Clinical investigation plan (CIP)

High-Intensity Focused Ultrasound (HIFU), a novel method for treatment of Cutaneous Neurofibromas in Neurofibromatosis Type 1: Safety and efficacy

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Preface

This document is aimed at giving authorities the rationale, objectives, design and proposed analysis, methodology, monitoring, conduct and record-keeping of a clinical investigation for ablative treatment of cutaneous neurofibromas (cNF) by a new ultrasound therapeutic methodology developed for targeted treatment of skin lesions called high intensity focused ultrasound (HIFU). The document is structured to follow the outline template for clinical investigation plans stated in Annex A of ISO14155:2020.

The study is conducted in parallel in two centers, one in Copenhagen, Denmark and one in Gothenburg, Sweden; each center having special explorative tasks e.g. lesional control by noninvasive imaging using 20 MHz ultrasound imaging assessment (Copenhagen) and by punch biopsy (Gothenburg).

An addition, specific assessment of the ethical aspects of the investigation is included in Appendix 1. This assessment follows and discusses specific issues required by the national ethical committees in the two countries where the centers are located. For Denmark ethical issues are governed and approved by “De videnskabsetiske medicinske komiteer (VMK)”, while “Etikprövningsmyndigheten” governs ethical approvals in Sweden.

List of abbreviations and acronyms

NF1	–	Neurofibromatosis Type I
cNF	–	Cutaneous Neurofibromas
AE	–	Adverse Event
CIP	–	Clinical Investigation Plan (according to ISO14155:2020).
ClinRo	–	Clinician Reported Outcomes
CRF	–	Case Report Form
DK	–	Denmark
GCP	–	Good Clinical Practice
HIFU	–	High Intensity Focused Ultrasound
IB	–	Investigators Brochure
MDD	–	European Council Directive 93/42/EEC of 14 June 1993 concerning medical devices
MDR	–	Regulation (EU) 2017/745 Regulation of the European Parliament on medical devices
NFD	–	Nominal Focal Depth
OCT	–	Optical Coherence Tomography
PRO	–	Patient Reported Outcome
QoL	–	Quality of Life
SAE	–	Serious Adverse Event
SE	–	Sweden
US	–	Ultrasound Scanning

1 Identification of CIP

Protocol ID	cNF-HIFU2101. CIV-21-09-037759
Study type	Multicenter (two-center) open-label study in humans (hospital outpatients)
Study device	TOOsonix System ONE-M A 20 MHz focused ultrasound device for controlled ablative treatment of cNF
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Table 1. Planned activities in the clinical investigation. The investigation consists of a single treatment session (Visit 2) with 3 subsequent follow-up evaluations. The first data analysis and report on primary end-points will be done as an interim assessment after the 3-month visit has been completed by all participants (Visit 4). Primary and secondary end-points are concluded in a final report after all participants have completed the 9-month visit (Visit 5).

Visit number Event Name	1 Pre-study	2 Day 0	3 7 ± 3 days (1 week)	4 12 ± 2 weeks (3 months)	5 24 ± 2 weeks (6 months)	6 39 ± 3 weeks (9 months)
	Consent, Screening and Enrollment	Pre-treatment documentation, treatment, and post-treatment assessments	Evaluation of wound generation	Evaluation of healing and surveillance for any new lesions	Evaluation of healing and surveillance for any new lesions	Evaluation of healing and surveillance for any new lesions
Clinical evaluation	X	X	X	X	X	X
Inclusion and registration	X					
Informed consent	X					
Photo documentation 2D		X[1]	X	X	X	X
Photo documentation 3D		X[1]	X	X	X	X
Ultrasound Imaging Assessment (Bispebjerg Hospital)		X[1]	X	X	X	X
HIFU treatment		X[2]				
Participant questions and scoring		X[1]	X	X	X	X
Medical team questions and scoring		X[1]	X	X	X	X
Adverse Event Reporting		X[1]	X	X	X	X
Biopsy of 1-3 treated cNF[3] (Sahlgrenska Sjukhuset)			X	X		
Biopsy of 1 untreated cNF (Sahlgrenska Sjukhuset)			X			

1 = Data collection should occur immediately before treatment and again within one hour after treatment
2 = A minimum of 5 cNF 2-5 mm in size are required for enrollment. A minimum of 3 and a maxumum of 9 cNF will be treated and assessed across visits 2-6
3 = The minimum number of cNF to be biopsied is 3 (two treated and one control). The maximum number of cNF to be biopsied is 7. The biopsies will occur for cNF that are between 2-5 mm in size at visits 3 and 4. There will be one untreated cNF biopsied at visit 3. A minimum of one treated cNF will be biopsied at both visit 3 and visit 4.

1.1 Summary of the clinical investigation

The overall objective of this study is to evaluate the safety, tolerability, and efficacy of cutaneous neurofibromas (cNF) removal in adults with Neurofibromatosis Type 1 (NF1) using high-intensity focused ultrasound (HIFU).

With an incidence of 1/2500 to 1/3500, Neurofibromatosis Type I (NF1) is among the most common single-gene inherited conditions worldwide (1, 2). The genetic condition predisposes to multiple forms of histologically benign and malignant neoplasms. The most common tumor across all people with NF1 is cutaneous neurofibroma (cNF), impacting up to 99% of adults (3-7). These tumors can number in the hundreds to thousands on a given individual, most often appear between late school age and in the second decade, and increase in number and size across the lifespan (3-5, 8-12).

In many patients, cNF are socially debilitating, painful and impede activities of daily living (4, 5, 10-14). Multiple different appearances of cNF exist as reported from patients and clinicians. These can range from barely visible flat nodules with subtle discoloration, to large and pedunculated masses. One proposed classification system to describe the clinical appearance of cNF assigns one of five subcategories: nascent, flat, sessile, globular, or pedunculated (15). Nascent lesions (very small dispersed tumors) and peduncular neurofibromas (protruding tumors with a narrow base) are not subject to study as they are not logic candidates for HIFU treatment at this early stage. The typical appearance of the cNF-categories relevant for treatment in this study is illustrated in Fig. 1.

Despite the remarkable prevalence of cNFs in people with NF1 and their documented influence on quality of life (QoL), current treatment is limited to surgical removal or the use of various devices that cause tissue destruction showing mixed, but largely low-efficacy success, to date (11, 16-18).

While blade-based surgical removal allows complete removal of the lesion(s), this method is impractical to address the many tumors affecting a typical adult with NF1, thus forcing patients to select specific tumors to remove. Additional drawbacks of surgical resection include variable scarring, and the time required for the procedure and healing. Alternative approaches that enable the removal of larger numbers of lesions per session include electrosurgical ablation, laser-based treatments, and radiofrequency ablation all reported to have variable outcomes(11, 15-17, 19-22).

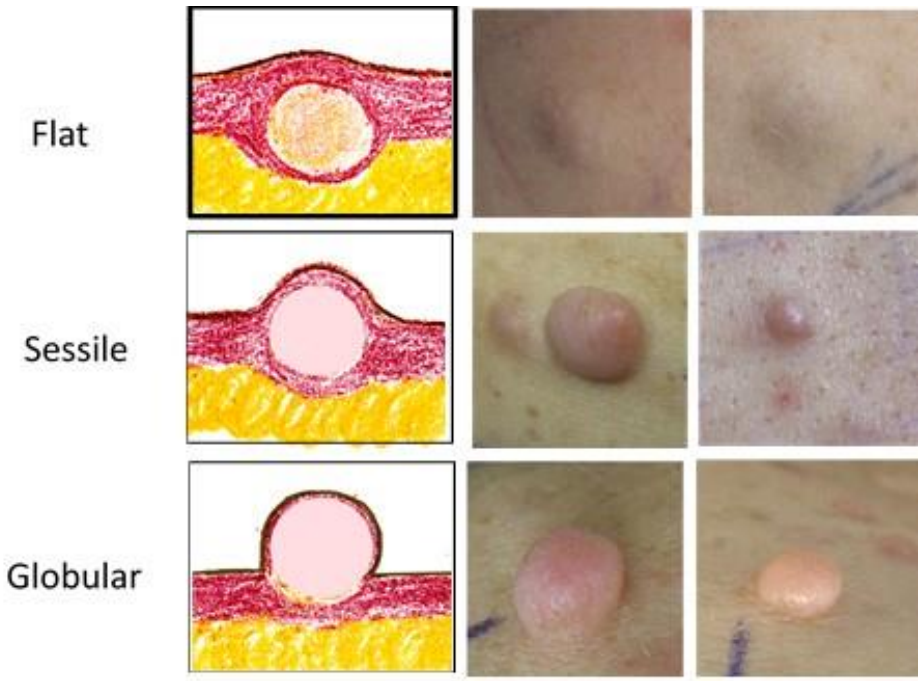

Fig. 1. Three subtypes of cutaneous neurofibromas considered for the current study.

The investigational device used in this study is a Danish developed system. As the first of its kind in the world, it is capable of controlled and targeted thermo-mechanical treatment to small intradermal volumes containing neoplastic cells, without inflicting damage to the surrounding tissue (23-26).

The device is proposed to be used in a non-ablative modality, where treatment does not damage the skin's basement membrane, and the skin surface thereby remain intact. Healing after treatment will thus be facilitated by internal cell transport through the vasculature, and risks of post-treatment complications or visible scarring will be minimized (23-25, 27).

The current protocol presents an investigation with an evaluation of the safety and efficacy profile 3 months after a single 3-5 minute treatment. Subsequent follow-up of secondary endpoints is done every third month until the end of the study 9 months after the treatment. The activities of the investigation are summarized in Table 1, and a graphic presentation of the patient-flow and actions are given in Fig. 2.

Provided that the expected positive clinical results can be verified, this new method could have potential for widespread use as an important replacement or compliment to existing methods for treatment of cNF.

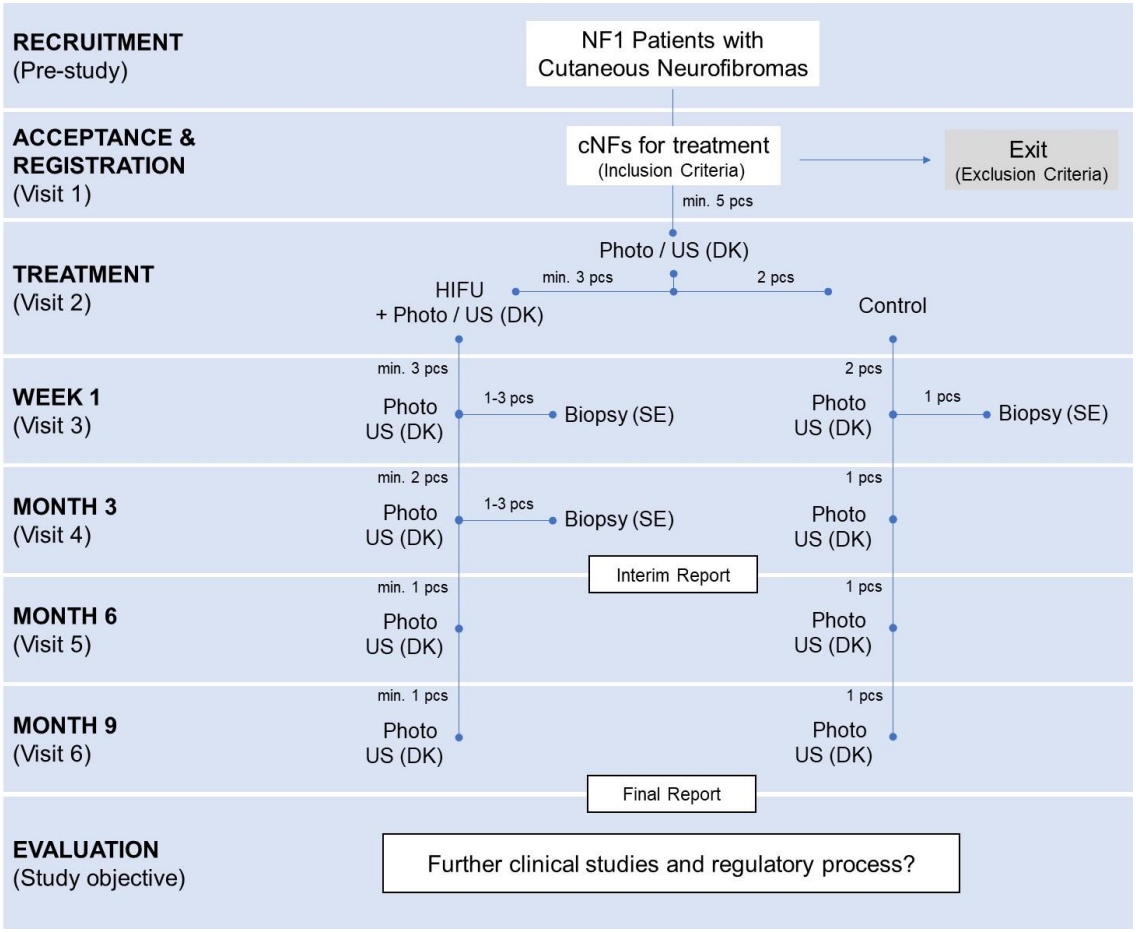

Fig. 2. Study flow chart. The study is an initial tolerance-study with the objective to investigate if a new therapeutic modality is safe, efficient and feasible, and thereby should be investigated further in larger future studies. cNF to be treated can be anywhere on the body with the exception of face, shoulders and the breast.

2 Identification and description of the investigational device

2.1 System ONE-M

The investigational device, called TOOsonix System ONE-M, is described in detail in the investigator's brochure, which includes details on model, type, software, accessories, the manufacturer, and the regulatory status of the device authorized for clinical studies on humans. The device is shown in Fig. 3.

Fig. 3. TOOsonix System ONE-M; 20 MHz focused ultrasound applied to skin with a selection of handpieces (depth of focal point in the skin 0.8, 1,3, 1,8 and 2,3 mm). Treatment is surveyed with a camera incorporated in the handpiece.

The operating principle of focused ultrasound has been used for decades for non-invasive treatment of lesions in internal organs (28-33). Acoustic energy is generated in a transducer with a concave geometry allowing the emitted sound to concentrate in a high-energy focal zone inside the body, where a thermal lesion can be positioned in the target of interest chosen to be treated.

Depending on instrument construction and setting, short pulses can produce a controlled thermal insult in the target. This target could be; e.g. a kidney stone or a cancer as exemplified by a breast cancer, thyroid cancer or a prostatic carcinoma resulting in thermal necrosis and destruction of the target. Focused ultrasound can also be used for treatment of intracranial targets, such as centers responsible for essential tremor and Parkinson's disease (26, 31, 34). The HIFU operating principle, which illustrates the application to skin, is shown in Fig. 4.

The convergent ultrasound beam, on its passage through tissues to reach the focal point, with proper settings, will not exert damage to the passage medium of tissue between transducer and target. Thus, the necrotizing or ablating effect can be set to match a desired level in the skin without causing effects in the adjacent tissue above, below and around.

Focused ultrasound devices, including the device under study, are designed to produce a focal heating in a focal point reaching up to 65 °C. This is a level that is high enough to produce cellular death in the focal zone, but low enough not to transmit major caloric energy to the surrounding tissue (23, 24).

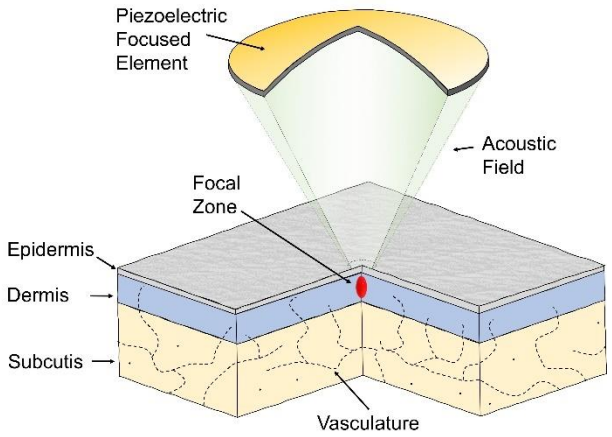

Fig. 4. Schematic illustration of the HIFU principle with concentration of the ultrasound energy inside the skin in a focal point exposed to a thermal insult causing cell death.

Treatment of internal organs with focused ultrasound has been documented in clinical research, and the method is now used around the world in oncology clinics of excellence (26). Treatment of internal targets in the body requires deep penetration and therefore need to operate with longer wavelengths at lower frequencies (from 500 kHz to 5 MHz), where attenuation of ultrasound is relatively low. For the treatment of dermal lesions, much smaller wavelengths at higher frequencies (> 15 - 20 MHz) is however needed, since targets, such as skin tumors, have small dimensions and need selective treatment with a small and well-controlled focal point both vertically and horizontally (23).

As the TOOsonix device is the first device operating at 20 MHz, the resolution allows for selective treatment of small sized targets in the skin. The device has shown efficacy in other diseases limited to the skin in which the goal was to eradicate the neoplasm without damaging the epidermis. There is therefore potential that the TOOsonix device can address multiple sizes and forms of cNF without the burden of scarring.

Practical operation of the investigational device is comparable to conventional dermatological lasers, which are covered in the formal specialist courses dermatologists must pass. An introductory training in focused ultrasound is needed, with practical training performed on some suitable tissue-like objects. TOOsonix A/S is offering introductory information, theoretically and practically, at the start of study, including instruction in the use of different hand pieces with different penetration depths.

During operation, a treatment dose of typically 150 milliseconds duration is applied to the skin under real-time supervision using the integrated camera showing a magnified image of the skin surface. Repeated ultrasound dosing is administered in close proximity to each other and positioned to cover the entire skin lesion in a “shoulder-by-shoulder” pattern. At the time of a dose-activation, a circular shockwave-effect on the outer skin is displayed, and it can be monitored and adjusted such that an applied dose is not too weak and not too strong.

Depending on the choice of hand piece (focal depth) used on the investigational device, the treatment can be selected to be ablative, with transport of dead cells into a superficial wound crust through a damaged basement membrane, or non-ablative with internal transport of dead cells through the body’s vasculature.

The device will be applied to cNFs that are intradermal. Visible cNFs commonly vary in diameter from 1 mm up to 1 cm in size. cNFs are, irrespective of size, typically covered by a roughly 150 µm

thick epidermis and 200-500 µm thick rim of dermis. Neurofibromas are by histology composed of Schwann cells, and are variable connective tissue components in a loose and poorly defined structure.

The treatment in this study is thus aimed at use in the non-ablative modality, where cell death in the dermis is created without damaging the basement membrane, subsequently minimizing the risk of broad tissue damage to dermal collagen, and thereby reducing unintended scar formation after healing.

To obtain the non-ablative treatment modality, hand pieces with nominal focal depth (NFD) depth of 1.8 mm or 2.3 mm will likely be the preferred standard in this study, while allowing for discretion of the investigator to use any available hand piece deemed appropriate based on the pre-treatment assessment of the thickness and invasion level of the tumor in the skin. Two more superficial hand pieces with NFD of 0.8 mm and 1.3 mm are available with the standard device accessories from the manufacturer.

The device displays the instrument settings and the number of shots applied during a treatment course, thereby relieving the operator of extraneous distractions. Additionally, the device works by ultrasound as the sole modality, relieving the patient the undue burden of additional medicines, electromagnetic radiation, or other more invasive measures.

3 Justification of the design of the clinical investigation

In this study, the TOOsonix System ONE-M is applied to cutaneous neurofibromas (cNF) consistent with neurofibromatosis type I (NF1).

Given the very low number of available dermatologic agents for the treatment of cNF, and the lack of data about the specific performance of these instruments and formal clinical assessment, this study will systematically assess the TOOsonix device for tolerability, biologic effect and efficacy as assessed by patient reported outcome and image assessment before and after treatment. This data will help determine if additional study of the TOOsonix device is warranted for cNF in people with NF1.

The investigational device is a Danish developed system aimed specifically at high frequency operation (20 MHz). At this high frequency, the device, as the first of its kind, allows controlled and targeted treatment of small intradermal volumes, thus with limited damage to the surrounding tissue.

The investigational device has passed pre-clinical studies in experimental laboratory models and in a safety study on Göttingen minipigs as reported in the IB and published in the literature (23, 24).

The pre-clinical experiments showed the following key results and conclusions:

- The 20 MHz ultrasound output is a well-defined ultrasound cone with a sharp focal point as demonstrated in laboratory measurements and in tissue-mimicking phantom gel simulating skin.
- The produced thermal lesion is highly reproducible from dose to dose.
- The depth of the focal point can be chosen and positioned as intended depending on the selected hand piece.
- The temperature in the focal point reproducibly matches 65 °C.

- The extension/severity of the heat effect in a phantom and read as necrotizing effect and heat lesion in the pig study was tunable within the range of available instrument settings, and reproducible.
- Depending on device setting, non-ablation subclinical effects can be induced, alternatively ablation/necrotizing effects, the former being relevant for cNF therapy.
- Post-treatment wound and healing studied in the pig followed over time (12 weeks) depended on the applied ultrasound energy.
- Isolated single dosing had much lower, or no, necrotizing effects as compared to multiple shoulder-by-shoulder dosing applied to a larger field.
- Histology after three months indicated mild fibrosis in pigs treated with higher ultrasound energy, however, clinical scar formation was not visible, or only barely visible.
- Studies indicated the depth of the ultrasound lesion in the dermis are well controlled by way of proper selection of instrument settings, and the choice of hand piece.
- Depending on dose, a transient wheal and flare reaction, with redness and edematous swelling, occurred within minutes and faded within about 30 min. This reaction is comparable to skin reactions from dermatological lasers. The reaction is considered to be caused by traumatic histamine release.
- Ultrasound treatment in clinical use may be associated with instant pain and possibly itch.
- Studies indicated no unexpected adverse events, and no systemic safety concerns.

The skin lesions observed in the pig models and human treatments therefore justifies the present protocol addressing treatment of human cNF, which are superficial skin lesions of small size, and easy to cover with visually guided shoulder-by-shoulder applied ultrasound shots.

This study is the original and the first systematic study of 20 MHz focused ultrasound applied to NF1 associated cutaneous neurofibromas. The method has the potential to open a new venue of clinically useful tumor mitigation therapy in people with NF1 where there is a major unmet need.

4 Benefits and risks of the investigational device, clinical procedure, and clinical investigation

The anticipated benefit of the 20 MHz HIFU modality in treatment of benign neurofibromas is to develop and confirm a new targeted therapy with a high benefit-risk ratio compared to existing surgical methods.

Cutaneous NFs for treatment will be selected in agreement with patients. Visible skin areas will be avoided since scar-formation resulting from HIFU treatment of cNF is not yet documented. Specifically, lesions on the face, shoulders and breasts will not be treated as these areas are especially vulnerable to development of scaring.

Participants shall be informed about potential risks and discomforts of the HIFU treatment before the treatment is applied. Persons having psychological or neurologic risk of pain are not included in the study. Persons with any acute or chronic medical condition, which in the opinion of the investigator, could interfere with this study are also not included in the study.

At the Danish center, the study will include ultrasound imaging assessment before and after HIFU treatment. This method is non-invasive and known to be without risks to the patients.

At the Swedish center, the study includes a minimum of 3 and a maximum 7 small punch biopsies taken from control and treated lesions during the study. Topical anesthesia will be used according

to normal routine before biopsy. Biopsies taken on cNFs are part of the routine treatment regime, and the overall risk is known to be minor and serious adverse events are very unlikely.

Focused ultrasound applied as short doses producing 65 °C heat in a cancer is widely documented in the literature as having a necrotizing effect on internal cancer tissue followed by clinical healing or cancer reduction (28-34). It is expected that the HIFU lesions in skin are equally sensitive to ultrasound generated heat.

The expectation regarding treatment of skin is therefore high, since these tumors are small and superficial, and therefore visible and easy to diagnose and target. The treatment effect can be dosed precisely under real-time visual supervision from the investigational device.

Anticipated local adverse effects are immediate wheal and flare reactions at the treatment site, and complications of wound healing, including the potential risk of bacterial infection and scarring. The former is significantly reduced when treatment is administered in the proposed non-ablative modality, where the skin barrier remains intact, and bacterial access thereby remains closed. Risk of scarring after healing of insults to the lower half of the dermis is known, but also depends on anatomical site and constitutional factors (24). Compared to potential scarring from healing of open surgical wounds the visual result is however considered to be very beneficial.

The treatment carries very low potential risk of systemic adverse effects except for pain on treatment due to heating in the treatment zone. Pain has an instant onset, but is very short, since the preferred pulse duration is only 150 milliseconds. However, participants shall be instructed about pain of treatment before treatment is provided.

Histamine release in the treated area may cause wheal-and-flare associated with itch on the treated site, however, traumatic wheals are short-lived and fade over 20-30 min (24). HIFU is, in comparison to light, not expected to interfere with the effect or metabolism of circulating drugs.

Finally, HIFU is a physical acoustic wave that can be transmitted through coupled media only. Unlike in laser treatments, users or patients are therefore not at risk of any unintentional exposure. The treatment can thus be conducted in a normal clinic setting without use of eye-protection PPE etc.

The results of this research may gain further understanding of use of these treatments in providing a new tool for treating cNF and potentially helping prevent growth of such lesions. There may or may not be direct benefits to participants in this study, however previous trials showed improvement in lesion appearance/clearance, as well as high patient satisfaction.

5 Objectives and hypothesis of the clinical investigation

The hypothesis of this study is, that small and highly localized acoustic focal zones produced by a new HIFU device non-invasively can induce acute cell-death in cutaneous neurofibromas (cNF) without disrupting the skin's basement membrane. The healing of the treated volume will subsequently be facilitated by internal cell-transport through the vasculature without creation of open wounds. This therapeutic modality will thus give less post-treatment complications and less visible scarring compared to surgical procedures that are currently the only standard treatment for removal of cNFs in patients carrying the Neurofibromatosis Type 1 genetic condition.

The objective of the study is therefore to document that 20 MHz focused ultrasound is safe and effective in the treatment of NF1 associated cNF with high reduction of the measured volume of each treated tumor after 3 months, and with follow up registration of potential regrowth or development of new tumors in the treated and untreated regions at 6 and 9 months.

5.1 Primary endpoint - Tolerability

- The grade of any adverse events (AE) requiring medical intervention within three months of treatment should be low. Device-based treatment will be considered tolerable if less than 30% of participants treated have an "Overall AE Score" larger than Grade 2 AE at the 3-month follow-up (visit 4) as assessed via Common Terminology Criteria for Adverse Events (CTCAE), U.S. Department of health and human services, Ver 5.0, Nov 27, 2017.

- The rate and nature of spontaneous healing of the treated cNF lesion and any safety related event including potential wound formation and wound healing should be equivalent to expectations from alternative method(s). Safety evaluation includes rating of treatment associated sequele by end of study, e.g. dyspigmentation and scarring and overall investigator rating compared to expectations.

5.2 Secondary endpoints – Efficacy, safety and feasibility

- Efficacy: Rate of clearance, e.g. reduction of tumor mass of treated cNFs based on 2D and 3D photography or ultrasound imaging assessment at 3 and 9 months parallel to assessment of the degree of dyspigmentation with hypermelanosis or hypomelanosis of the lesion and the perilesional untreated skin.
- Safety: Itch and pain from the treatment measured on a 0-10 point numerical scale.
- Safety: Wheal and flare reaction and inflammatory responses are measured on a graded scale.
- Safety: Any objective adverse effect or event, local or systemic, related to the treatment and the investigational device is measured (wound and course of wound healing, scar formation, instrumental hazards) is categorized immediately after treatment and at all follow-up visits.
- Feasibility: Feasibility is evaluated both quantitatively (time spent on treatment) and qualitatively depending on the object considered and cover elements that may compromise or favor HIFU relative to other treatments employed for lesions in clinical practice or from the literature. Feasibility assessment will not produce a direct comparison with alternative methods since the study has no arm with an alternative method. The evaluation comprise the following essentials:
    1) Time and resource spent on the treatment session using the device,
    2) Investigators' assessment of the practice of using the device and the treatment,
    3) Participants'' assessment of satisfaction with the treatment and the device.

  All skin toxicities will be grated according to the classifications in Common Terminology Criteria for Adverse Events (CTCAE), U.S. Department of health and human services, Ver 5.0, Nov 27, 2017.

5.3 Exploratory endpoints – Diagnosis and measurement methods

A core question to be addressed in this study is the effects of the thermal energy delivered to the treated cNF. Specifically, the study will seek to understand if the entire cNF tumor receives equal thermal energy by the method, if there are differences in dose relative to depth of the lesion, and what the inflammatory response within and around cNF is at one week (in the early stage of reaction) and after 3-6 months when coagulation and connective tissue repair are expected to be completed.

The inflammatory infiltrate observed after one week will indicate the severity and extension of the HIFU thermal effect on the cNF, and it can be assesses if the entire lesion was given full treatment or not. This data will be necessary to select recommended instrument setting and dose interval in future efficacy studies.

Both centers will assess the tumor's horizontal extension and skin elevation including typing (flat, sessile, globular) before and after treatment using 2D and 3D digital images as a part of the secondary efficacy endpoint. Additional exploratory end-point for qualitative and quantitative evaluations of the above characteristics and response to the method will be based on existing expertise and routine at the respective centers.

5.3.1 Ultrasound imaging assessment and characterization of treated CNF (Denmark)

Ultrasound imaging assessment will be used to assess the vertical thickness and depth of tumors at each time point of the study using a 20 MHz B-Mode ultrasound scanner. The scanner allows non-invasive measurement of tumor depth, cross-section area and quantitative characterization of e.g. various inflammatory responses. Measurements made on the tumors before HIFU, immediately after treatment, and at all follow-up visits will be noted directly in the study CRF. The combined conclusions regarding the use of ultrasound imaging assessment as a method for assessing the safety and efficacy of the HIFU treatment of cNF will be summarized by the center investigator in the final study report.

5.3.2 Biopsy of Cutaneous Neurofibroma (Sweden)

In Sweden, treated and reference untreated cNF from the same anatomical region will be subjected to biopsy and histological evaluation as a means of characterizing the above post-treatment characteristics. Small punch biopsies are preferred, but the investigator may decide to perform a surgical biopsy, or a shave biopsy, depending on participant's preference and the aim to possibly remove the entire lesion with the biopsy.

After the first three patients have been treated and have had the 3-month biopsy completed, the tissue will be assessed by histopathologists to assess if the pathology is stationary or not. The combined conclusions regarding the use of biopsy as a method for assessing the safety and efficacy of the HIFU treatment of cNF will be summarized by the center investigator in the final study report.

6 Design of the clinical investigation

6.1 General

The study is a two-center, open-label study in humans (hospital outpatients).

The two centers are both located in EU, and thus follow the same overall regulatory framework for clinical research and approvals.

The center located in Denmark, Department of Dermatology, Bispebjerg Hospital Region Copenhagen Capital, is a department under the Copenhagen University. The department provides advanced dermatological treatment, and is the largest dermatology department in Denmark.

The center located in Sweden, Sahlgrenska University Hospital is a non-profit University hospital owned and managed by Västra Götaland Region. It is a secondary referral center for dermatological patients and serves a population of about 750 000 in and around city of Gothenburg.

6.2 Selection and grouping of tumors

Tumors to be included in the study will be selected and documented in the treatment session (visit 2) via collaboration between the participant and the treating physician. According to the willingness of the participant there may be 1-3 triplets of tumors representing all three categories (flat, sessile and globular) which would make a total of 3-9 cNF tumors treated and 3-7 cNF tumors biopsied per participant. The minimum number of cNF to be treated is three. The maximum number of cNF to be treated is 9. The minimum number of cNF to be biopsied is 3 and the maximum number of cNF to be biopsied is 7 to limit discomfort or inconvenience associated with healing. Ideally, paired sets of tumors representing all three categories (flat, sessile and globular) will be included. However, it is acknowledged that this may not be feasible to identify all three categories of tumors in pairs in each participant. It will be noted in the CRF which cNF is the control/reference and which are treated and sampled. The selected tumors will be documented by 2D and 3D photos.

6.3 Characterization of treatment response

The two centers will use identical clinical criteria of diagnosis of cNF which is a clinical diagnosis made in a person with NF1 who has skin neoplasms involving the dermis (see Fig. 1).

The cNFs to be treated will initially be described relative to anatomical site, tumor size at baseline, cNF subtype (Fig. 1), pigment, hair, and associated symptoms and signs at the time of treatment. The rationale for selection of tumors will be described (pain, itch, disfigurement). The selected tumors will be documented by 2D and 3D photos.

The centers will furthermore use two different methods to document the base-line state of the selected cNFs, and the response and result of the treatment immediately after treatment, and in the subsequent follow-up visits. Each method is well established in the respective department routine. In addition to the digital images, in Denmark cNF will be measured with 20 MHz pulsed ultrasound imaging and in Sweden a selection of cNF tumors will be biopsied (see section 6.2 and 6.3.2).

6.3.1 Denmark - High-frequency ultrasound cross-sectional ultrasound imaging.

In Denmark, measurements of the baseline state of cNFs to be treated, and subsequent response to the treatment will be based on 20 MHz pulsed ultrasound imaging.

20 MHz ultrasound assessment has been used in many fields in dermatology for measurement of skin and tumor thickness and for characterization of inflammatory skin diseases since its introduction in 1979. Tumors and inflammation create low echogenic vertical profiles in the dermis, which can be measured by ultrasound imaging, including an analysis of the image density registered as low echogenic pixels (27).

The DermascanC, from Cortex Technology, Hadsund, Denmark will be used. Ultrasound imaging assessment with this system creates real-time, cross-sectional images to a depth of up to 4 mm with axial resolution about 25 µm and lateral resolution about 70 µm. The ultrasound pulses are of low energy and involves no safety concerns related to persons being scanned, and has no therapeutic effect confounding the HIFU treatment.

Scanning is non-invasive and not associated with any pain or discomfort. In this study the manual method of recording in vivo distances and thickness will be applied, and not the automatic facility having less accuracy (27). An example of an ultrasound imaging of a dermal tumor (not cNF) with qualitative measurement of the cross-sectional area is shown in Fig. 5.

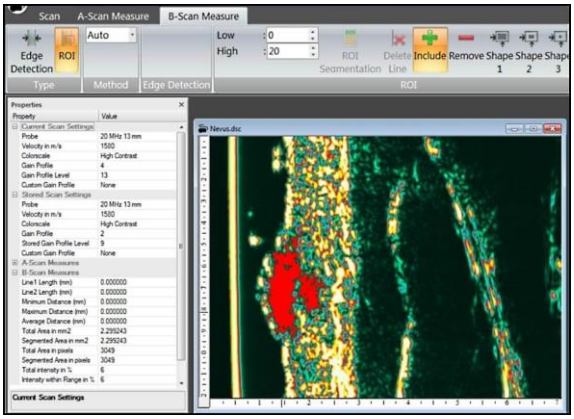

Fig. 5. Example of measurement of a tumor cross-section using the Cortex DermaScan C device (not cNF). Source: https://cortex.dk/scientific-ultrasound-dermascan-c/

Ultrasound imaging assessment will be performed on all treated and control cNFs included in the evaluation at baseline (visit 2), one week after treatment (visit 3), 12 weeks after treatment (visit 4), 24 weeks/6 months (visit 5) and 39 weeks/9 months (visit 6). This will be done concurrent with 2D and 3D photos (Table 1).

6.3.2 6.3.2 Sweden – Biopsy

In Sweden, patients will undergo biopsy of a minimum of 3 cNFs (1 control/reference, 1 cNF biopsied one week after treatment, and 1 cNF at 12 weeks after treatment) and a maximum of 7 (1 control and 6 treated cNF) to characterize the baseline and treated response at two time points (1 and 12 weeks after treatment) (**Fig. 6**).

The control biopsy will be taken one week after the treatment (visit 3). Biopsies of the treated tumors will be taken one week after treatment (visit 3) and 3 months after treatment (visit 4). The pathologic analysis of these tumor samples will illustrate the short-term inflammatory response and mid-term healing effects respectively. If the patient is willing to have more tumors treated and biopsied, a maximum of 1 control biopsy and up to 6 biopsies from treated tumors per patient will be taken throughout the study. The schedule for biopsy characterization is illustrated in Fig. 6.

Tumors subject to biopsy are lost for clinical follow up, thus, up to nine lesions per patient will preferably be included in the study in the Swedish center. cNFs selected for biopsy must be 2-5 mm in diameter. Biopsy is preferably taken as a punch biopsy, but the investigator may decide to perform a surgical biopsy or a shave biopsy depending on participant's preference and the aim to possibly remove the entire lesion with the biopsy.

The tissue collected via biopsy will be assessed with standard histology and immunohistochemistry.

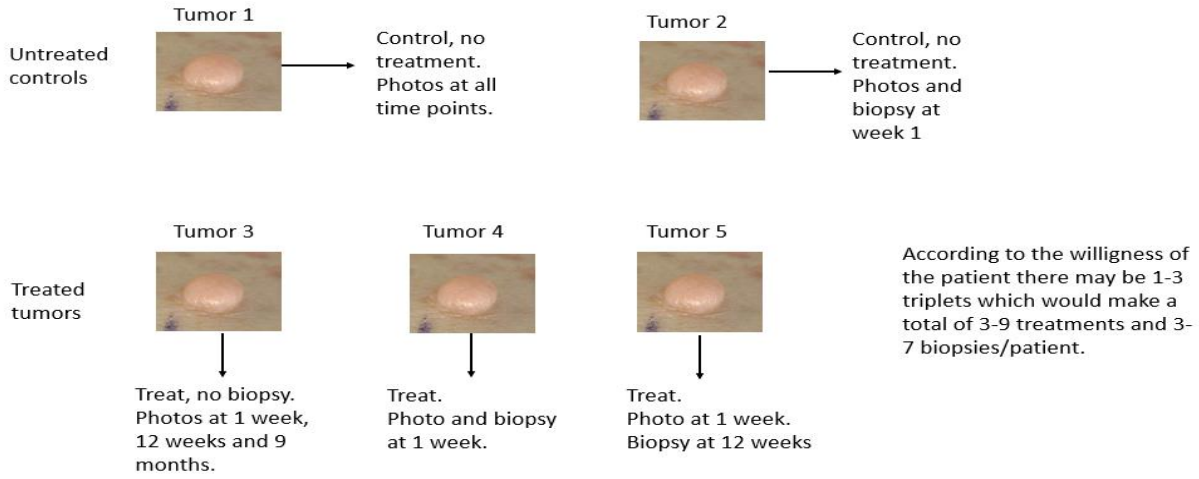

Fig 6. Schema for cutaneous neurofibroma biopsies.

6.4 Investigational device

The investigational device, TOOsonix System ONE-M, was CE-marked as an electrical medical device according to MDD as a Class I device in July 2020. The device therefore conforms to the relevant safety requirements needed for human treatments in the harmonized IEC standards covering medical devices. Type testing was performed by TÜV SÜD, with a range of supporting evaluations and tests performed by internal and external parties.

The device is indicated for a range of aesthetic procedures, and has furthermore been used in case-based treatments, on the doctor's clinical responsibility, for selected clinical volunteers that could not helped by other treatments.

Recently a GCP clinical Investigation on removal of non-nodular basal cell carcinoma using the device was submitted and conditionally approved by the Danish medical and ethics authorities. The study is planned to start in the autumn 2021 after all final relevant contracts and have been signed.

During operation, the HIFU device is not in contact with the participant except for the touch from the tip of the hand piece held against the skin surface, with a standard ultrasound gel used as coupling medium between skin and probe. There is no electrical contact between device and participant. The contact involves no physical insult, cut or injection, and no breakage of the skin barrier. Probe tip and cables are validated allergen free and biocompatible.

No comparator device is included in the study, and no medicinal product is involved.

6.5 Participants

Study participants are recruited among the hospital patients of the two centers, invited out of the routine offering them different treatment options. Further recruitment may be made by direct referral of eligible patients after contact to other relevant hospital departments and centers or patient associations in these the respective countries.

Diagnosis of their cNF is according to standard routine of the hospitals. Confirmation of the neurofibromatosis syndrome by biopsy is not used in the routine since the disease is characteristic and commonly diagnosed by simple means e.g. history and clinical signs. Patients may have been evaluated genetically or not. Participants' enrolment as hospital patients continues along with their participation in the study.

Because most patients with NF1 and cNF wish many of their cNFs treated, there is no upper limit of number to be treated according to the protocol. The patient and the physician will decide the cNFs to be treated.

6.5.1 Inclusion criteria and selection

- Males and females 18 years of age or older.
- Have a clinical diagnosis of NF1 based on the most recent published criteria (35) (Appendix 2)
- Patients must be seeking active treatment for cNF.
- Patients must have ≥ 5 cNF that are visible and measure a minimum of 2 mm in size. These must be in areas amenable to treatment and surveillance.
- Patients with Fitzpatrick Type I to VI skin-type
- Able and willing to comply with all visits, treatments, evaluations, schedules, and requirements.
- Patients shall have received oral and written study information, accepted participation and signed the informed consent document.
- Patients who are willing and mentally and physically capable to understand and follow the treatment and follow-up schedule including post-treatment care instructions.
- Patients who are willing to have photographs and images taken of the treated lesions to be used anonymously or coded in evaluations and publications.

6.5.2 Exclusion criteria for participant selection (non-inclusion criteria)

- Patients who are undergoing other treatment modalities or investigational agents for their cNF lesions.
- Individuals who cannot give informed consent or adhere to study schedule.
- Patients who are actively tanning during the course of the study.
- Patients with adverse reactions to compounds of any external agent in use.
- Patients with known allergy to injectable anesthetics (relevant for biopsy only).
- Patients with any condition which, in the Investigator's opinion, would make it unsafe (for the participant or study personnel) to treat the participant as part of this research study.
- Patients, where target treatment may cause the acoustic beam to enter the eye.
- Pregnant females, due to possible discomfort with the procedure even though the procedure is localized and there is no new drug.
- Patients with tendency for keloid and hypertrophic scar formation.
- Patients with impaired wound healing.
- Patients with any other acute or chronic condition which, in the opinion of the investigator, could interfere with the conduct of the study.

6.5.3 Criteria and procedures for participant withdrawal or lost to follow up

- Exclusion criteria identical to non-inclusion criteria, however, appearing during the study.
- Patients acquiring disease of higher priority contraindicating their further participation or making participation not possible in practice.
- Patients' decision to leave the trial, irrespective of reason.
- Patients' non-adherence to the study schedule.
- Unforeseen hazards or significant clinical adverse events making continuation of the study not ethical.

6.5.4 Withdrawal from the Study

Each participant has the right to withdraw from the study at any time without prejudice. The investigator may discontinue any participant's participation for any reason, including failure to comply with the protocol (as judged by the investigator such as compliance below 80%, failure to maintain appointments, etc.).

Should a participant withdraw from the study, the reason(s) must be stated on the case report form, and a final evaluation of the participant should be performed.

Patients who go off treatment must be followed for AEs for at least 30 days from treatment or biopsy.

Reason for withdrawal from study is documented in the case report form. Withdrawals after HIFU treatment and before the 3-month visit are replaced and not included in the analysis of results (but registered as dropouts). Withdrawals after 3 months are replaced on investigators discretion.

6.5.5 Study Termination Criteria

The study may be terminated for multiple potential reasons. Some of these reasons include:

- Majority of participants experience severe and unbearable pain, on investigator's decision based on a minimum of 3 participants.

- Unbearable immediate wheal and flare reaction with severe itch and swelling and urticaria disseminating to larger skin areas experienced by a minimum 3 patients, on investigator's decision as indicated above.
- Any systematic (general or local) acute response to the treatment deemed by the investigator to be a clinically significantly different clinical or psychological reaction or adverse event than expected.
- The efficacy of the treatment is in the majority of participants at the 3-month interim assessment (visit 4) deemed to be unacceptably poor by the investigator or the sponsor. Unacceptable efficacy is defined as cure rate below 20% at the 3-month visit, based on a sample of minimum 8 participants in each group of the study.

6.5.6 Participant enrollment and handling

Every effort will be made to arrange routine clinical follow-up for all participants, independent of how many study-associated visits they have completed.

Enrolment into study will take place at the two participating hospital departments (Department of Dermatology, Bispebjerg University Hospital and Department of Dermatology and Venereology, Sahlgrenska University Hospital).

The total duration of the study from enrolment of first participant to end of data collection is expected to be 1.5 years.

Expected total duration of each participants' participation is 9 months, with milestone evaluation after every 3 months.

In total 20 participants will be enrolled (target enrolment is 10 participants at Department of Dermatology, Bispebjerg University Hospital and 10 participants at Department of Dermatology and Venereology, Sahlgrenska University Hospital). Enrolment is not stratified. Limits can be changed based on the coordinating investigators discretion if recruitment to one study group turns out being not possible or according to experience unrealistic. Enrolment is based on participants remitted to the centers for diagnosis and treatment of NF1. Participants with urgent treatment need are not enrolled.

Participants of the study continue their course and normal registration in the hospital department. Each study visit is registered and documented in the hospital file parallel to registrations in the case reports of the study.

Histology taken at the Swedish center will be labeled using the study codes which do not contain the personal identification number of the study person. The samples will be used only for research purpose and not for patients care and treatment. The samples will be analyzed separately by a pathologist and the PI in Sweden. After the analyses the samples will be stored in Biobank Väst in Sahlgrenska Universitetssjukhuset,

6.6 Procedures

The study will be conducted according to the requirements of Good Clinical Practice (GCP) as per ISO 14155:2020.

The study personnel will inform the participants verbally and in a participant information document in the respective local language on the experimental procedure to be utilized, and assure the participants that their decision regarding participation in the study will have no bearing on the quality

of medical care received, and that their decision whether to participate in the study is strictly voluntary.

During the initial interview, the participants will be assured that they are free to change their mind and will be allowed to participate in the study or withdraw from the study with no adverse effect on their standard medical care. Participants will have at least 24 hours to consider their consider their participation.

The study personnel will obtain written Informed Consent prior to the participants' participation in any study procedure.

The investigational device is described in detail in the investigator's brochure. The device is used in the study as described in the brochure. The two devices used in the study are identical and validated to be so by the equipment supplier.

The standard choice of hand piece will typically be those with a focal point depth of 1.8 or 2.3 mm. The hand piece depth shall match the estimated thickness of the tumor best possible. The hand piece with focal depth 1.8 mm will be preferred for treatment on the extremities, where the skin is known to be relatively thin. For treatments on the trunk, where skin is known to be thicker, the hand piece with focal depth of 2.3 mm will be preferred. The choice will be guided by tumor size and thickness of the overlying skin, location, and the investigator can decide to use hand piece best suited for the case. The tumor shall preferably be exposed to ultrasound down to the bottom of the tumor, but not so deep as to increase risk of post-treatment scarring. The selected hand piece applied for each tumor is documented in the CRF.

Prior to the HIFU treatment, the selected area will be sterilized with an alcohol swab and the selected area will be outlined with a pen where necessary. The tumor will be documented by a photo, repeated immediately post treatment, and at the planned visits after 1-week, 3 months and 9 months.

HIFU treatment will primarily be administered with shot-duration of 150 milliseconds, as this has been demonstrated in an animal safety study and in human treatments to be effective, and at the same time sufficiently short to avoid disturbing effects from movement of the hand piece during energy delivery.

HIFU power will be selected dependent on treatment site and skin response in initial test shots applied to the tumor. Power levels between 4 to 9 W acoustic power is the range of operation of the device. The acoustic energy dosage will, thus, be in the range of 0.6 to 1.3 J/shot.

On-going ex-vivo results will be used to validate these treatment guidelines and potentially adjust accordingly. Adjustments, if relevant, will not exceed existing certified clinical settings or existing standard limitations of the device.

The investigator can decrease or increase the energy level guided by the visual assessment of the observed treatment dose effect in the tumor. A HIFU-dose shall produce an immediate visible whitening or contraction of a circular area sized about 1-2 mm around the target center. This is observed through the integrated dermoscope allowing direct control of the treated field. Dosing is applied as shoulder-by-shoulder covering the entire tumor and 2 – 3 mm of perilesional skin, immediately around the tumor, with 1-2 mm between each dose. The investigator can choose to treat the surrounding (non-thickened) skin with lower energy to save normal tissue and minimize the post-treatment wound.

The treatment of a cNF, which is in the range of 2 - 12 mm in horizontal width, is expected to require approximately 25 - 50 shots spaced nearby each other, “shoulder-by-shoulder”. Elapse between shots is some 2 - 3 seconds. The total direct time for a treatment is, thus, estimated to be not more than 5 minutes, including small pauses to monitor the treatment effect and the need of adjustment of device setting. The total time for the treatment session is estimated to be in the range 20 – 40 min.

The equipment supplier is responsible for control and maintenance of the investigational device during the study including check of the energy output.

The equipment supplier has a third device in stock available for the study in case of instrument breakdown of a study device during study.

Clinical recording of the study includes clinical assessment of healing and therapeutic effect on the tumors. Such assessment follows clinical criteria described in textbooks, guidelines and clinical consensus in the specialty of dermatology. Prior to study, the two investigator sites have coordinated their clinical assessment method.

The chosen 9-month total study period is considered acceptable for observation of tolerance and curative effect of HIFU for cNF with observation of primary therapeutic effect after 3 months. Assessments includes monitoring of the wound healing phase, observation of late recurrences, and observation of scarring.

The study includes no follow up medication, but the study can involve use of wound healing dressings and other remedies in the wound healing phase on investigators individual recommendation. The study involves no standard recommendation on post treatment care, which is anticipated to be simple.

After the study participants are followed in the respective hospital departments according to their needs and the department routines.

Participants who drop out of the study before the 3-month visit voluntarily or for non-medical reasons, including non-adherence, will be replaced by new participant(s). Withdrawals after 3 months are replaced on investigators discretion with considerations to the impact of the overall investigation schedule.

Patient with incomplete cure documented by ultrasound imaging assessment or biopsy and histology after 3 months or subsequent control visit (indicated by clinical suspicion of reoccurrence) will be offered a second treatment based on the standard treatment offered by the center. The specific treatment will be decided by the investigator and the patient based on the extent and severity of the recurrence.

The study will conclude when all participants have completed the 9-month study period, when all data are on file, and the statistical analysis is concluded. Interim analysis of data collected after 3 months and later is allowed, per investigator’s decision.

If the study design is deemed inferior, the protocol can be amended.

The study produces no physical test samples relevant for purposes outside the study.

6.7 Monitoring plan

The study will be monitored separately in the two centers. In Denmark, it is intended that the study is monitored by GCP-enheden ved Københavns Universitetshospital. In Sweden, the study is intended to be monitored by Gothia Forum for Clinical Studies. Both are units serving the respective regional healthcare researchers.

As the monitoring unit in the region of the sponsor's center, the Danish GCP unit will have the responsibility to coordinate a shared monitoring plan, where each party monitors the investigation center. The monitoring plan will be made with due consideration to the centers designated tasks and reflect considerations to specific risks in the tasks performed.

An overview of monitoring activities and focus-areas are given in Table 4.

Table 4. Monitoring activities in the two monitoring units used by the investigation centers.

Investigation activity	**Stage / Visit**	**GCP Unit KU, DK**	**Gothia Forum, SE**
Monitoring plan	Before	X	(x)
Initiation of study	Before	X	X
Recruitment	Before	X	X
Screening and inclusion/exclusion	Visit 1	X	X
Selection and documentation of lesions for treatment	Visit 2	X	X
HIFU Treatment	Visit 2	X	X
Post treatment documentation and evaluation by investigator and patient	Visit 2	X	X
Follow-up visits with documentation and evaluation by investigator and patient	Visit 3 - 6	X	X
Ultrasound scanning – Procedure and analysis from measurements	Visit 2 – 6	X	
Biopsies – Procedure and analysis from pathological report	Visit 2 – 4		X
CRF entry and REDcap	Visit 1 - 6	X	X
Statistical analysis	After		X
Study Reporting	After	X	
Close of study – Archive readiness	After	X	X

7 Statistical design and analysis

The number of patients recruited for participation in the study will be balanced with due consideration to ethical aspects and the statistical background for conclusive statements regarding the primary endpoint.

As mentioned above, the primary endpoint of the study is to document the tolerability and safety profile of the treatment. As the main measurable endpoint, the grade of overall adverse effects following treatment is best suited for evaluation of participants in the study. The measurement of this endpoint is done subjectively by the investigator according to a conventional grading system, and is measured directly after treatment and at all subsequent control visits. The input template for measurement is illustrated in Fig. 7.

Investigator overall scoring of treatment side effect severity (one X)					
Absent / Normal	**Mild**	**Moderate**	**Severe**	**Life-threatening or disabling**	**Death**
Grade 0	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5

Fig 7. The primary end-point of the study is the investigator's grading of adverse effects following treatment as measured directly after treatment and in all subsequent patient follow-up visits.

According to the defined endpoint, the minimum number of patients must therefore be calculated to support a sufficiently high power for the statement that not more than 30% of patients will experience an overall side-effect of Grade 3 or above based on Common Terminology Criteria for Adverse Events (CTCAE), U.S. Department of health and human services, Ver 5.0, Nov 27, 2017.

Secondary endpoints regarding efficacy, feasibility etc. are also measured and analyzed throughout the study, and form important data for analysis, but are not included in the calculation of the minimum number of patients.

To obtain an initial indication of the necessary number of participants needed in the study, a simple estimation based on the conventional calculation of sample size to estimate a proportion or apparent prevalence with specified precision can be used.

A simple binary outcome is considered:

- Accept: Participants have Grade 2 side effects or lower
- Reject: Participants have Grade 3 side effects or higher

Based on substantial previous clinical experience with the study device (used for other skin treatments), severe side effects and adverse events are very rare, and an estimated proportion of 90% having Grade 2 or lower side-effects is therefore a reasonable starting point for the calculation (P=0.90).

As the measurement of side-effects by the investigator is subjective and with a quite course resolution, a total error can be set to 30% (e = ±0.15), roughly reflecting the interval between the six possible gradings. A standard confidence interval of 95% can be used (Z = 1.96).

At these inputs, the minimum population for the investigation can be calculated as follows:

$$N = (Z^2 \times P \times (1 - P))/e^2 = (1.96^2 \times 0.90 \times (1 - 0.90)/0.15^2) = 15.37$$

At 16 participants in the study, it is therefore possible to say with 95% confidence that the results of treatments will fall within the acceptable group. The minimum number of participants at various other proportions is shown in Table 2.

Table 2. Calculated minimum population in an investigation for obtaining statistical significance at a cure rate of p and with a error of ±15%

p	0,95	0,90	0,85	0,80	0,75	0,70	0,65	0,60	0,55	0,50
N	9	16	22	28	33	36	39	41	43	43

In a second step, power curves can be calculated against an alternative base-line of equal proportions having acceptable and rejectable grades of side-effects (the null hypothesis).

Fig 8. shows the power curve for the study containing the above minimum 16 participants as a function of the actual obtained passed AE-profiles. At the hypothesis of 90% having an acceptable AE-profile after HIFU treatment the power is calculated to be 71%.

Similarly, curves correlating the number of necessary participants at fixed power-levels of 80% and 90% at the hypothesized 90% having an acceptable AE-profile after HIFU treatment can be calculated. As shown in Fig. 9, the study will require 20 participants if a power of 80% is needed, while 26 participants will be needed to reach a power of 90%.

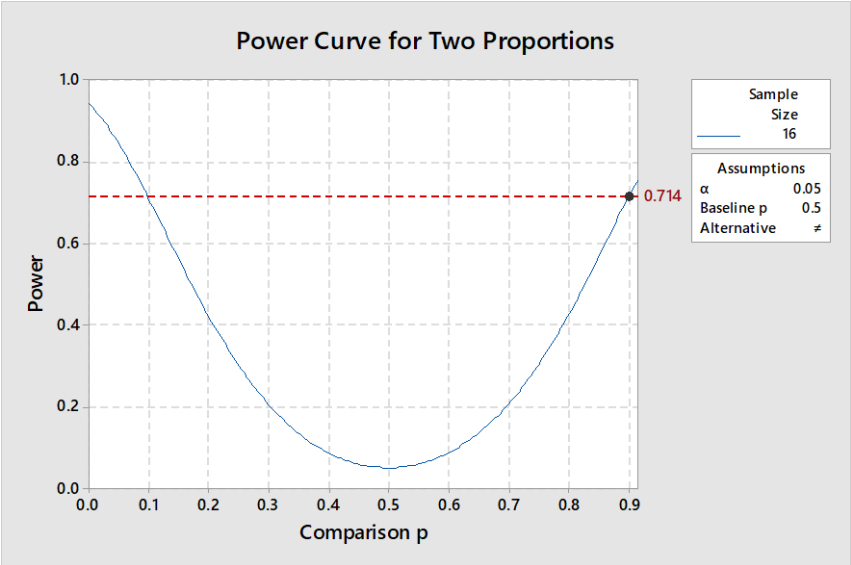

Fig. 8. Power curve showing the probability of being able to detect difference between HIFU treatment with 90% passed AE profile against a baseline treatment with 50% acceptable proportion. At the projected succes rate of 90%, the difference in cure rate can be detected with a 95% confidence level and power of above 71% if the population in the investigation is 16 participants.

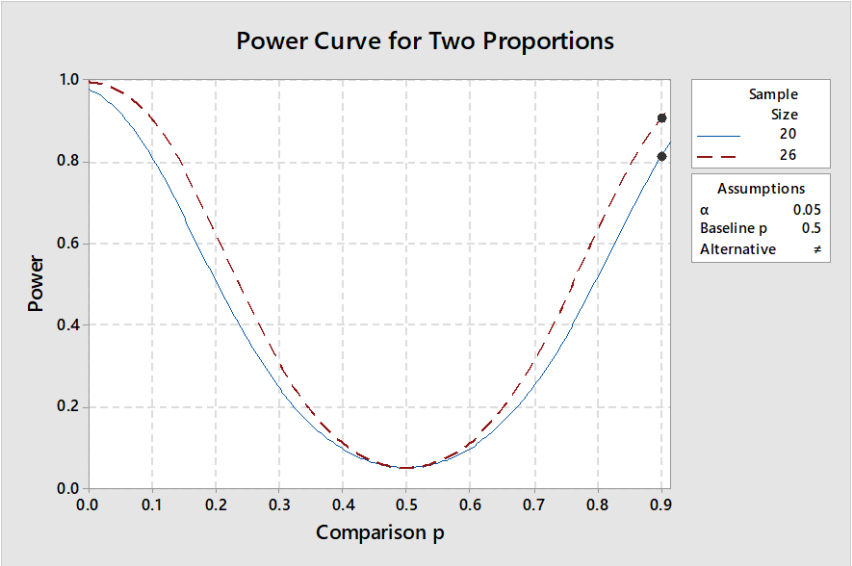

Fig. 9. Power curves showing the necessary population to obtain power levels of 80% and 90%. At the projected acceptable AE-profile of 90%, the hypothesis can be confirmed with a power of 80% probability if the population is 20 participants and a poer of 90% if the population in the investigation is 26 participants.

In conclusion, the statistical analysis shows that participation as low as 16 patients will give an acceptable power, provided the AE profile is in the expected range. At worse AE-profiles, or requirements for a higher power-level in comparison with alternatives, the required population size naturally increases, and populations above 20 will typically needed.

In consideration of the status as an initial tolerability and feasibility study performed on a relatively young population group with low expected drop-out, it is considered that a total of **20 participants distributed among the two centers** will be a well-balanced population taking ethical, clinical and practical issues into due consideration.

It should finally be mentioned that each participant will present a minimum of 4 tumors for treatment, and sometimes significantly more than this. The total number of treated tumors in the study will thus be high. Under certain assumptions, this allows for pooling of data on separate tumors during the data-analysis phase, rather than looking at combined reactions and results on participant-level only. The CRF in the study accommodates such individual data collection.

8 Data management

The study shall respect all legal requirements regarding data protection of the participant, with the necessary exceptions specified in the study consent form. As a study conducted by two centers in

the EU, the overall framework of the European Regulation on General Data Protection Regulation 2016/679 (GDPR) with its respective national adaptations will govern data management.

In Denmark, all information about participants is subjected to “Lov om behandling af personoplysninger” and “Sundhedsloven”, and these will be complied. The approval from Danish Data Proctection Agency (dataanmeldelse i Region Hovedstaden) will be applied.

In Sweden, all information about participants is particpanted to The General Data Protection Regulation of the EU, and “25 kap. 11 § 5 p. offentlighets- och sekretesslagen (2009:400) (OSL)”, and these will be complied. The approval from Etikprövningsmyndigheten will be applied.

Each participant will be informed that representatives from the respective national data protection agency and Medicines Agency may inspect their medical journals and trial records, in all confidentiality. The requirements to ensure anonymity of data, data security and confidentiality of data will be explained to the participants and complied.

All information will be treated with strict confidentiality and stored as confidential material according to the respective national regulations. Only the principal- and coordinating investigators will have access to the information.

Study results will be reported in anonymous form. The investigator will keep identification lists of all participants. This list will include full name and social security number.

The recorded data will be kept in a Case Report Form and an individual journal will be created for the investigation. The information will only be available for inspection for authorized representatives from the relevant authorities upon request (national GCP Unit, Data Protecting Agency, Medicines Authority).

During the investigation, paper-based CRF will be used. All information according to protocol and collected during the study must be entered in the appropriate field of the CRF. The investigator, or designated representative, should complete the appropriate CRF fields as soon as possible after information is collected. The information must match the information that exists as source documents in the clinic chart, hospital chart, and/or investigator’s files. An explanation should be given for all missing data. REDCap will be used for storage of such information afterwards.

The investigators in each center will retain investigational records, copies of CRF and source documentation for the maximum period required by the regulatory authorities.

The center in Denmark will be appointed as data-processor. Only pseudonymized data will be transmitted from the center in Sweden to Denmark for combined processing and reporting. A cooperation agreement including encrypting of the files and relevant provisions for data transfer and management will be made between the two centers to ensure that transfer of such data is in compliance with GDPR and the legislative requirements in both countries.

It is the coordinating and principal investigators at each center responsibility to assure the accurate completion, review, and approval of all CRFs, and the timely completion and submission of all adverse event forms.

In the case of a personal data breach, the relevant investigator(s) must without undue delay and, where feasible no later than 72 hours after having become aware of it, notify the personal data breach to the respective national Data Protection Agency, unless the personal data breach is unlikely to present a risk to the affected individuals.

Reporting must be done as per the national guidelines, e.g. via electronic log-in to the relevant portals.

9 Amendments to the CIP

If the sponsor intends to introduce modifications to a clinical investigation that are likely to have a substantial impact on the safety, health or rights of the subjects or on the robustness or reliability of the clinical data generated by the investigation, it shall notify, within one week, the relevant national authorities in Denmark and Sweden of the reasons for and the nature of those modifications.

The sponsor shall include an updated version of the relevant documentation as part of the notification. Changes to the relevant documentation shall be clearly identifiable.

The relevant authorities in Denmark and Sweden shall assess any substantial modification to the clinical investigation.

The sponsor may implement the modifications at the earliest 38 days after the notification unless:

a) A medical authority in Denmark or Sweden has notified the sponsor of its refusal based on the grounds referred to in MDR Article 71(4) or on considerations of public health, subject and user safety or health, of public policy, or
b) An ethics committee in Denmark or Sweden has issued a negative opinion in relation to the substantial modification to the clinical investigation, which, in accordance with national law, is valid for that entire country.

The relevant authorities concerned may extend the period for decision by a further seven days, for the purpose of consulting with experts.

10 Deviations from clinical investigation plan

Deviations to the CIP are not allowed unless they affect the subjects' rights, safety and well-being, or affect the scientific integrity of the investigation.

Deviations needed to protect the rights, safety and well-being of human subjects under emergency circumstances may proceed without prior approval of the sponsor and the relevant authorities.

Deviations, which are systematic, necessary, and significant to the study, i.e. affect the subjects' rights, safety and well-being, require a protocol amendment with submission and approval from the relevant authorities.

Insignificant, minor and unsystematic deviations to the CIP incurred to maintain the scientific integrity of the investigation, need notification in the case report only.

All deviations must be documented and reported to the sponsor, and must be included in the case report of the day of the visit or the deviation. All deviations shall made available for study monitor's inspection.

11 Device accountability

The sponsor is responsible for registration of the accounts of the device, properly documented. Delivery, placement and return of device has no special risk of hazard and needs no special procedure. No special risk of fire or contamination is noted.

12 Statements of compliance

The sponsor carries responsibility that statements signed by the participating centers/investigators on the fulfilment of the study in accordance with the Declaration of Helsinki, regional and national regulations and any relevant authority or legal requirement. The statements are kept in a study master file.

The equipment supplier shall provide insurance of hazards and accidents related to the use of the device in the study. Hazards and accidents independent of the device and related to the hospital setting are covered by the standard insurance of the hospital. Any hazard or accident in a participant is reported in the participants' patient file belonging to the hospital, and as adverse event in the case report.

13 Informed consent process

Candidates for the investigation are hospital patients referred to the participating departments for a cNF tumor. They all are registered in the standard electronic hospital files.

Potential candidates are informed orally and in writing about the investigation using the standard information of the study submitted to Lægemiddelstyrelsen and the Danish National Committee for Health Research Ethics.

Oral information is provided at an information-meeting with the participating investigator(s). The candidate will be informed in advance that he/she is allowed to bring an assessor for the meeting. The meeting will be held in a room that will only be used for this purpose to avoid unnecessary disturbances. The written information includes investigation participation document as well as relevant information folders from the respective national authorities. In Denmark this includes the document "Dine rettigheder som forsøgsperson" from "De Videnskabsetiske Medicinske Komiteer (VMK)". In Sweden this includes printed information for research persons which will be accepted by Etikprövningsmyndigheten pror to the study.

The informed consent form will include all the elements that are required according to the respective national legislation. If the participant wishes to participate, a new appointment will be booked where the doctor and the participant sign the consent. The new appointment and time of reflection will be within 2-7 days after the first appointment (allowing more than the required 24 hours of reflection time), but if the patient needs more time of reflection this can also be accommodated. If the patient wishes to sign the content at the first appointment this is also acceptable. If the patient fulfills all in- and exclusion criteria the patient will be included.

After the information-meeting, all active participants will be updated with any new and important information or change in the study that can have influence on the participants' willingness to participate or safety. The approved documents will be updated accordingly.

Oral information is repeated on the inclusion visit (visit 1 in the CRF), with opportunity for the patient to ask questions. The consent form is signed after the patient has declared the full understanding of the information and the study concluded as acceptance to enter study. In the oral information individual aspects of special relevance to the participant shall be emphasized.

Participants who are not able to understand the information cannot be included in the study. The signed and qualified acceptance of the participant to volunteer for the study is filed in the case report form book.

14 Adverse events, adverse device effects, and device deficiency.

14.1 Adverse Events

An adverse event (AE) is 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.

14.2 Serious Adverse Events

A serious adverse event (SAE) is any 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. hospitalization or prolongation of patient hospitalization,
    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

14.3 Device deficiency

A device deficient is 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.

14.4 Reporting to national authorities

The sponsor shall report, without delay to the respective national Medicines Agency all of the following:

a) any SAE that has a causal relationship with the investigational device or the investigation procedure or where such causal relationship is reasonably possible.

b) any device deficiency that might have led to a SAE 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 (a) and (b).

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.

Analysis of the reporting requirements for AE's and SAE's, i.e. whether reporting should be sent to the respective medical authority, will be conducted as per the guidelines given by the Medical Device Coordination Group (MDCG) in "MDCG 2020-10 Guidance on safety reporting in clinical investigations". Reporting will be done electronically using the templates included in the appendix to this guideline (MDCG 2020-10/2).

Records and reports of events shall include accurate and relevant information on type of event, date, treatment, resolution, assessment of seriousness and relationship to the investigational device and the whole procedure.

The investigational device applies ultrasound generated focused heat to the outer skin, being the mode of action. Unexpected stronger burn than anticipated according to instrument setting is a potential adverse event, which may result in more severe necrosis with delayed wound healing. However, such event is due to the construction of the transducer and the hand piece, and in any case limited to the skin, and therefore primarily a local tolerance issue.

Pain associated with the treatment is foreseeable and an expected adverse event, however, unlikely to be significantly worse than pain associated with other instrumental treatments of skin in general use such as surgical interventions, laser treatment and photodynamic treatment of skin cancer.

The two study centers are hospital departments with immediate access to emergency treatments.

15 Vulnerable population

The study does not include identified vulnerable participants, see inclusion and exclusion criteria.

16 Suspension or premature termination of the clinical investigation

The study is suspended if serious adverse events occur related to the investigational device, and the decision taken by the primary investigator and the sponsor to either terminate the study or continue the study depending of the clarification of the case. Continuation of study may require a protocol amendment.

The study is unblinded and no issue re. premature unblinding is relevant.

Participants having device-related adverse events are followed up upon investigator's discretion with control and medical treatment as required in the individual case.

17 Publication policy

The study will be registered in the European registry (www.clinicaltrials.gov) for ongoing clinical trials.

The result of the study shall be published in a peer-reviewed journal with the consent of all participating investigators, regardless if the overall results of the study is positive or negative.

The research publication shall be submitted to a journal within 6 months after the final study report is available.

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