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Universitätsklinik für
Plastische- und Handchirurgie
Co-Direktoren und Chefärzte:
Prof. Dr. med. Mihai A. Constantinescu
Prof. Dr. med. Esther Vögelin

Clinical Study Protocol

A diagnostic sensitivity study to analyse the ability of intradermally administered indocyanine green (ICG) and near infrared fluorescence imaging (NIRFI) to transcutaneously identify sentinel lymph nodes (SLNs) in malignant melanoma and Merkel cell carcinoma - after intradermally administered injection of technetium 99m and traditional lymphoscintigraphy (LS) - a clinical, prospective, single-arm, single-blind, single-centre Phase II study

Short Title:

Lymph node **I**dentification in skin **M**alignancy using **I**ndocyanine green **T**ranscutaneously (**LIMIT**) Study

Study Type:	Clinical trial with Investigational Medicinal Product (IMP), Medical Device (MD)
Study Categorisation:	Risk category A
Study Registration:	ClinicalTrials.gov, Swiss National Clinical Trials Portal (SNCTP) (intended)
Study Identifier:	Not applicable
Sponsor:	Prof. Dr. med. Mihai Constantinescu Department of Plastic and Reconstructive Surgery Inselspital, University Hospital of Bern, University of Bern Freiburgstrasse 10 3010 Bern Switzerland
Investigational Product:	V3 HD3D Infrared fluorescence camera (Visionsense Iridium system)
Protocol Version and Date:	V6.0, 09.12.2019

Universitätsklinik für
Plastische- und Handchirurgie
Co-Direktoren und Chefärzte:
Prof. Dr. med. Mihai A. Constantinescu
Prof. Dr. med. Esther Vögelin

Signature Page(s)

Study number	ClinicalTrials.gov, Swiss National Clinical Trials Portal (SNCTP) (intended)
Study Title	A diagnostic sensitivity study to compare intradermally administered indocyanine green (ICG) and near infrared fluorescence imaging (NIRFI) with intradermally administered technetium 99m and traditional lymphoscintigraphy (LS) in the trans-cutaneously identification of sentinel lymph nodes (SLN) in malignant melanoma – a clinical, prospective, single-arm, single-blind, single-centre Phase II study

The sponsor and principal investigator have approved the protocol version 6, 09.12.2019, and confirm hereby to conduct the study according to the protocol, current version of the World Medical Association Declaration of Helsinki, ICH-GCP guidelines or ISO 14155 norm if applicable and the local legally applicable requirements.

Sponsor:
Prof. Dr. med Mihai Constantinescu

Bern, 09.12.2019 _____
Place/Date Signature

Principal Investigator:
Dr. med. Radu Olariu

Bern, 09.12.2019 _____
Place/Date Signature

Local Principal Investigator at study site:
I have read and understood this trial protocol and agree to conduct the trial as set out in this study protocol, the current version of the World Medical Association Declaration of Helsinki, ICH-GCP guidelines or ISO 14155 norm if applicable and the local legally applicable requirements.

Site:	Department of Plastic and Reconstructive Surgery Inselspital, University Hospital of Bern, University of Bern Freiburgstrasse 10 3010 Bern Switzerland
Principal investigator:	Dr. med. Radu Olariu

Bern, 09.12.2019

Place/Date

Signature

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STUDY SYNOPSIS

Sponsor / Principal Investigator	Sponsor: Prof. Dr. med. Mihai Constantinescu Principal Investigator: Dr. med. Radu Olariu
Study titel:	A diagnostic sensitivity study for the analysis of the ability of intradermal indocyanine green (ICG) and near infrared fluorescence imaging (NIRFI) to transcutaneously identify sentinel lymph nodes (SLNs) in malignant melanoma and Merkel cell carcinoma after intradermal injection of technetium 99m and traditional lymphoscintigraphy (LS) - a clinical, prospective, single-arm, single-blind, single-centre Phase II study
Short titel / Study-ID:	The ability of indocyanine green near infrared fluorescence imaging (ICG - NIRFI) to identify LS-positive SLN under the skin.
Protocol-Version and Date:	V 6.0, 09.12.2019
Study registration:	Clinicaltrials.gov und SNCTP
Study category and rationale:	Category A: Medical devices and diagnostic sensitivity study. The primary objective is to evaluate a new technique in transcutaneous SLN identification against the current gold standard.
Klinische Phase:	Phase II

Background and rationale:	Switzerland has the highest rate of new melanomas in Europe (19.2 per 100,000). Melanomas have the worst prognosis of all skin cancers. The current treatment depends on the histological diagnosis after a biopsy and is primarily related to the tumor thickness (Breslow Score), the tumor cells in division (mitosis rate), the substance defect of the skin (ulceration), the occurrence of regression, and the age of the patients. The initial treatment is performed by surgical removal with a safety margin of macroscopically healthy skin around the tumor. If the tumor thickness is more than 1 mm or more than 0.7 mm associated with a high mitosis rate in younger patients, ulcerations, regression or Clark Level IV / V, then current melanoma guidelines suggest that the patient undergoes sentinel lymph node biopsy (SLNB) as this is most likely the first site where metastases spread. Merkel cell carcinoma is a very aggressive, neuroendocrine skin tumor with a mortality rate of about 33% after 3 years. Due to the frequent lymphatic metastases, SLNB is highly recommended in all patients in order to better assess their prognosis. The gold standard technique to identify SLNs is to inject the radioisotope Technetium-99m around the primary tumor into the skin. The patient is then scanned to determine the position of the SLN after approximately 30 and 120 minutes. Other teams have attempted to identify transcutaneous SLN with ICG and NIRFI, but have concluded that ICG fluorescence technique is not reliable in patients with high BMI or a primary tumor with lymph drainage in the axillary lymph node region. This study aims to evaluate a medical device that uses an improved technology compared to previous studies (stereoscopic 3D high definition for both fluorescence and visible light imaging). Our hope is that by applying similar principles SLNs can be identified through the use of transcutaneous fluorescent dye injections and NIRFI.
Aim:	To investigate whether SLNs in melanomas and Merkel cell carcinomas can be identified transcutaneously by near infrared fluorescence imaging (NIRFI) as well as by gold standard (in this case LS).
Result:	That the SLN identified by NIRFI is the same as that identified by lymphoscintigraphy.
Study design:	A clinical, prospective, single-arm, single-blind, monocentric, diagnostic sensitivity study.

Inclusion and exclusion criteria:	Inclusion criteria: - Patient has signed the consent form - Patients who have tumors with any of the following characteristics: - Malignant melanoma a) Breslow score ≥ 1 mm b) Breslow score ≥ 0.7 mm with ulceration c) Breslow score ≥ 0.7 mm with regression d) Breslow score ≥ 0.7 mm with Clark Level IV/V e) Breslow score ≥ 0.7 mm with mitosis-rate ≥ 1/mm2 in young patients - Merkel cell carcinoma Exclusion criteria: - Age < 18 years - Pregnancy and lactation (perform pregnancy test on women of fertile age [defined as not sterilized, hysterectomized and/or more than 12 months postmenopausal]). - Allergy to ICG and iodine - previous chemotherapy, radiation or surgery of the lymph nodes to be examined - the patient is not able to give his consent to the study - simultaneous participation in other intervention studies

Measurements and procedures:	The aim of the study is to evaluate whether the V3 Visionsense 3D HD camera is capable of detecting transcutaneous SLNs. The treatment will be standard and will only differ with two protocol changes. Patients will not be informed of the location of the SLN identified by the LS. After the intradermal ICG injection has been administered to the patient under general anesthesia, the patient is examined according to protocol with the VisionSense camera, depending on the location of the primary tumor: - <u>Tumor of head and neck:</u> - The scan is performed from the primary tumor via the ipsilateral lymphatic tract to identify the preauricular, retroauricular, anterior cervical LNs (Zone I - IV) and posterior cervical LNs (Zone V) - The same procedure is used on the contralateral side - T<u>umor of the trunk:</u> - The scan is performed from the primary tumor in the direction of the ipsilateral armpit and through the follow ipsilateral inguinal region - The same procedure is used on the contralateral side - <u>Tumor of the upper extremity:</u> - The scan is performed from the primary tumor in the direction of the ipsilateral armpit - There is no need to scan other LN regions - <u>Tumor of the lower extremity:</u> - The scan is performed from the primary tumor in the direction of the ipsilateral inguinal region - If the tumor is located below or distally to the knee, the LN in the hollow of the knee will also be scanned - If the primary LN is above or proximal of the knee, the LN in the hollow of the knee won't be scanned

	The surgeon will then be unblinded with regard to the result of the LS and begin the operation. The surgeon will ONLY perform an SLK biopsy on the LNs identified by the LS. All LNs identified by the VisionSense 3D HD Camera and do not agree with the results of the LS are not biopsied. The surgery will be performed according to the standard surgical procedure with a combination of gamma probe navigation and fluorescence imaging.
Study product / intervention:	V3 HD3D VisionSense NIRFI Camera: This is used for about 10-15 minutes to scan the patient preoperatively. The LS is used as a control examination to compare the result of the camera.
Control intervention (if necessary):	Tc 99m based lymphoscintigraphy
Number of study participants and rationals:	After calculating the power analysis, 93 patients are required. With this sample size we would have a width of 10% at a sensitivity of 60%. This is done under the assumption that each patient will have at least one SLN, but often more, so that the power of the study is increased at the end.
Duration of study:	The duration of the study will be about 27 months. In the last four years, our clinic has performed an average of 84 (SD 5.4) SLNBs per year.
Study schedule:	Start: September 2017 (planned) End: June 2020 (planned)
Investigator(s):	Prof. Dr. med. Mihai Constantinescu, Director (Sponsor) Dr. med. Radu Olariu (Principal Investigator) Clinic for Plastic and Reconstructive Surgery Inselspital, University Hospital Bern, University Bern Freiburgstrasse 10 3010 Bern Switzerland
Study center:	Clinic for Plastic and Reconstructive Surgery Inselspital, University Hospital Bern, University Bern Freiburgstrasse 10 3010 Bern Switzerland
Statistical calculation:	The required sample size was determined with the Bruderer`s formula for sensitivity 60% (93 lymph nodes). The data set to be analyzed includes the identification of the location of the SLK using the camera and the identification using the gold standard (LS).

GCP Declaration:	This study is conducted in accordance with the Protocol, the current version of the Declaration of Helsinki, the ICH-GCP or ISO EN 141558 (where applicable) and all national legal and regulatory requirements.

Investigated disease, health status

Black skin cancer and its metastasis in lymph nodes.

Summary of the study

Switzerland has one of the highest skin cancer rates in Europe. The most malignant form of skin cancer is called black skin cancer or malignant melanoma. This type of skin cancer can spread to other parts of the body, can be very aggressive and is particularly difficult to treat in advanced stages. One of the most important factors in the treatment of black skin cancer is to detect the early spread of the cancer.

Malignant melanoma spreads through the lymph vessels in the lymph nodes of certain areas of the body. Typically the neck, armpits or groin are affected. The first lymph node to which the cancer spreads is called the sentinel lymph node. It is important to identify it so that a biopsy can be taken and examined under the microscope. The examination will show whether the cancer has spread or not. Currently, the best method to find this lymph node is to inject a radioactive dye into the skin around the tumour. This dye is transported via the lymph vessels into the sentinel lymph nodes. In order to see where the lymph node is located in the body, several special scintigraphic images are required after 30 minutes and 2 hours after the injection.
Although the procedure is important for the success of the treatment, each patient is exposed to radiation. A new type of dye has been successfully used in other cancers to find the sentinel lymph node. It is the fluorescent dye indocyanine green (ICG), which can be seen with special cameras without exposing the patient to radiation.

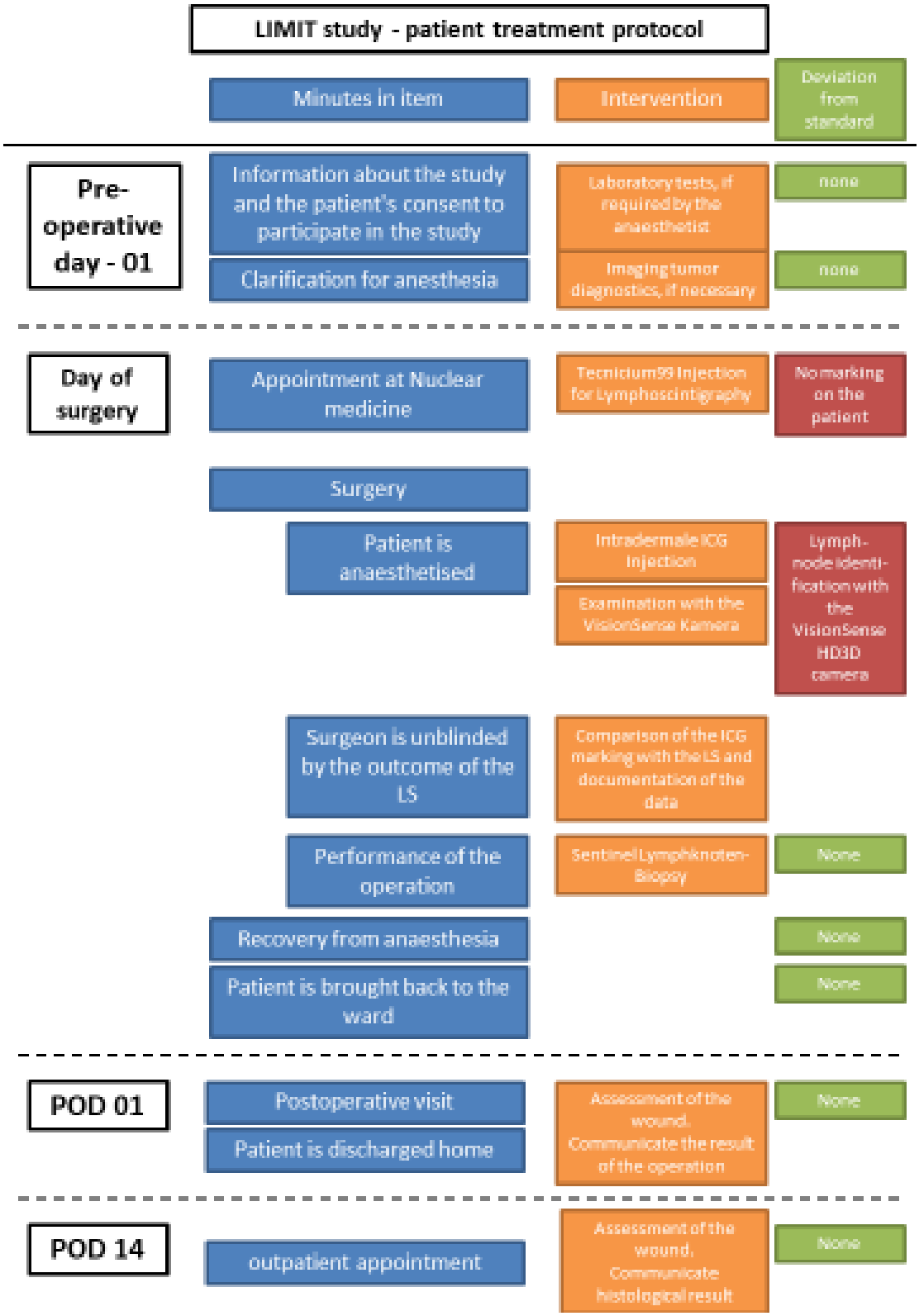
This fluorescent dye (ICG) is also injected around the skin where the cancer is located shortly before the operation. The ICG, like the radioactive dye, is transported through the lymph vessels and stains the sentinel lymph node. The surgeon can then transcutaneously identify the stained lymph node using the V3 HD3D VisionSense NIRFI camera.

The aim of this study is to find out whether fluorescent dye injections can replace radioactive injections. This would reduce the unnecessary exposure of patients to radioactive materials that can be harmful.

ABRAVIATIONS

AE	Adverse Event
BMI	Body Mass Index
CA	Competent Authority (e.g. Swissmedic)
CEC	Competent Ethics Committee
CI	Confidence Interval
CRF	Case Report Form
CTU	Clinical Trials Unit
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture
GCP	Good Clinical Practice
HD	High Definition
HFG	Humanforschungsgesetz (Law on human research)
HMG	Heilmittelgesetz
IB	Investigator's Brochure
ICG	Indocyanine green
ICH	International Conference on Harmonisation
IMP	Investigational Medicinal Product
IR	Infrared
ISO	International Organisation for Standardisation
KlinV	Verordnung über klinische Versuche in der Humanforschung *(in English: ClinO, in French OClin)*
LR	Likelihood Ratio
LRH	Loi fédérale relative à la recherche sur l'être humain
LN	Lymph node
LK	Lymphknoten
LS	Lymphoscintigraphy
MD	Medical Device
NIH	National Institute of Health
NIRFI	Near Infrared Fluorescence Imaging
NPV	Negative Predictive Value
OClin	Ordonnance sur les essais cliniques dans le cadre de la recherche sur l'être humain *(in German : KlinV, in English : ClinO)*
OR	Odds Ratio
PI	Principal Investigator
PPV	Positive Predictive Value
SLK	Sentinellymphknoten
SLKB	Sentinellymphknotenbiopsie
SLN	Sentinel Lymph Node
SLNB	Sentinel Lymph Node Biopsy
SNCTP	Swiss National Clinical Trials Portal
SOP	Standard Operating Procedure

Study flow chart



1. STUDY ADMINISTRATIVE STRUCTURE

1.1 Sponsor

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1.4 Statistician ("Biostatistician")

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1.5 Laboratory
Not applicable.

1.6 Monitoring institution
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1.7 Data Safety Monitoring Committee
Not applicable.

1.8 Any other relevant Committee, Person, Organisation, Institution
Not applicable.

2. ETHICAL AND REGULATORY ASPECTS

Before the study will be conducted, the protocol, the proposed patient information and consent form as well as other study-specific documents shall be submitted to a properly constituted Competent Ethics Committee (CEC) in agreement with local legal requirements, for formal approval. Any amendment to the protocol must as well be approved (if legally required) by this institution.
The decision of the CEC concerning the conduct of the study will be made in writing to the sponsor before commencement of this study. The clinical study can only begin once approval from all required authorities has been received. Any additional requirements imposed by the authorities shall be implemented.

2.1 Study registration
The study will be registered in the Clinical Trials Registry Platform of the National Institute of Health (NIH) – ClinicalTrials.gov. In addition, the trial will be registered in the Swiss National Clinical Trials Portal (SNCTP).

2.2 Categorisation of study
Category A: Clinical trial with a medical device that bears a conformity marking, and is used in accordance with the instructions for use.

2.3 Competent Ethics Committee (CEC)

The responsible investigator will ensure that approval from the Competent Ethics Committee (CEC) of the Kanton Bern, Switzerland is sought for the clinical study.
If immediate safety and protective measures have to be taken during the conduct of the clinical trial, the investigator shall notify the CEC of the measures, and of the circumstances necessitating them, within 2 days. The investigator shall submit intermediary reports on the safety of the participants to the CEC once per year (Annual Safety Reports). The regular end of the research project will be reported to the CEC within 90 days upon completion of the project and the premature end or interruption of the research project will be reported within 15 days. The final study report will be submitted within one year after study end. Amendments will be reported according chapter 2.10.

2.4 Competent Authorities (CA)

Not applicable.

2.5 Ethical Conduct of the Study

The study will be carried out in accordance to the protocol and with principles enunciated in the current version of the Declaration of Helsinki[5], the guidelines of Good Clinical Practice (GCP) issued by ICH[6,7], in case of medical device: the European Directive on medical devices 93/42/EEC[8] and the ISO Norm 14155 and ISO 14971[9,10], the Swiss Law and Swiss regulatory authority's requirements[11-13]. The CEC will receive annual safety and interim reports and be informed about study stop/end in agreement with the local requirements.

2.6 Declaration of interest

The VisionSense 3DHD Camera will be supplied by Rumed GmbH. None of the Sponsors have any financial association with the company or development of the medical device.

2.7 Patient Information and Informed Consent

All participants of the study will be provided a participant information sheet and a consent form describing the study and providing sufficient information for the participant to make an informed decision about his or her participation in the study.
The patient information sheet and the consent form will be submitted to the CEC for review and approval. The formal consent of a participant, using the approved consent form, must be obtained before the participant is submitted to any study procedure.
The participant should read and consider the statement before signing and dating the informed consent form, and should be given a copy of the signed document. The consent form must also be signed and dated by the investigator (or his designee) and it will be retained as part of the study records.

2.8 Participant privacy and confidentiality

The investigator affirms and upholds the principle of the participant's right to privacy and that they shall comply with applicable privacy laws. Especially, anonymity of the participants shall be guaranteed when presenting the data at scientific meetings or publishing them in scientific journals.
Individual subject medical information obtained as a result of this study is considered confidential and disclosure to third parties is prohibited. Subject confidentiality will be further ensured by utilising subject identification code numbers to correspond to treatment data in the computer files.

For data verification purposes, authorised representatives of the sponsor, or the CEC may require direct access to parts of the medical records relevant to the study, including participants' medical history.

2.9 Early termination of the study

The sponsor may terminate the study prematurely according to certain circumstances, for example:

- ethical concerns,
- insufficient participant recruitment,
- when the safety of the participants is doubtful or at risk, respectively,
- alterations in accepted clinical practice that make the continuation of a clinical trial unwise,
- early evidence of significant discrepancies in diagnostic accuracy of the study

2.10 Protocol amendments

Substantial amendments are only implemented after approval of the CEC.
Under emergency circumstances, deviations from the protocol to protect the rights, safety and well-being of human subjects may proceed without prior approval of the sponsor and the CEC. Such deviations shall be documented and reported to the sponsor and the CEC as soon as possible.
All Non-substantial amendments are communicated to the CEC within the Annual Safety Report.

3. BACKGROUND AND RATIONALE

3.1 Background and Rationale

Switzerland has the highest incidence of melanoma in Europe (19.2 per 100,000)[1] and this carries the poorest prognosis of all the skin cancers. The current treatment is dependent on the histological diagnosis following a biopsy and is determined primarily on tumour thickness (Breslow score), the rate the cancerous cells are dividing (mitotic rate), presence of ulceration, presence of regression, and age of patient. Initial treatment is through surgical excision with a safety margin of macroscopically normal skin surrounding the tumour. If the tumour thickness is more than 1 mm or more than 0.7 mm in association with high mitotic rate in younger patients, ulceration, regression or Clark level IV / V, the current cancer network guidelines suggest that the patient is offered an SLN biopsy as this is most likely to be the first site any metastasis spreads to. Merkel cell carcinoma is a highly aggressive neuroendocrine skin tumor with a mortality of approximately 33% at 3 years[2]. Because of the frequent lymphatic metastasis, a SLNB is highly recommended in all the patients to better assess their prognosis. The current gold standard technique to identify SLNs is to inject the radioisotope Technetium 99m intra-dermally around the location of the primary tumour. The patient is then scanned using multiple X rays to detect the location of the SLN at approximately 30 and 120 minutes. Other teams have tried to transcutaneously identify SLNs with ICG and NIRFI, but they concluded that ICG fluorescence technique is not reliable in patients with a high BMI or in patients with a primary tumor draining in the axillary node field.[3] This study aims to evaluate a medical device that uses improved technology compared to previous studies (stereoscopic 3D high definition for both fluorescence and visible light imaging). Our hope is that by applying similar principles, SLNs may be identified transcutaneously using fluorescent dye injections and NIRFI.

3.2 Investigational Product (treatment, device) and Indication

The medical device used is the Visionsense™ VS3 Iridium – Stereoscopic High Definition Visualisation System which includes:

- High Definition Infrared Fluorescence Scope
- High Definition 3D Camera
- Laser Light Source used to generate the florescence excitation illumination
- Camera Control Unit
- Display Monitor

Manufacturer: Visionsense Ltd, 20 Hamagshimim St., Tikva, 49348 Israel
Name or number of the model/type:
VS_3 – Visionsense Stereoscopic High Definition (3DHD) Camera (SN: 374-3050)
VS_3 Iridium – Visionsense Infrared (IR) Fluorescence Digital Miniature Microscope (MMS-IR) (SN: 378-4007)
Its use is based on ICG fluorescence visualisation transcutaneously with the device specific infrared fluorescence visualisation system. The population for which the medical device is intended comprises the patients eligible for SLNB:

- Malignant melanoma patients having one of the following characteristics:
    a. Breslow score ≥ 1 mm
    b. Breslow score ≥ 0.7 mm associated with ulceration

c. Breslow score ≥ 0.7 mm associated with regression
d. Breslow score ≥ 0.7 mm associated with Clark Level IV / V
e. Breslow score ≥ 0.7 mm associated with mitotic rate ≥ 1/mm^2 in young patients

- Merkel cell carcinoma

All the members of the team that are going to use the device will be trained how to use it before the beginning of the study.

3.3 Preclinical Evidence
Not applicable.

3.4 Clinical Evidence to Date
The medical device has been used in plastic and reconstructive surgery to assess viability of flaps through their perfusion.[14]

3.5 Medical Device: Rationale for the intended purpose in study (pre-market MD)
The Visionsense Iridium provides 3D high definition (HD) for both fluorescence and visible light imaging, powerful and uniform fluorescence excitation illumination, real-time fusion of IR and visible light images and a compact camera size.

3.6 Explanation for choice of comparator
Lymphoscintigraphy is the current gold standard method for detection of the sentinel lymph nodes.

3.7 Risks / Benefits
There is no additional risk to patients.
There is no immediate benefit to patients, however the long-term aim is to prove the hypothesis that SLNs can be successfully identified using this technology and thus reduce the exposure to radiation and improve the patient pathway.
There are no expected threats of competing trials to this study.

3.8 Justification of choice of study population
SLNB is a staging tool for patients with clinically node-negative primary cutaneous malignancies and no evidence of distant metastasis. It is used to determine the histologic status of the nodes of the regional nodal basin(s) draining the primary site. If the SLN is negative, the rest of the nodes in the basin are presumed to be negative. The status of the SLN has been shown to be the strongest predictor of outcome in melanoma, in patients fulfilling the inclusion criteria mentioned above.[15] SLNB detects Merkel cell carcinoma in one third of patients whose tumors would have otherwise been clinically and radiologically under-staged and who may not have received treatment to the involved node bed. There is a significant benefit of adjuvant nodal therapy, but only when the SLNB was positive. Therefore, SLNB is important for both prognosis and treatment of Merkel cell carcinoma patients.[16]

No vulnerable patients will be included in this study.

4. STUDY OBJECTIVES

4.1 Overall Objective

To determine whether the VisionSense NIRFI technology can transcutaneously identify SLNs as effectively as LS.

4.2 Primary Objective

The study seeks primarily to determine ability of the VisionSense NIRFI technology to transcutaneously identify SLNs as effectively as LS.

4.3 Secondary Objectives

To determine if anatomical location of SLN has an effect on the ability of VisionSense NIRFI to detect SLNs.
To determine whether patient factors (e.g. BMI, sex, age, etc.) impact the ability to transcutaneously identify SLNs.

4.4 Safety Objectives

Not applicable.

5. STUDY OUTCOMES

5.1 Primary Outcome

Correlation of SLN(s) identified by LS vs. VisionSense NIRFI.

5.2 Secondary Outcomes

Correlation of SLN(s) identified by LS vs.VisionSense NIRFI in specific anatomical locations and in defined patient groups (e.g. groups defined based on BMI, sex, age).

5.3 Other Outcomes of Interest

Not applicable.

5.4 Safety Outcomes

Not applicable.

6. STUDY DESIGN

6.1 General study design and justification of design

This prospective, single-blinded, single-centre, single-arm, not randomised diagnostic sensitivity study is intended to analyse the ability of intradermally administered ICG and NIRFI to transcutaneously identify sentinel lymph nodes in malignant melanoma and Merkel cell carcinoma that are found positive with intradermally administered Technetium 99 and traditional lymphoscintigraphy. A number of 93 patients eligible for sentinel lymph node biopsy with lymphoscintigraphy (current gold standard method for SNLB) are going to be also examined at the time of operation with ICG and NIRFI. The lymph nodes identified by lymphoscintigraphy will not be marked on the patients and the images obtained in lymphoscintigraphy will not be initially uploaded in the hospital's system, so the surgeons will be the blinded persons in the study. They will first try to identify the lymph nodes with ICG and NIRFI and then use gamma probe signals to locate the radioactive lymph nodes from lymphoscintigraphy and also examine the images recorded from lymphoscintigraphy that are going to be uploaded in the system during the operation time.
The complete sequence of the study is provided in the flow chart.

6.2 Methods of minimising bias

The lymph nodes identified by lymphoscintigraphy will not be marked on the patients, therefore the surgeons won't be influenced when identifying the lymph nodes with ICG and NIRFI. In addition, the surgeon will not have access to the images recorded from lymphoscintigraphy since they are going to be uploaded in the hospital's system only after the surgeon has tried to identify intraoperatively the lymph nodes with ICG.

6.2.1 Randomisation

Not applicable.

6.2.2 Blinding procedures

The surgeons performing SLNB will be the blinded persons. The results of the lymphoscintigraphy will not be marked on the patient's skin as usual and no preoperative access to the images will be granted to the surgeons.

6.2.3 Other methods of minimising bias

In order not to influence the surgeons, the patients are advised not to discuss the lymphoscintigraphy results with them and this will be reinforced during the consent procedure.

6.3 Unblinding Procedures (Code break)

Not applicable.

7. STUDY POPULATION

7.1 Eligibility criteria

Individuals fulfilling all of the following <u>inclusion</u> criteria are eligible for the study:

- Informed consent as documented by signature
- Individual must present with any of the following cancer types:
    - Malignant melanoma with one of the following characteristics:
        a. Breslow score ≥ 1 mm

b. Breslow score ≥ 0.7 mm associated with ulceration
c. Breslow score ≥ 0.7 mm associated with regression
d. Breslow score ≥ 0.7 mm associated with Clark Level IV / V
e. Breslow score ≥ 0.7 mm associated with mitotic rate ≥ 1/mm^2 in young patients

- Merkel cell carcinoma

The presence of any one of the following exclusion criteria will lead to exclusion of the individual:

- age < 18 years
- pregnancy and breastfeeding (pregnancy test to be performed for women of child-bearing potential, defined as women who are not surgically sterilized/ hysterectomized, and/ or who are postmenopausal for less than 12 months)
- known allergy to ICG or Iodine
- previous chemotherapy, radiotherapy or surgery to the lymph nodes of interest
- lack of capacity to provide informed consent
- current enrolment in any other interventional study

7.2 Recruitment and screening

Patients who fulfill the inclusion criteria for the study will be sent an information packet detailing the background rationale of the study and the details of the surgical procedure. Prior to surgery a member of the study group will confirm the patient's willingness to participate in the study. If they agree, they will formally consented regarding procedures, risks and benefits of the study.

7.3 Assignment to study groups

Not applicable.

7.4 Criteria for withdrawal / discontinuation of participants

The participants are withdrawn from the study if they withdraw their consent, if the operation cannot take place anymore for medical reasons, if they are proven to have distant metastases following radiological staging or if there are no LNs on lymphoscintigraphy.

8. STUDY INTERVENTION

8.1 Identity of Investigational Products (treatment / medical device)

In all patients of our study a transcutaneous ICG lymphography is performed by injection of ICG intradermally around the scar of the primary excision of the tumour and transcutaneous assessment of fluorescence with the Visionsense™ VS3 – Stereoscopic High Definition Visualisation System (VS3-3DHD).

8.1.1 Experimental Intervention (treatment / medical device)

The Medical device used is the Visionsense™ VS3 – Stereoscopic High Definition Visualisation System (see image). Its use is based on ICG fluorescence visualisation transcutaneously with the device specific Infrared (IR) fluorescence visualisation system. The system is made up of a display and hardware tower and an infrared camera as well as connecting cables.

8.1.2 Control Intervention (standard/routine/comparator treatment / medical device)

Tc 99m based lymphoscintigraphy.

8.1.3 Packaging, Labelling and Supply (re-supply)

Not applicable.

8.1.4 Storage Conditions

Storage according to standard procedures in the facilities of the Plastic Surgery Operation theatre.

8.2 Administration of experimental and control interventions

8.2.1 Experimental Intervention

After induction of anaesthesia, standard procedures for injection of ICG intradermally are performed according to product description and hospital SOPs.
After ICG injection the possible draining lymphatic basins from the primary scar (i.e. axillae, inguinal, popliteal and neck regions) are scanned with the VS3-3DHD camera and all transcutaneous fluorescence spots recorded in the CRF. The scanning is discontinued after a maximum of 15 minutes regardless of findings.

After this the surgeon is unblinded to the results of the lymphoscintigraphy and the further procedures of sentinel lymph node dissections are performed according to surgical intervention standard procedures.

8.2.2 Control Intervention
Tc 99m based lymphoscintigraphy.

8.3 Dose / Device modifications
Not applicable.

8.4 Compliance with study intervention
There is no possibility of non-adherence to the intervention since all study procedures are done once under general anaesthesia after obtaining informed consent.

8.5 Data Collection and Follow-up for withdrawn participants
Should patients choose to retrospectively withdraw their consent to further study participation, all data collected up to the time point of withdrawal will still be analysed in coded form. For withdrawn patients standard follow-up will be pursued.

8.6 Trial specific preventive measures
There are no specific contraindications regarding the use of the VS3-3DHD device.

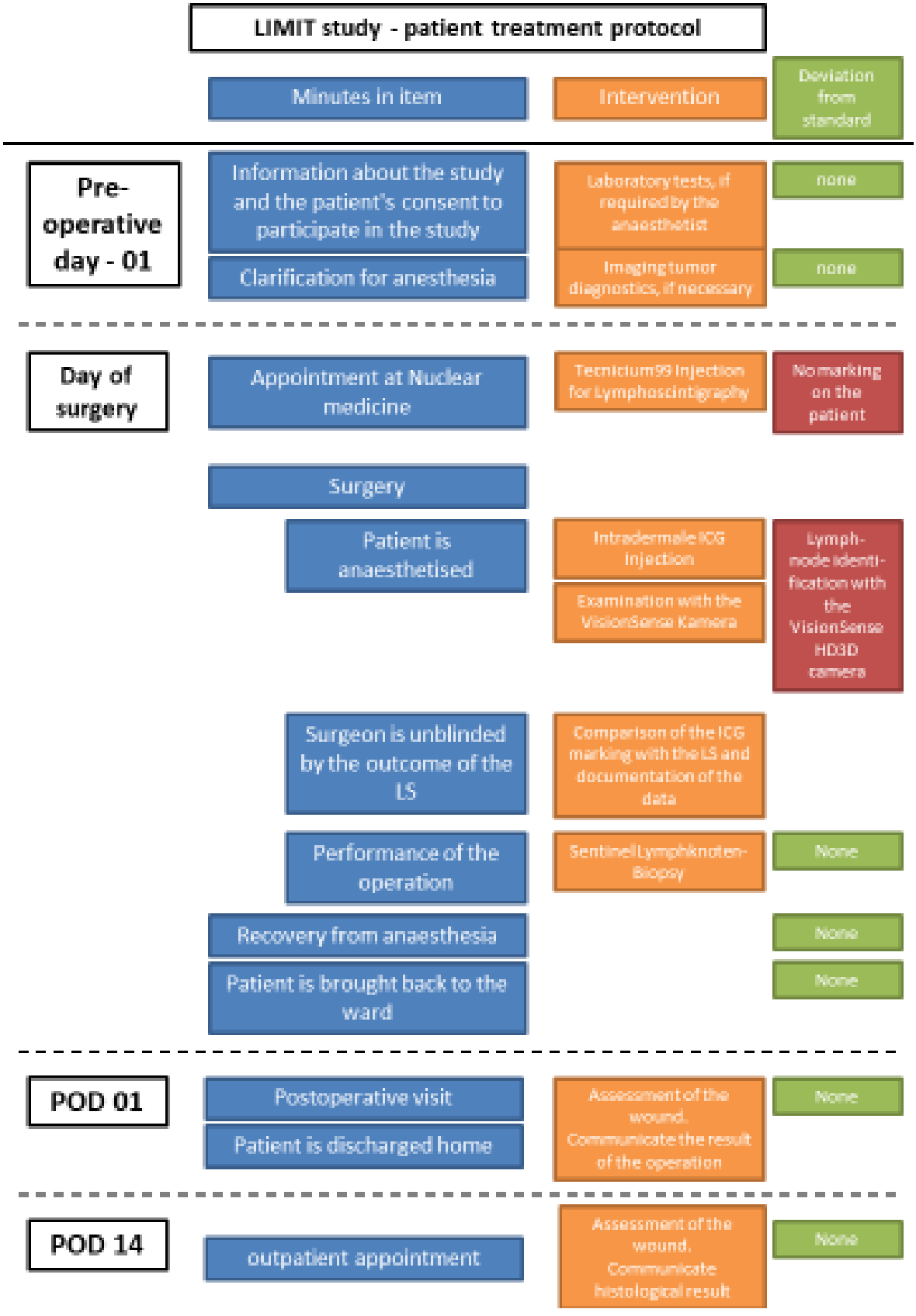
8.7 Concomitant Interventions (treatments)
There are no specific treatments associated with the use of the medical device.

8.8 Study Drug / Medical Device Accountability
Device maintenance plan according to the hospital's technical-medical departments SOP's.

8.9 Return or Destruction of Study Drug / Medical Device
After the study is completed, the VisionSense devices will be returned to the manufacturer.

9. STUDY ASSESSMENTS

9.1 Study flow chart(s) / table of study procedures and assessments



9.2 Assessments of outcomes

9.2.1 Assessment of primary outcome

The primary end-point is determined once the responsible surgeon has either identified or failed to identify a SLN transcutaneously (as per protocol described in chapter 9.3). At this stage the surgeon is unblinded and the results will be compared to the LS findings; all data will be recorded for analysis.

9.2.2 Assessment of secondary outcomes

Secondary outcomes will be made once the study period is completed. Data relating to the ability of SLNs to be successfully identified transcutaneously will be grouped anatomically or according to patient characteristics such as BMI, sex, and age and statistically analysed to determine if certain anatomical regions or patient groups have different sensitivity rates.

9.2.3 Assessment of other outcomes of interest

Not applicable.

9.2.4 Assessment of safety outcomes

Not applicable

9.2.5 Assessments in participants who prematurely stop the study

Any patient whom chooses to terminate the study early will receive the same post-operative (standard) care and follow-up as those included in the study.

9.3 Procedures at each visit

9.3.1.0 <u>Patient booked for sentinel lymph node biopsy.</u>

- Patient identified according to national guidelines that they should be offered a sentinel lymph node (SLN) biopsy
- Patient demographics and skin tumour details checked to ensure they fulfil the inclusion/exclusion criteria of the trial
- Patient information pack sent to home address detailing the study and aims of the clinical trial

9.3.2.1 <u>Patient admission.</u>

9.3.2.1.1 *Informed consent and patient agreement of participation in study*

- Patient admitted to dermatology or plastic surgery ward
- Pre-operative consultation explaining the surgical procedure including the risks and benefits of surgery
- Patient consent to be obtained for both the surgical procedure and for participation in study

9.3.2.1.2 *Pre-operative consultation with anaesthetic department*

- Routine pre-operative assessment
- Routine blood tests as requested by anaesthetic team including pregnancy testing for relevant patients
- Electrocardiogram monitoring where indicated
- **This information will not be recorded for the purpose of this study**

9.3.2.1.3 *Radiological cancer screening (where applicable):*

- Patients will complete radiological cancer screening depending on the type and size of tumour
- This may involve ultrasonography of anatomical lymph node basins for local evidence of regional metastasis or plain chest radiograph with x-ray or computed tomography (CT)
- **This information will not be recorded for the purpose of this study**

9.3.3.0 <u>Day of surgery.</u>

9.3.3.0.1 *Nuclear medicine appointment:*

- The patient will attend a nuclear medicine appointment for the lymphoscintigraphy scan (LS) on the morning of the planned surgery
- This will be performed in accordance with the standardised technique of injecting four small volumes (0.1 - 0.4 ml) of Technicium^{99} around the site of the primary tumour
- The patient will then have scans taken at approximately 30 and 120 minutes following injection to locate the SLN(s)
- The patient **will not** have the location of the SLN marked on his or her skin with permanent marker
- The patient will be asked **not to disclose** the location of the detected SLN to the surgeon or any other member of the surgical team to ensure that the surgeon is blinded to the result and limit the degree of bias in the study
- **This information will not be recorded for the purpose of this study**

9.3.3.0.2 *Patient called to the operating room:*

- The patient will be called to the operating room at the time of surgery
- A member of nursing staff will accompany the patient.
- The patient will be anaesthetised under full general anaesthetic

9.3.3.0.3 *Intra-dermal Indocyanine Green (ICG) injection:*

- The responsible surgeon will inject 0.2 – 0.4 ml of ICG intra-dermally around the site of the primary tumour
- The surgeon will wait for a period of 5 – 15 minutes to allow for ICG to be absorbed and passed into the lymphatic system
- After this period, the surgeon will use the VisionSense HD3D camera to attempt to locate the SLN after confirming the camera is functioning correctly by commencing the scan at the site of ICG injection, identified lymph nodes (LN) will be marked with a permanent skin marker in RED.
- The scan will proceed in the following manner depending on the site of the primary tumour:
  - Head and neck tumours:
    - The scan will proceed from the primary tumour to the ipsilateral lymphatic chains to cover the pre-auricular LNs, posterior-

auricular LNs, anterior cervical LNs (Level I – IV) and posterior cervical LNs (Level V)
- The same procedure will be performed on the contra-lateral side

- Trunk and thorax tumour:
  - The scan will proceed from the primary tumour towards the ipsilateral axilla followed by the ipsilateral inguinal region.
  - The same procedure will be performed for the contralateral side
- Upper extremity tumour:
  - The scan will proceed from the primary tumour towards the ipsilateral axilla
  - There is no need to scan other LN basins
- Lower extremity tumour:
  - The scan will proceed from the primary tumour towards the ipsilateral inguinal region.
  - If the tumour is located below or distal to the knee, the popliteal LN basin will be scanned.
  - If the primary tumour is above or proximal to the knee the popliteal LN basin will not be examined

9.3.3.0.4 *Un-blinding of the surgeon to the LS result:*

- The nuclear medicine department will be telephoned and asked to upload the scans and radiology report of the LS onto the electronic PACS system
- The surgeon will visualise the location of the LS scan and locate them on the patient using a gamma probe using a BLUE permanent skin marker
- **The results will be recorded in the CRF**

9.3.3.0.5 *Commencement of the surgical procedure*:

- The surgeon will **ONLY** perform the SLN biopsy on the LNs identified by LS
- Any LNs identified by the VisionSense HD3D camera which do not correlate with the LS results will **NOT** be biopsied
- The operation will be carried out in accordance with the standard operative procedure using a combined approach of gamma probe navigation and fluorescent imaging
- **This information will not be recorded for the purpose of this study**

9.3.3.0.6 *Patient recovery from surgical procedure:*

- Following the surgical procedure, the patient will be woken from general anaesthesia
- The patient will be transferred to the general recovery unit and monitored routinely
- Once the patient vital parameters have been met and their pain controlled, they will be transferred back to the ward
- **This information will not be recorded for the purpose of this study**

9.3.3.0.7 *Post-operative surgical consultation:*

- Once the patient has been re-admitted to the ward the responsible surgeon will make a routine post-operative visit of the patient

- The surgical wounds will be inspected for any early operative complications
- The patient will be informed of the surgical procedure and findings, including the results of the clinical trial
- **Any adverse events will be recorded in the CRF**

9.2.4.0 Post-operative day one

9.3.4.0.1 *Second post-operative consultation:*

- The responsible surgeon will visit the patient on a second occasion the day following surgery
- The surgical wounds will be inspected again to monitor for any early surgical complications
- The patient will have the results of the surgical procedure explained to them for a second time and be asked if they have any questions regarding the procedure or follow up
- **Any adverse events will be recorded in the CRF**

9.3.4.0.2 *Patient discharge:*

- Once the patient has fulfilled standard discharge criteria they will be prepared for discharge
- A prescription for analgesia will be provided

10. SAFETY

10.1 Drug studies
Not applicable.

10.2 Medical Device Category C studies
Not applicable.

10.3 Medical Device Category A studies

10.3.1 Definition and Assessment of safety related events

There are no known patient safety related concerns regarding the use of the device. The device was certified with the CE 0086 marking by the British Standards Institution according to ISO 13485 and adheres to following normatives.

Safety norms:
IEC/EN-60601-1 (2006)
IEC/EN60601-2-18 (2009)
IEC/EN60825-1-2 (2014)

EMI-Norms:
IEC/EN60601-1-2 (2007)

10.3.2 Reporting of Safety related events

Category A study - there is no safety related event reporting necessary.

Reporting to Sponsor-Investigator:
Health hazard that require measures are reported to the Sponsor-Investigator within 24 hours upon becoming aware of the event:

Pregnancies
Reporting is not needed, because pregnancy is an exclusion criteria

Reporting to Authorities:
In Category A studies it is the Investigator's responsibility to report to the local Ethics Committee

- Health hazards that require measures within 2 days

11. STATISTICAL METHODS

This study is a diagnostic sensitivity study designed to analyse the ability of a new diagnostic method in comparison to the current "gold standard" diagnostic procedure. Gold standard diagnostic procedures are the current best-practice for diagnosing a disease. Sensitivity measures inherent validity of a diagnostic test against a gold standard. We want to ensure that the data will yield estimates of sensitivity that have good precision. Study sample size requirements can be calculated based on a clinically acceptable degree of precision, the hypothesized values of sensitivity and the estimated prevalence of disease in the target population.

11.1 Null Hypothesis

We will not do any hypothesis testing. The aim of the study is to determine the sensitivity of VisionSense NIRFI to detect SLNs in comparison to the current gold standard of LS.

11.2 Determination of Sample Size

Sample size at the required absolute precision level for sensitivity can be calculated with Buderer's formula.[4] With an expected sensitivity of 60% and assuming a maximum clinically acceptable width of the 95% CI of 10% (i.e. 50 - 70%) we will require 93 LNs identified by LS. All of the patients will have at least one positive LN in LS, but there could also be cases with more than one LN per patient, increasing the precision of the sensitivity estimate. As it is not possible to make a reasonable assumption about the number of LNs per patient and the correlations of the measurements within a patient, we will recruit 93 patients.

11.3 Statistical criteria of termination of trial

Not applicable. This is a diagnostic sensitivity test, we need to examine all the required LNs to determine the sensitivity before deciding whether this method works or not.

11.4 Planned Analyses

11.4.1 Datasets to be analysed, analysis populations

All eligible patients will be included in the analysis.

11.4.2 Primary Analysis

The statistical analysis will be performed by a statistician at the end of the study (i.e., when all patients are included). Baseline characteristics will be reported as mean and standard deviation or median and interquartile range, and number and percentage of patients for continuous and categorical variables,respectively.
The sensitivity (i.e. the proportion of NIRFI and LS positive LN among LS positive LN) will be reported with a 95% confidence interval and adjusted for multiple observations per patients using generalized estimating equations (GEE)[17] or the method by Rao & Scott.[18]
The number of LS-negative LN that are NORFI positive will be reported.

11.4.3 Secondary Analyses

Data relating to the ability of SLNs to be successfully identified transcutaneously by NIRFI will be grouped anatomically and according to patients' characteristics (e.g. BMI, sex, and age) and afterwards analysed to determine if certain anatomical regions or patient groups have different sensitivity rates.

11.4.4 Interim analyses
An interim analysis will be performed after 60 examined patients to analyze how many LNs could be successfully identified transcutaneously by NIRFI until then and if there is a significant difference in the number of identified LNs with the standard methods.

11.4.5 Safety analysis
The number and type of adverse effects will be listed and reported.

11.4.6 Deviation(s) from the original statistical plan
In principle we do not expect any deviation. Eventual deviations will be justified only when the statistician will suggest a different analysis. Any deviation will be communicated to the data monitoring committee and to the research team in a joint meeting.

11.5 Handling of missing data and drop-outs
Only patients with positively identified LNs in LS will be included in the study and there will be no missing data from the LS.
We do not expect any or only a small number of missing NIRFI data. However, patients with missing data in NIRFI will be excluded from the main analysis. For a sensitivity analysis, we will assume that all missing NIRFI results will be negative. The number of missing data will be reported together with the reasons behind their absence.

12. QUALITY ASSURANCE AND CONTROL

12.1 Data handling

The responsible investigator will maintain appropriate medical and research records for this trial, in compliance with ICH-GCP and regulatory and institutional requirements for the protection of confidentiality of subjects. The principal investigator, sub-investigator, and clinical research nurses or coordinators will have access to the records. The responsible investigator will permit regulatory agencies to examine clinical records for the purposes of quality assurance reviews and evaluation of the study safety and progress.

12.1.1 Case Report Forms

The CRF will be electronic. All data requested on the eCRF must be recorded and the recorded data should be consistent with the source documents or the discrepancies should be explained. The investigator should ensure the accuracy, completeness, and timeliness of the data reported in the eCRF and all other required reports. Generally, the eCRF should be completed within one week of completion of a participant's visit.

12.1.2 Specification of source documents

Source data must be available at the site to document the existence of the study participants. Source data must include the original documents relating to the study, as well as the medical treatment and medical history of the participant.
For all data captured in the eCRF, the location of the source will be documented on a list of source documents (source data location list), which will be stored in the investigator site file at each study site.
If certain data are directly entered into the eCRF (and are thus considered as source data) this must be specified on the source data location list accordingly.
Any change or correction to source data should be dated, initialed, and explained (if necessary) and should not obscure the original entry.

12.1.3 Record keeping / archiving

All data acquired from this study will be kept for a minimum of 10 years from the completion or premature termination of the trial.

12.2 Data management

12.2.1 Data Management System

The CRFs in this trial are implemented electronically using a dedicated electronic data capturing (EDC) system (REDCap). The EDC system is activated for the trial only after successfully passing a formal test procedure. All data entered in the CRFs are stored on a Linux server in a dedicated mySQL database.
Responsibility for hosting the EDC system and the database lies with CTU Bern.

12.2.2 Data security, access and back-up

The server hosting the EDC system and the database is kept in a locked server-room. Only the system administrators have direct access to the server and back-up tapes. A role concept with personal passwords (site investigator, statistician, monitor, administrator etc.) regulates permission for each user to use the system and database as he/she requires.
All data entered into the CRFs are transferred to the database using Secure Sockets Layer (SSL) encryption. Each data point has attributes attached to it identifying the user who entered it with the exact time and date. Retrospective alterations of data in the database

are recorded in an audit table. Time, table, data field and altered value, and the person are recorded (audit trail).
A multi-level back-up system is implemented. Back-ups of the whole system including the database are run internally several times per day and on external tapes once a day. The back-up tapes are stored in a secure place in a different building.

12.2.3 Analysis and archiving
At interim and final analyses, data files will be extracted from the database into statistical packages to be analysed. The status of the database at this time is recorded in special archive tables.
The study database with all archive tables will be securely stored by CTU Bern for at least 15 years. The sponsor also keeps the Trial Master File and interim/final reports for at least 10 years.

12.2.4 Electronic and central data validation
Data is checked by the EDC system for completeness and plausibility. Furthermore, selected data points are cross-checked for plausibility with previously entered data for that participant.

12.3 Monitoring
For quality control of the study conduct and data retrieval, the study site will be visited on-site by an appropriately trained and qualified monitor. Any findings and comments will be documented in site visit reports and communicated to the investigator and the sponsor as applicable. The investigator will support the monitor in his/ her activities. Prior to study start (first participant enrolled) a plan detailing all monitoring-related procedures will be developed.
All source data and relevant documents will be accessible to monitors and questions of monitors are answered during site visits.

12.4 Audits and Inspections
Since this is a single-center investigator-initiated trial, the sponsor does not plan to perform any audits. If the CA (Swissmedic) or CEC performs an inspection site staff will support the inspectors in their activities, study documentation and the source data/ source documents will be accessible to inspectors and study staff will answer questions from inspectors as needed. All involved parties must keep the participant data strictly confidential.

12.5 Confidentiality, Data Protection
Direct access to source documents will be permitted for purposes of monitoring and inspections. The investigator ensures anonymity of the patients; patients will not be identified by names in any documents leaving the study site. Signed informed consent forms and patient enrollment log will be kept strictly confidential to enable patient identification at the site.

Each lymph node is identified by a unique consecutive patient-lymph node identification number (Format PxxLNx, eg. P01LN1, P01LN2, P02LN1, etc.). A list with the identification numbers will be kept in the investigator site file at the study site, with access restricted to study personnel only.

12.6 Storage of biological material and related health data
Not applicable.

13. PUBLICATION AND DISSEMINATION POLICY

The trial will be registered in online trial databases (ClinicalTrials.gov and SNCTP) and as such the trial protocol will be publicly accessible. The results of the trial will be published in subject matter related peer reviewed scientific journals with significant impact in the medical scientific community. The results of the trial will also be presented at the meetings of Swiss and international societies of Plastic Surgery, Dermatology and during meetings organized by the Dermatologic Tumor Center Inselspital Bern. Since the use of the medical device is according to its intention there are no trade secret issues involved. Final decision on authorship of the manuscript lies with the sponsor.

14. FUNDING AND SUPPORT

14.1 Funding

All patient related procedures are funded by the health insurance companies since the sentinel lymph node biopsy is a procedure included in the standard care list. No additional accost will be incurred to the insurance company as a result of this study.

The use of the VS3-3DHD device is funded by the University Clinic for Plastic Surgery.

The other trial costs will be supported by a grant of the “Bernische Krebsliga” (see attached grant approval letter)

14.2 Other Support

Not applicable.

15. INSURANCE

Not applicable (Category A study with only minimal risks and burdens).

16. REFERENCES

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17. APPENDICES

1. Medical Devices: IB (according to ISO 14155)

2. Medical Devices: Assurance of producer

3. Medical Devices: List of norms (vollständig eingehaltene, teilweise eingehaltene)

   Safety norms:
   IEC/EN60601-1 (2006)
   IEC/EN60601-2-18 (2009)
   IEC/EN60825-1-2 (2014)

   EMI-Norms:
   IEC/EN60601-1-2 (2007)

4. Grant Approval Letter