was selected (i.e., 50% of the effect size identified in the porcine model). Assuming a one tailed test, a Type I error rate of 0.05, and a Type II error rate of 0.80, a sample of 20 was determined with an additional 10 amputation patients recruited in the event that there is any protocol- or data-related issues. Data analysis will be performed after half of the patients are enrolled to re-calculate sample size needed to determine a difference.

We anticipate that 20 subjects will be accrued locally. We anticipate that 20 patients will be enrolled at Brigham and Women's Hospital. A total of 40 patients will be enrolled.

12.0 Recruitment Methods

Patients, 18 years of age or older who present to DHMC who are scheduled to undergo a lower extremity amputation (either below knee, through knee or above knee) will be considered for study enrollment. 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. 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.

A Potential subjects consenting to undergo a surgical procedure, will be informed by their surgeon or another member of their Orthopaedic care team that there is a research study for which they might be interested in participating. Following this discussion, either at the same visit or a subsequent visit the patient will be approached by a member of the research study team who will present the study and inquire if the patient would like to participate. 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. Following consent, inclusion and

exclusion criteria will be reviewed for all patients. Patient who meet the appropriate eligibility criteria will be enrolled in the study.

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 at no cost to confirm pregnancy status before administration of ICG.

13.0 Withdrawal of Subjects*

Participants will be removed from the protocol if:

1) Study imaging is not completed for any reason.
2) The subject withdraws consent.
3) The subject has an occurrence of a significant clinical event that precludes imaging.
4) 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.

14.0 Risks to Subjects*

Risk of ICG injection: ICG (Indocyanine Green) will be administered intravenously three times: once after exposure, once after osteotomy and once after soft tissue stripping. 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 on request at no cost.

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.

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).

15.0 Potential Benefits to Subjects*

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 amputation.

16.0 Data Management* and Confidentiality

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.

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.

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.

17.0 Provisions to Monitor the Data to Ensure the Safety of Subjects*

This is a study with minimal risk

18.0 Provisions to Protect the Privacy Interests of Subjects

The information collected for this study will be used only for the purposes of research. It includes optical images, clinical images (i.e., CT, MRI), and surgical outcomes. Information on your gender, age, diagnosis, etc., will be collected in order to characterize some basic demographics of the subject population enrolled in the study. The data collected for this will be maintained indefinitely.

The identities of the people in this study will be carefully protected. Specifically, the subjects' identity is coded into a study number and most of the data and analysis occurs with information that is archived only by study number. Information which identifies of the participants in the study is maintained in binders as required by the FDA, and these

binders will be kept in the office of research team. The office is kept locked when not in use, and the study team is trained in the importance of maintaining your confidentiality. Research data, if sent electronically to members of the research team, is de-identified and only secure file transfer methods are used.

The health information of the subjects can be only used by the research team member who is involved in the research. The research team includes the study director plus others working on this study at Dartmouth-Hitchcock Medical Center and elsewhere

The information collected for this study may be used by researchers or officials of the following institutions.

- Dartmouth College
- Mary Hitchcock Memorial Hospital
- Dartmouth-Hitchcock Clinic
- Dartmouth-Hitchcock Medical Center

Identifiable data collected for this study will be used for research purposes which are determined to be reasonable and in line with expectations by a review committee. Once data collected for this research study is no longer identifiable, the data may be used or disclosed for other purposes.

19.0 Compensation for Research-Related Injury

This is an imaging study with minimal risk. We do not anticipate any injury or sickness happen due to the study. If in any case subjects are injured or become ill as a result of research procedures, the subjects will be provided with medical treatment, but the following organizations do not plan to pay for this treatment.

- Mary Hitchcock Memorial Hospital
- Dartmouth-Hitchcock Clinic
- Dartmouth-Hitchcock Medical Center
- Trustees of Dartmouth College

20.0 Economic Burden to Subjects

Subjects will not be charged for any procedures conducted solely for research purposes, nor will they be charged for the SPY Elite® System (nor Pentero or SPY PHI) assessment.

Insurance plans will not be billed for research procedures that are not the usual care for subject's condition. Some of the medical care that the subjects will receive during this

study is the usual care a doctor would recommend for subjects' condition. Subjects or subjects' insurance plan are expected to pay for the costs of this usual medical care.

21.0 Consent Process

To obtain informed consent, study personnel will adhere to the following procedures: (1) present study information in a manner that is understandable to the patient. a Spanish IRB approved translated copy of the consent will be used when appropriate (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 and that their participation is voluntary; and (5) complete the consent process and obtain signatures from the patient and research team. The process of obtaining and documenting informed consent will be completed in accordance with Good Clinical Practice. 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

Non-English Speaking Subjects

This study will consent patients for whom English is their second language. This will pertain to only individuals whose native language is Spanish. Prospective subjects who present for traumatic amputation surgery will be consented with the assistance of a Spanish speaking representative from DHMC Interpreter Services. Spanish speaking individuals will be given the Informed consent that has been translated to Spanish to review and discuss via interpreter. Subjects willing to participate will sign and date the Spanish version of the consent and be given a copy for their records. The interpreter will sign the Spanish consent as a witness to the process. Prospective subjects who present for traumatic amputation surgery will be

22.0 Process to Document Consent in Writing

The consent form documentation process will follow SOP (HRP-091). This is an imaging study with minimal risk. The signed consent form will be uploaded to DHMC EDH.

Both the English version and the Spanish version of the consent form will be submitted for approval.

23.0 Setting

All consenting and imaging will be carried out in the orthopedic department or surgical unit in DHMC. The imaging processing will be carried out in Thayer biomedical imaging Lab located in Williamson 7F.

24.0 Resources Available

The Department of Orthopaedic Surgery at DHMC, chaired by David Jevsevar, MD, includes the Section of Orthopedics, Section of Physical Medicine and Rehabilitation, the Spine Center, and the Center for Shared Decision Making. Clinical activity consisted of approximately 55,000 ambulatory visits and 5,500 operative cases in 2014 with $215 million total revenue. Each new patient and surgical patient is tracked for appropriate care and patient reported outcomes (PROs). The effort led to a $24 million award from the Centers for Medicare and Medicaid Services (CMS) to create the High Value Health Care (HVHC) Innovation Center, to implement shared-decision-making and PROs in 19 major medical centers across the US. DOS is distinguished nationally for research through the Spine Patient Outcomes Research Trial (SPORT) – the largest orthopedic surgery clinical trial ever funded by the NIH with over $30M from NIAMS since inception in 2001.

Dr. Gitajn is the principle investigator for the study. Dr. Gitajn has multiple NIH and DoD funded projects. She is actively engaged in research in orthopaedic trauma surgery and clinical translation of early-stage medical devices and imaging systems.

The Dynamic Surgical-Guidance Laboratory at Dartmouth-Hitchcock is directed by Assistant Professor Jonathan Elliott and is connected to Dartmouth-Hitchcock Medical Center, Norris Cotton Cancer Center, Center for Comparative Medicine and Research, Rubin Research Building and the state-of-the-art Williamson Translational Research Building by a shared hallway. Dr. Elliott leads a team of researchers including two postdocs, two graduate students, and a dedicated research engineer and is responsible for an annual research budget of over $1 million.

All study members, including clinicians, faculty members, staff and students, will be approved for this protocol before engaging in any activities related to this project, and will therefore be required to complete CITI training and be cleared by the Department of Orthopaedics before physically attending to any of the study procedures.

25.0 Multi-Site Research*

This study will be conducted at two clinical sites,

- Dartmouth-Hitchcock Medical Center
- Brigham and Women's Hospital

We anticipate that 20 subjects will be accrued locally. We anticipate that 20 patients will be enrolled at Brigham and Women's Hospital. A total of 40 patients will be enrolled.

This distribution of patients is subject to change during monitoring of rate enrollment at each clinical site.

All data will be communicated in a HIPPA-compliant manner using a HIPPA-compliant database for clinical data (REDCap) and a HIPPA-compliant storage drive for imaging data. Clinical and imaging data will be uploaded to the appropriate HIPPA-compliant site by the local research team at each institution and all data will be analyzed at either Dartmouth-Hitchcock Medical Center or Dartmouth College/Thayer School of Engineering. De-identified patient information using a Study ID number will be utilized when possible.