Sentinel lymph node localisation with an ultra-low dose of superparamagnetic iron oxide nanoparticles in patients with breast cancer

Protocol: SUGBG-2022002

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Sponsor: Sahlgrenska University Hospital

Principal Investigator: Fredrik Wärnberg

1 SIGNATURES

1.1 Principal investigator's Agreement

This confidential document is the property of the sponsor. No unpublished information contained in this document may be disclosed without prior written approval of the sponsor. I have read the attached protocol entitled "Sentinel lymph node localisation with an ultra-low dose of superparamagnetic iron oxide nanoparticles in patients with breast cancer", and agree to abide by all provisions set forth therein.

I agree to comply with the International Conference on Harmonization (ICH) Tripartite Guideline on Good Clinical Practice (GCP), ISO14155, and applicable national or regional regulations/guidelines.

I agree to ensure that Financial Disclosure Statements will be completed by:
• me (including, if applicable, my spouse [or legal partner] and dependent children)
• my co-investigators (including, if applicable, their spouses [or legal partners] and dependent children) at the start of the study and for up to one year after the study is completed, if there are changes that affect the financial disclosure status.

Signature

Fredrik Wärnberg

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Principal investigator Department of Surgery Sahlgrenska University Hospital SE-413 45 Gothenburg, Sweden	Date (DD Month YYYY)

1.2 Sponsor´s agreement

I approve the attached protocol entitled ”Sentinel lymph node localisation with an ultra-low dose of superparamagnetic iron oxide nanoparticles in patients with breast cancer”, and agree that the study is conducted at the Department of Surgery, Sahlgrenska University Hospital, Sweden.

Signature

Erik Johnsson

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Chair, Department of Surgery Sahlgrenska University Hospital SE-413 45 Gothenburg, Sweden	Date (DD Month YYYY)

2 INVESTIGATORS

2.1 Principal investigators

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3 LIST OF ABBREVIATIONS AND KEY TERMS

3.1 List of abbreviations

AE	Adverse Event
CT	Computed Tomography
EC	Ethics Committee
CRF	Case Report Form
GCP	Good Clinical Practice
IB	Investigators Brochure
MDR	Medical Device Regulation
MPA	Medical Products Agency
MRI	Magnetic Resonance Imaging
PMCF	Post Market Clinical Follow-up
SAE	Serious Adverse Event
SDIEQ	Skin Discoloration Impact Evaluation Questionnaire
SLN	Sentinel Lymph Node
SLNB	Sentinel Lymph Node Biopsy
SOC	Standard of Care
SPC	Summary of Product Characteristics
SPIO	Superparamagnetic nanoparticles of Iron Oxide
Tc99m	Technetium99m
USADE	Unanticipated Serious Adverse Device Effect

3.2 List of key study terms

Terms	Definition of terms
Investigational period	Period of time where major interests of protocol objectives related to defined endpoints are observed, and usually where the test device or comparative device (sometimes without randomization) is given to a subject and continues until the last observation after completing administration of the test device or comparative device.
Treatment number	Number assigned to each subject who has completed all screening assessments successfully at baseline and is willing to be included in the study.
Enrolled subject	Subjects included in the study.
Source data	All information in original records and certified copies of original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents (original records or certified copies).
Source documents	Original documents, data, and records including source data.
Subject	An individual who participates in a clinical trial, including screening but not necessarily given study device (enrolment).
Withdrawal	Subject enrolled but did not complete the study for any reason.

4 SYNOPSIS

Title of Study	Sentinel lymph node localisation with an ultra-low dose of superparamagnetic iron oxide nanoparticles in patients with breast cancer
Planned Study Period	4 years, 6 months Enrolment period: 18 months (Q1/2023 – Q3/2024). Follow-up period: 3 years (Q3/2024 – Q3/2027)
Study Objective(s)	The overall aim is to demonstrate that the use of superparamagnetic iron oxide nanoparticles (SPIO) as a tracer in an ultra-low dose (0.1ml) is non-inferior for sentinel lymph node (SLN) detection in patients with breast cancer compared to the dual technique using Tc^{99m} +/- blue dye, and to evaluate MRI breast artefacts and skin staining over time. Primary objective: To compare SLN detection rates between Magtrace® 0.1ml and the dual routine technique with radioactive tracer (Technetium^{99m}, Tc^{99}) +/- blue dye Secondary objectives: A. To follow MRI breast artefacts for two years B. To follow SPIO and blue dye skin staining for three years
Design and Methodology	A prospective cohort study where all research persons have a SLN biopsy using both SPIO and the dual technique
Number of Subjects Planned	220 patients, based on a non-inferiority design with a 4% margin with each patient being their own control
Endpoints	Primary endpoint: SLN detection rate for Magtrace® 0.1ml and for the dual technique (Tc^{99} +/- blue dye) measured as per cent of patients where a SLN is identified using either the magnetic or the dual technique Secondary endpoints: A. MRI Magtrace® artefacts at 3-6, 12 and 24-36 months postoperatively measured as per cent of patients with remaining artefacts evaluated by blinded central review. B. Skin staining due to injected Magtrace® and blue dye at 4 weeks, 12, 24 and 36 months measured as per cent of patients with remaining brown or blue skin staining and stain size, evaluated by the investigator and the Skin Discoloration Impact Evaluation Questionnaire (SDIEQ) C. Rate of device-related AEs and SAEs D. Numbers of nodes detected and removed for Magtrace® 0.1ml and for the dual technique (Tc^{99} +/- blue dye) E. Concordance between detected sentinel lymph nodes by Magtrace® 0.1ml or by the dual technique (Tc^{99} +/- blue dye)

Selection Criteria	Inclusion Criteria 1. Age above 18 years 2. Planned for sentinel lymph node biopsy at (or after) breast surgery 3. Signed and dated written informed consent before the start of specific protocol procedures Exclusion Criteria 1. Pregnant or breast-feeding 2. Iron overload disease 3. Known hypersensitivity to iron, dextran compounds or blue dye. 4. Inability to understand given information and give informed consent or undergo study procedures
Discontinuation Criteria	Subjects must be discontinued from the study for the following reasons: 1. Inappropriate enrolment (violation of Inclusion / Exclusion Criteria) 2. Withdrawal of consent 3. Due to safety reasons judged by the Investigator

5 INTRODUCTION

5.1 Background

Breast cancer is the most frequent cancer in women with approximately 10,000 new patients in Sweden yearly (The National Board of Health and Welfare of Sweden, www.socialstyrelsen.se). The sentinel lymph node (SLN) is the first lymph node that receives lymphatic drainage from the primary tumour site. Sentinel lymph node biopsy (SLNB) is an established technique for tumour staging in breast cancer patients [1]. The SLN is usually detected by the dual technique of injecting a radioactive isotope (Technetium^{99m}, Tc^{99}) and a blue dye (Patent bleu®) in the breast. The SLN is identified by a handheld gamma-probe and the dye stains the SLN with a blue colour which makes it easy to visualize during surgery.

A new SLN tracer containing superparamagnetic nanoparticles of iron oxide (SPIO/Magtrace®) has shown similar detection rates as for the dual technique [2-4]. Magtrace has been injected interstitially (intra/peri-tumourally or subcutaneously behind the areola) before surgery and the SLNs are detected during surgery with a handheld probe (SentiMag®) that measures the strength of the magnetic field created by the magnetic tracer.

After the use of SPIO/Magtrace® in breast cancer there is a risk of magnetic resonance imaging (MRI) artefacts after breast conserving surgery, as well as a risk for long-lasting skin staining after the injection. A feasibility study in melanoma, the MAGMEN study, have reported promising results using a ultra-low dose of 0.1 mL Magtrace®, that was injected intradermally [5]. In a feasibility study including 30 patients with breast cancer, we have shown that the SLN was detected in all 30 patients with a similar intradermal injection of 0.1ml SPIO/Magtrace® (results not yet published). This ultra-low dose of Magtrace® would potentially reduce the drawbacks of SPIO related MRI artefacts and skin staining.

6 STUDY OBJECTIVES AND DESIGN

6.1 Study objectives

The overall aim is to evaluate the use of superparamagnetic iron oxide nanoparticles (SPIO) as a tracer in an ultra-low dose (0.1ml) is non-inferior for sentinel lymph node (SLN) detection in patients with breast cancer compared to the dual technique using Tc^{99m} and blue dye, and to evaluate MRI breast artefacts and skin staining over time.

Primary objective:

SLN detection rate for Magtrace® 0.1ml and for the dual technique (Tc99 +/- blue dye) measured as per cent of patients where a SLN is identified using either the magnetic or the dual technique

Secondary objectives:

A. To follow MRI breast artefacts for two to three years
B. To follow SPIO and blue dye skin staining for three years

6.2 Study endpoints

Primary endpoint:

SLN detection rate for Magtrace® 0.1ml and for the dual technique (Tc^{99} +/- blue dye) measured as per cent of patients where a SLN is identified using either the magnetic or the dual technique

Secondary endpoints:

A. MRI Magtrace® artefacts at 3-6, 12 and 24-36 months postoperatively measured as per cent of patients with remaining artefacts evaluated by blinded central review.

B. Skin staining due to injected Magtrace® and blue dye at 4 weeks, 12, 24 and 36 months measured as per cent of patients with remaining brown or blue skin staining and stain size, evaluated by the investigator and the Skin Discoloration Impact Evaluation Questionnaire (SDIEQ)[8]

C. Rate of device-related AEs and SAEs

D. Numbers of nodes detected and removed for Magtrace® 0.1ml and for the dual technique (Tc^{99} +/- blue dye)

E. Concordance between detected sentinel lymph nodes by Magtrace® 0.1ml or by for the dual technique (Tc^{99} +/- blue dye)

6.3 Risk-benefit evaluation

The magnetic technique is already approved for SLN diagnostics in breast cancer. The results are comparable to the clinically routine use of Tc^{99} and blue dye. The risk of using a lower dose of Magtrace® is that the SLN is not detected. An ultra-low dose of Magtrace® for identifying the SLN has been explored in a feasibility study with 30 patients and in all patients the SLN was detected. Also, in this study Tc^{99} +/- blue dye will be used in parallel as a comparison and back up minimising the risk of an unsuccessful SLNB.

For the study participants the study will result in an extra injection that can be painful but only for a short time. Skin staining from the Magtrace® can occur but as Magtrace® is currently used as a routine tracer in Sweden in a ten times higher dose, the benefit of testing a lower dose is greater than the risk of a small skin staining. The skin staining is also one of the outcomes that will be followed.

In a subgroup of included persons, two to three breast MRIs will be performed after 3-6, 12 and 24-36 months. MRI is painless and does not cause ionising radiation, but some people find the tight space discomforting. This part of the study is optional and up to the research person´s. The magnetic field could affect metal objects which are inside the body. Therefore, persons with surgical implants (e.g., insulin pump, or pacemaker) will be excluded from this part of the study.

Participating in the study also takes extra time and personal data is to be handled. The risk of breaking their integrity is considered as small in this study. All patients are given information about both the benefits and risks of the research. They can then voluntarily decide whether they want to participate or not, and they can withdraw their consent at any time.

In summary, the risk of being included in this trial is assessed to be low, and we have not identified any ethical problem in a broader perspective. The study participants contribute to advancing research on SLN and staging technology, which may be useful for other patients in the future.

6.4 Study design and procedures

6.4.1 Overall study design

This is a prospective cohort study comparing an ultra-low dose of 0.1 mL Magtrace® with the dual technique with Tc99 +/- blue dye for SLN detection with a non-inferiority design. There is no randomisation but instead every person is their own control, i.e., the patient will have the SLN identified with both tracer methods. This study design has already been used in several international studies and 220 persons will be included at four study sites, during 18 months.

Skin staining will be documented at the first follow up at four weeks with measurements of size of staining performed and documented with a photograph if staining is present for either SPIO or blue dye. Skin staining will be followed for three years after surgery. Those with a remaining skin staining at the routine 12 months follow up will be followed by telephone interview or physical visit at 24 and 36 months.

Furthermore, up to 50 persons will be included for secondary objective A, for evaluation of MRI artefacts in the operated breast at 3-6, 12, and 24 months. All MRI and corresponding mammograms will be evaluated centrally regarding artefacts and breast density.

6.4.2 Procedures

An intradermal dose of 0.1ml Magtrace® will be injected up to 30 days before surgery. The injection should be in the skin over the tumour, or at the border of the areola. A pre-operative photo of the breast will be taken, including any staining.

Before surgery Tc^{99} is injected in the breast as per routine practice. Blue dye is optional and if used, it is injected after induction of anaesthesia. During surgery, SLNs are first identified with the SentiMag®-probe. The dual technique, with the gamma probe +/- blue dye will be used afterwards, confirming each removed SLN and for detection of additional SLNs. For each tracer, the following parameters will be recorded in the CRF: transcutaneous magnetic and radioactive signals in breast and axilla, number of nodes, the colour of the nodes, signals in vivo and ex vivo for each SLN, residual transcutaneous signals at the end of surgery in breast and axilla. A lymph node is considered a SLN if it contains more than a 10% activity compared to the maximum activity of the highest scoring lymph node (first SLN) with either tracer. Each SLN will be sent separately for routine histopathology with the specific question concerning microscopically detected SPIO. For all patients, the pathologists are requested to report the lymph node status separately.

At the first post-operative visit (approximately 4 weeks after surgery) blue and brown skin staining will be recorded by photography including a centimetre scale, and any residual magnetic signal will be measured in the breast. In those patients with a remaining skin staining this procedure will be repeated at 12 months, and after 24 and 36 months by telephone interview without a photo or a physical visit. Patients will be asked about how they experience the staining according to a 5-point Likert scale (Skin Discoloration Impact Evaluation Questionnaire (SDIEQ))[8].

In up to 50 patients, a postoperative routine MRI of the breast, with contrast medium will be performed at 3-6, 12 and 24-36 months after surgery to evaluate the presence of SPIO artefacts. MRI artifacts and mammographic density will be recorded in a separate CRF by the radiologists and then transferred to our CRF.

6.4.3 Flow-chart/time and events schedule

	Screening	Day		Week	Months			
		-30 to 0	0	4 +/-2	3-6 +/-1	12	24	36
Eligibility assessments								
Informed Consent	X							
Inclusion/Exclusion Criteria	X							
Clinical Procedures								
Photography[a]	X			X		X	X*	X*
Administration of Magtrace[b]		X						
Administration of Tc99 +/- blue dye			X					
Surgery			X					
Residual magnetic activity[a]			X	X		X	X	X
MRI breast*					X	X	X**	X**

*Optional, **One MRI between 24 and 36 months, [a] Follow up is ended when the stain is gone. [b]Magtrace injection 0-30 days before surgery.

7 STUDY POPULATION

Patients planned for breast surgery and SLNB, will be considered for enrolment.

7.1 Inclusion criteria

1. Age above 18 years
2. Planned for sentinel lymph node biopsy at (or after) breast surgery
3. Signed and dated written informed consent before the start of specific protocol procedures

7.2 Exclusion criteria

A patient meeting ANY of the following criteria is not eligible for study participation:

1. Pregnant or breast-feeding
2. Iron overload disease
3. Known hypersensitivity to iron, dextran compounds or blue dye.
4. Inability to understand given information and give informed consent or undergo study procedures

7.3 Subject enrolment

Subject eligibility will be established before enrolment. Subjects will be enrolled strictly sequentially, as subjects are eligible for study participation. After enrolment the subject will be given a unique subject number per site, consisting of three letters for the study, the following three letters for the site and then a lot number, i.e., MAGGBG001. If a subject discontinues from the study, the subject number will not be reused, but the subject may be replaced.

7.4 Discontinuation criteria for individual subjects

The subject is free to withdraw from the study for any reason and at any time without giving reason for doing so and without penalty or prejudice to their treatment. The investigator is also free to terminate a subject's study treatment at any time if the subject's clinical condition warrants it.

Discontinuation Criteria for Individual Subjects:
Subjects must be discontinued from the study for the following reasons:

1. Inappropriate enrolment (violation of Inclusion / Exclusion Criteria)
2. Withdrawal of consent
3. Due to safety reasons judged by the Investigator.

The reasons for discontinuation should be recorded in the case report form (CRF). Persons discontinued from the study will be asked to return for an "End of Treatment Visit" and will thereafter be taken care of and followed at the discretion of the treating physician. Persons who discontinue the study will be replaced. Data collected up to the moment of withdrawal will be a part of the study analysis if accepted by the person.

7.5 Premature termination of the study

The sponsor has the right to terminate the trial prematurely if there are any relevant medical or ethical concerns, or if completing the trial is no longer feasible. If such action is taken, the investigator must inform all subjects and ensure appropriate care and follow up. The reasons

for terminating the trial must be documented in detail and the Ethics Committee must be given a written explanation.

Premature termination of the trial will be considered if:

1. The risk-benefit balance for the trial subjects' changes markedly.
2. The sponsor considers that the trial must be discontinued for safety reasons.
3. Due to futility

8 STUDY TREATMENTS

8.1 Description of investigational product(s)

Magtrace® (Endomagnetics Limited) is the magnetic tracer that is intended and calibrated for use with the SentiMag® device to identify SLN. It is a blackish-brown sterile suspension of SPIOs coated with carboxydextran, in a saline solution, with an iron concentration of 28mg/ml. The carboxydextran coating prevents agglomeration while maintaining biocompatibility. The Z-averaged particle diameter, including the organic coating, is 60nm (<0.25 polydispersity). The diameter enables the SLNs to selectively filter out the particles. After injection the particles drain naturally to the lymph nodes via the lymphatic system where they are physically filtered, trapped, and accumulate. This allows them to be used as a lymph node marker, which can be identified by the SentiMag® device. Magtrace® can be injected up to 30 days before SLNB. There is no evidence of anaphylaxis with interstitial tracer injection. Sentimag and Magtrace are CE-approved for SLN localisation.

Tc^{99} consists of a sterile aqueous suspension of Technetium-99m labelled to human albumin aggregate particles and is used for radioactive detection of sentinel nodes. Blue dye, (Bleu Patenté V Sodique Guerbet, 25mg/ml) is a blue dye that is approved for use as a sentinel node tracer. Both are used within the context of a general license from the MPA after a license application (Licensmotivering avseende Generell humanlicens) since many years, and this is regularly updated.

8.2 Dose and administration

In this trial a low dose of Magtrace® 28mg/ml (0.1ml) will be injected intracutaneously at one time-point. This is different from the recommendation of 1-2 mL in the subcutaneous tissue. The rationale is that the lymphatic outflow from an intracutaneous injection is much higher, and a lower dose is then needed. This is the same type of injection that is currently used with Tc^{99} and blue dye. The injection should be done slowly with a thin needle in the most superficial part of the skin, so that the tip of the needle is visible and that the Magtrace® creates a small spider web in the skin as it enters the lymphatic vessels.

8.3 Packaging, labelling, and handling of investigational products(s)

Magtrace® will be purchased by the official distributor in Sweden according to current clinical routine and stored according to manufacturer's instructions. As control, the routine use of Tc^{99} and blue dye will be used according to current clinical routine. Magtrace® will be packaged, delivered and stored according to manufacturer's instructions and current clinical routine and accompanied with the approved study IFU.

9 SAFETY AND TOLERABILITY

Safety reporting in clinical investigations of medical devices shall be performed in line with the requirements of the Regulation (EU) 2017/745 – Medical Device Regulation (MDR) Article 80(2). The sponsor shall report, without delay to all Member States in which the clinical investigation is being conducted, all of the following by means of the electronic system referred to in MDR Article 73:
a. any serious adverse event that has a causal relationship with the investigational device, the comparator or the investigation procedure or where such causal relationship is reasonably possible;
b. any device deficiency that might have led to a serious adverse event if appropriate action had not been taken, intervention had not occurred, or circumstances had been less fortunate; c. any new findings in relation to any event referred to in points 1 and 2.

The period for reporting shall take account of the severity of the event. Where necessary to ensure timely reporting, the sponsor may submit an initial report that is incomplete followed up by a complete report.

9.1 Investigational device

The definition of an investigational device is a device that is assessed in a clinical investigation, MDR Article 2(46). An investigational device can be a non-CE marked device or a CE marked device. The definition does not differentiate between different regulatory statuses of devices. However, the reporting requirements are different depending on whether the clinical investigation is done for purposes described in Article 62, 74 or 82. The definition is understood to cover also the devices investigated in PMCF investigations, even if they are not subject to notification per Article 74.2.

9.1.1 Adverse Event (AE)

An adverse event is defined as any untoward medical occurrence experienced by or worsening of a pre-existing condition of a subject during this trial in relation to the investigational device or study procedure. The assessment whether a detected adverse event is related to the investigational product or procedure is done by the specific site investigator.

Any untoward medical occurrence, unintended disease or injury or any untoward clinical signs, including an abnormal laboratory finding, in subjects, users or other persons, in the context of a clinical investigation, whether or not related to the investigational device. This definition includes events that are anticipated as well as unanticipated events, and also includes events occurring in the context of a clinical investigation related to the investigational device, the comparator or the procedures involved.

9.1.2 Serious Adverse Event (SAE)

A serious adverse event (SAE) is any adverse event that led to any of the following: a) death, b) serious deterioration in the health of the subject, that resulted in any of the following: i. life-threatening illness or injury, ii. permanent impairment of a body structure or a body function, iii. hospitalisation or prolongation of patient hospitalisation, iv. medical or surgical intervention to prevent life-threatening illness or injury or permanent impairment to a body structure or a body function, v. chronic disease, c) foetal distress, foetal death or a congenital physical or mental impairment or birth defect.

9.1.3 Device deficiency
Any inadequacy in the identity, quality, durability, reliability, safety or performance of an investigational device, including malfunction, use errors or inadequacy in information supplied by the manufacturer.

9.1.4 Unanticipated Serious Adverse Device Effect (USADE)
An Unanticipated Serious Adverse Device Effect (USADE) is an effect which by its nature, incidence, severity or outcome has not been identified in the current risk assessment. Procedures associated with the use of a device should be addressed in the risk assessment, which makes it possible to determine whether the procedure related SAEs are Unanticipated Serious Adverse Device Effect or not. SAEs related to procedures imposed by the clinical investigation plan but not with the use of the device should not be considered Serious Adverse Device Effects.

9.1.5 Causality
The relationship between the use of the medical device (including the medical - surgical procedure) and the occurrence of each adverse event shall be assessed and categorized. During causality assessment activity, clinical judgement shall be used and the relevant documents, such as the Investigator's Brochure, the Clinical Investigation Plan or the Risk Analysis Report shall be consulted, as all the foreseeable serious adverse events and the potential risks are listed and assessed there. The presence of confounding factors, such as concomitant medication/treatment, the natural history of the underlying disease, other concurrent illness or risk factors shall also be considered. The above considerations apply also to the serious adverse events occurring in the comparison group.

For the purpose of harmonizing reports, each SAE will be classified according to four different levels of causality: 1. Not related 2. Possible 3. Probable 4. Causal relationship

The sponsor and the investigators will use the following definitions to assess the relationship of the serious adverse event to the investigational device, the comparator or the investigation procedure.

1. Not related: Relationship to the device, comparator or procedures can be excluded when: - the event has no temporal relationship with the use of the investigational device, or the procedures related to application of the investigational device; - the serious adverse event does not follow a known response pattern to the medical device (if the response pattern is previously known) and is biologically implausible; - the discontinuation of medical device application or the reduction of the level of activation/exposure - when clinically feasible - and reintroduction of its use (or increase of the level of activation/exposure), do not impact on the serious adverse event; - the event involves a body-site or an organ that cannot be affected by the device or procedure; the serious adverse event can be attributed to another cause (e.g. an underlying or concurrent illness/ clinical condition, an effect of another device, drug, treatment or other risk factors); - the event does not depend on a false result given by the investigational device used for diagnosis, when applicable; In order to establish the non-relatedness, not all the criteria listed above might be met at the same time, depending on the type of device/procedures and the serious adverse event.

2. Possible: The relationship with the use of the investigational device or comparator, or the relationship with procedures, is weak but cannot be ruled out completely. Alternative causes

are also possible (e.g., an underlying or concurrent illness/ clinical condition or/and an effect of another device, drug or treatment). Cases where relatedness cannot be assessed, or no information has been obtained should also be classified as possible.

3. Probable: The relationship with the use of the investigational device or comparator, or the relationship with procedures, seems relevant and/or the event cannot be reasonably explained by another cause.

4. Causal relationship: the serious adverse event is associated with the investigational device, comparator or with beyond reasonable doubt when: - the event is a known side effect of the product category the device belongs to or of similar devices and procedures; - the event has a temporal relationship with investigational device use/application or procedures; - the event involves a body-site or organ that - the investigational device or procedures are applied to; - the investigational device or procedures have an effect on; - the serious adverse event follows a known response pattern to the medical device (if the response pattern is previously known); - the discontinuation of medical device application (or reduction of the level of activation/exposure) and reintroduction of its use (or increase of the level of activation/exposure), impact on the serious adverse event (when clinically feasible); - other possible causes (e.g. an underlying or concurrent illness/ clinical condition or/and an effect of another device, drug or treatment) have been adequately ruled out; - harm to the subject is due to error in use; - the event depends on a false result given by the investigational device used for diagnosis, when applicable;

In order to establish the relatedness, not all the criteria listed above might be met at the same time, depending on the type of device/procedures and the serious adverse event. The sponsor and the investigators will distinguish between the serious adverse events related to the investigational device and those related to the procedures (any procedure specific to the clinical investigation). An adverse event can be related both to procedures and the investigational device. Complications caused by concomitant treatments not imposed by the clinical investigation plan are considered not related. Similarly, several routine diagnostic or patient management procedures are applied to patients regardless of the clinical investigation plan. If routine procedures are not imposed by the clinical investigation plan, complications caused by them are also considered not related. In some particular cases the event may not be adequately assessed because information is insufficient or contradictory and/or the data cannot be verified or supplemented. The sponsor and the Investigators will make the maximum effort to define and categorize the event and avoid these situations. Where an investigator assessment is not available and/or the sponsor remains uncertain about classifying the serious adverse event, the sponsor should not exclude the relatedness; the event should be classified as “possible” and the reporting should not be delayed. Particular attention shall be given to the causality evaluation of unanticipated serious adverse events. The occurrence of unanticipated events related could suggest that the clinical investigation places subjects at increased risk of harm than was to be expected beforehand.

9.1.6 Reporting

All reporting will be performed using the MDCG 2020-10/2 Clinical Investigation Summary Safety Report Form v1.0 as provided by the MPA. It shall be transmitted to all NCAs where the clinical investigation is being performed. Once Eudamed is available and fully functional the obligations and requirements that relate to performing safety reporting via Eudamed shall apply from the date corresponding to six months after the date of publication of the notice

referred to in Article 34(3) of the MDR. Any reported events will also be sent to Endomag for their knowledge.

Contact details to sponsor: Professor Fredrik Wärnberg, Dept. of Surgery, Sahlgrenska University Hospital 413 45 Gothenburg Sweden Fax: +46 31-823931 Phone: +46 70 6146251 E-mail: fredrik.warnberg@vgregion.se

Reportable events: For the purpose of this guidance and based on the definitions above, the following events are considered reportable events in accordance with MDR Art. 80(2):

1. any serious adverse event that has a causal relationship with the investigational device, the comparator or the investigation procedure or where such causal relationship is reasonably possible;

2. any device deficiency that might have led to a serious adverse event if appropriate action had not been taken, intervention had not occurred, or circumstances had been less fortunate;

3. any new findings in relation to any event referred to in points 1 and 2.

Reporting timelines: Reportable events have to be reported to the sponsor immediately, but not later than 3 calendar days after investigation site study personnel's awareness of the event. Reportable events have to be reported to the competent authority by the sponsor of the clinical investigation. The timeline for these reports is based on:

1. For all reportable events which indicate an imminent risk of death, serious injury, or serious illness and that requires prompt remedial action for other patients/subjects, users or other persons or a new finding to it: Immediately, but not later than 2 calendar days after awareness by sponsor of a new reportable event or of new information in relation with an already reported event. This includes events that are of significant and unexpected nature such that they become alarming as a potential public health hazard. It also includes the possibility of multiple deaths occurring at short intervals. These concerns may be identified by either the NCA or the manufacturer.

2. Any other reportable events or a new finding/update to it: Immediately, but not later than 7 calendar days following the date of awareness by the sponsor of the new reportable event or of new information in relation with an already reported event

10 TERMINATION OF THE CLINICAL STUDY

The study end is defined as date of the last visit of the last subject participating in the study.

11 STATISTICS

11.1 Statistical methods

We estimate the SLN detection rate to be about 98% for both Magtrace® and the dual technique based on our earlier feasibility study where all patients had a successful SLN-biopsy with 0.1ml Magtrace® intracutanously. The detection rate for the dual technique is estimated based on our earlier studies, i.e., Nordic Trial, Monos and SentiDose trials. We set an acceptable difference in SLN-detection rate to 4%. If there is truly no difference between the dual technique and the experimental 0.1ml Magtrace®, then 386 patients would be

required in a randomised study to be 80% sure that the upper limit of a one sided 97.5% confidence interval will exclude a difference in favour of the dual technique of more than 4%. As every patient is their own control the sample size required is 193. Allowing for a dropout rate of approximately 10%, 220 patients will be recruited.

For the second endpoint skin staining, a subgroup of patients will be included in the analyses. Regarding skin staining, only patients undergoing breast conserving surgery will be included. As breast conserving surgery is done in approximately 85% of all patients, this cohort will include approximately 187 patients.

For the second endpoint, MRI artefacts, 50 of the 187 patients undergoing breast conserving surgery will be asked separately if they accept doing an MRI at 3-6, 12 and 24-36 months postoperatively. These patients will only be included in Gothenburg and Uppsala. They are recruited consecutively, and inclusion stops when 50 have accepted participation.

11.2 Drop-outs

Persons that drop-out from the study can be replaced.

12 OPERATIONAL AND ADMINISTRATIVE CONSIDERATIONS

12.1 Procedure for clinical study quality control

This trial will be conducted in accordance with the GCP standard ISO 14155, *Clinical investigations of medical devices for human subjects – Good clinical practice*, version SS-EN ISO 14155:2020)

12.1.1 Data Collection

All data on each subject generated according to the protocol must be recorded continuously in the CRF.

12.1.2 Data Management

Data management will be coordinated by the sponsor.

12.1.3 Specification of Source Documents

The investigator must keep source documents for each subject in the study. A document describing what has been classified as source data in the study should be included in the Investigator Site File (ISF). The investigator must ensure that all source documents are accessible for monitoring and other quality control activities.

The following documents are considered source, including but not limited to: Medical records, medical records from other department(s), or other hospital(s), or discharge letters and correspondence with other departments/hospitals, if subject visited any during the study period.
Source data must be available at the centre to document the existence of the study subjects and substantiate the integrity of study data collected. The following information (at least but not limited to) should be included in the source medical records:

1. Demographic data (age, sex, weight, and height)

2. Participation in the study and signed and dated Informed Consent Form
3. Visit dates
4. Key efficacy and safety data (as specified in the protocol)
5. Reason for premature discontinuation

12.1.4 Direct Access to Source Data/Documents

The investigator and the study site must accept auditing by the sponsor as well as inspections from the Independent Ethics Committee (IEC) and relevant regulatory authorities. The confidentiality of the subjects' identities shall be well protected consistent with local and national regulations when the source documents are subject to direct access.

12.1.5 Data storage and management

All data should be recorded, handled and stored in a way that allows its accurate reporting, interpretation and verification. All source data including informed consent, a copy of completed CRF if applicable, original protocol with amendments and the final report will be stored at study site for a minimum period of 15 years after termination of the trial, in accordance with Swedish regulation/law (Chapter 10, 3 § in LVFS 2011:19).

Staff designated by the Sponsor will review the data entered into the eCRFs by investigational staff for completeness and accuracy and instruct the site personnel to make any required corrections or additions. Queries are issued electronically. Designated investigator site staff is required to respond to the query and confirm or correct the data.

At the conclusion of the study, the occurrence of any protocol deviations will be determined. After these actions have been completed and the database has been declared to be complete and accurate, it will be locked and available for data analysis.

12.2 Clinical Study Monitoring

A study monitor will be appointed by the sponsor. The monitor will be appropriately trained and informed about the nature of the study, subject written information, GCP and applicable regulatory requirements. The monitor's qualifications will be documented.

The monitor will have regular contacts with the clinic to verify informed consents of participating subjects, to confirm that facilities remain acceptable, that the investigational team is adhering to the protocol, that data are being accurately recorded in the CRFs, to verify inclusion/exclusion criteria, study main endpoints, check AE/SAE reporting and that product accountability is being carried out. The investigator should ensure that all persons assisting with the trial are adequately informed and trained about the protocol, the investigational products(s) and their trial related duties and factions. The monitor will check that training has been performed and that this is documented. The monitor will also ensure source data verification (comparison of the data in the eCRF with the medical records and other source data). The monitor must have direct access to source data. The confidentiality of the subjects' identities shall be well protected consistent with local and national regulations when the source documents are subject to direct access. The extent of monitoring will be defined in a monitoring plan.

12.3 Audits and inspections

Authorized representatives of the sponsor, a Competent Authority or an Ethics Committee may perform audits or inspection at the center, including source data verification. The purpose of an audit or inspection is to examine all study-related activities and documents systematically and independently, to determine whether these activities were conducted, and data were recorded, analysed and accurately reported according to the protocol, Good Clinical Practice (GCP) and any applicable regulatory requirements. The monitor(s), the auditor(s), and the MPA(s) must be granted direct access to the subject's original medical records for verification of clinical trial procedures and/or data, without violating the confidentiality of the subject, to the extent permitted by the applicable laws and regulations.

12.4 Deviations from clinical investigation plan

An Investigator must not make any changes or deviate from the protocol, except to protect the life and physical well-being of a subject in an emergency. An investigator shall notify the sponsor, the reviewing ethics committee and the MPA of any deviation from the investigational plan to protect the life or physical well-being of a subject in an emergency, and those deviations which affect the scientific integrity of the clinical investigation. Such notice shall be given as soon as possible, but no later than 3 business days after the emergency occurred. All deviations from the investigational plan will be reported to the sponsor. Investigators must also adhere to ethics committee and MPA reporting requirements for institutional reporting of deviations. Deviations will be reviewed throughout the study. If necessary, corrective and preventive actions will be initiated by the sponsor. Principal investigator disqualification will be initiated if a principal investigator repeatedly or deliberately deviates from the protocol for reasons other than to protect the rights, safety and well-being of human subjects under emergency circumstances, if the investigator repeatedly or deliberately submits false information to the sponsor, ethics committee or, if applicable, MPA

12.5 Ethics and protection of subject confidentiality

12.5.1 Independent Ethics Committee

This protocol and the Subject Information Sheet and Informed Consent Form will be submitted to both the Swedish Ethical Review Authority and the Swedish Medical Products Agency according to the national laws and regulations. Prior to starting the study favourable opinion must be obtained in writing. No subject must be included in the study before the accredited Ethics Committee and the MPA has issued a favourable opinion.

12.5.2 Protocol Amendment and/or Revision

Any changes to the study, which arise after approval of the protocol, must be documented as protocol amendment or administrative amendments. Depending on the nature of the amendment and/or revision, either approval from the accredited Ethics Committee and the MPA or notification is required. The changes will become effective only after the approval of the sponsor, the regulatory authority and the accredited Ethics Committee and the MPA (if applicable). Written verification of the accredited Ethics Committee and the MPA and regulatory authority approval will be obtained before any amendment is implemented.

12.5.3 Ethical Conduct of Study

The investigator(s) and all parties involved in this study should conduct the study in adherence to the ethical principles based on the Declaration of Helsinki, Good Clinical Practice (GCP), ICH Guidelines (GCP standard ISO 14155, *Clinical investigations of medical devices for human subjects – Good clinical practice*, version SS-EN ISO 14155:2020).

12.5.4 Informed Consent of Subjects

The Principal Investigator at each centre will ensure that the subject is given full and adequate oral and written information about the nature, purpose and possible risks and benefits of the study. Subjects must also be notified that they are free to discontinue from the study at any time. The subject should be given the opportunity to ask questions and allowed time to consider the information provided.

Verbal and written informed consent must take place before any specific procedure related to the study is started. Signed and dated informed consent will be obtained from each patient in accordance with the principles of Good Clinical Practice (GCP). Documentation that the informed consent was signed and dated prior to study inclusion must be entered into the medical records at the time the informed consent is obtained. The original, signed Informed Consent Form (ICF) must be stored in the Investigator's Study File. A copy of the signed ICF must be given to the subject.

12.5.5 Subject Confidentiality

All patient data collected and processed for the purposes of this study will be managed by the sponsor with adequate precautions to ensure the confidentiality of those data, and in accordance with applicable national and/local laws and regulations on personal data protection. No patient identifiable data will be obtained. In any presentations of the results of this study; at meetings or in publications, the patients' identity will remain confidential.

12.6 Administrative matters

The sponsor will be responsible for all data registrations, statistical programming and analysis as well as statistical quality control and validation of programming and statistical analysis. The sponsor will be responsible for the collected data in the study.

12.6.1 Arrangement for Use of Information and Publication of the Clinical Study

The study will be registered on clinicaltrials.gov, the results from the trial will be presented at scientific symposia and congresses, and in a peer reviewed scientific journal. Authorship credit should be based on substantial contribution to conception and design, execution, or analysis and interpretation of data. All authors should be involved in drafting the article or revising it critically for important intellectual content and must have read and approved the final version of the manuscript. Authors should adhere to the practices of their research field and the guidelines of the International Committee of Medical Journal Editors (ICMJE)

12.6.2 Documents and Records Related to the Clinical Study

The investigator will archive all study data (e.g., Subject Identification Code List, source data, CRFs, and Investigator's File) and relevant correspondence. These documents are to be kept on file for at least 15 years after final study report, in case of audit or follow-up of subjects who participated in the study. The investigator agrees to obtain the sponsor's agreement prior to disposal, moving, or transferring of any study-related records. The sponsor will archive and retain all documents pertaining to the study according to local regulations.

12.7 Economics
There will be no financial compensation for study participants. Sponsoring of the trial from Endomagnetics Limited will be given as an institutional grant to cover costs for the research procedures involved in the trial.

12.8 Publications
After completion of the study, the results will be analysed, and a clinical study report will be prepared. Within one year after the end of the study, the sponsor will submit a final study report with the results of the study, including any publications/abstracts of the study, to the accredited Ethics Committee and the MPA.

The study results will be considered for publication in an international peer-reviewed journal and/or presented at scientific symposia and congresses. Both positive and negative results will be published.

12.9 Insurance
The study subjects are covered by the Swedish Patient Insurance.

13 STUDY ORGANIZATION
The study is conducted at Department of Surgery, Sahlgrenska University Hospital. Screening and enrolment of patients will be done from patients being referred for SLNB at the different study sites.

14 REFERENCES
1. Balch, C.M., *Detection of melanoma metastases with the sentinel node biopsy: the legacy of Donald L. Morton, MD (1934-2014).* Clin Exp Metastasis, 2018. **35**(5-6): p. 425-429.

2. Waanders, S., et al., *A handheld SPIO-based sentinel lymph node mapping device using differential magnetometry.* Phys Med Biol, 2016. **61**(22): p. 8120-8134.

3. Karakatsanis, A., et al., *Superparamagnetic iron oxide nanoparticles as the sole method for sentinel node biopsy detection in patients with breast cancer.* Br J Surg, 2017.

4. Karakatsanis, A., et al., *The Nordic SentiMag trial: a comparison of super paramagnetic iron oxide (SPIO) nanoparticles versus Tc(99) and patent blue in the detection of sentinel node (SN) in patients with breast cancer and a meta-analysis of earlier studies.* Breast Cancer Res Treat, 2016. **157**(2): p. 281-294.

5. Mirzaei N, Katsarelias D, Zaar P, Jalnefjord O, Johansson I, Leonhardt H, Wärnberg F, Olofsson Bagge R. Sentinel lymph node localization and staging with a low-dose of superparamagnetic iron oxide (SPIO) enhanced MRI and magnetometer in patients with cutaneous melanoma of the extremity - The MAGMEN feasibility study. Eur J Surg Oncol. 2022 Jan 1;S0748-7983(21)01439-6.

6. Andreas Karakatsanis, Kosmas Daskalakis, Peter Stålberg, Helena Olofsson, Yvette Andersson, Staffan Eriksson, Leif Bergkvist, Fredrik Wärnberg. The routine use of Super Paramagnetic Iron Oxide nanoparticles as the sole method for Sentinel Node Biopsy in patients with breast cancer. The MONOS study. Br J Surg. 2017 Nov;104(12):1675-1685.

7. Hersi AF, Pistiolis L, Dussan Luberth C, Vikhe-Patil E, Nilsson F, Mohammed I, Olofsson Bagge R, Wärnberg F, Eriksson S, Karakatsanis A. Optimizing Dose and Timing in Magnetic Tracer Techniques for Sentinel Lymph Node Detection in Early Breast Cancers: The Prospective Multicenter SentiDose Trial. Cancers (Basel). 2021 Feb 9;13(4):693.

8. Balkrishnan R, Kelly AP, McMichael A, Torok H. Improved quality of life with effective treatment of facial melasma: the pigment trial. J Drugs Dermatol 2004; 3(4): 377– 381