Study Protocol

Project title:

Tolerability of laser-assisted cisplatin+5-fluorouracil— an exploratory proof of concept study of topical combination chemotherapy for basal cell carcinoma

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Project Schedule (see Appendix 1 for details):

Study start:	1. March 2018
Study treatment and follow up:	1. March 2018 – 1. March 2019
Study termination:	1. March 2019

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1. Background

Basal cell carcinoma (BCC) is the most common form of skin cancer, affecting more patients than all other cancers combined (1). Keratinocyte-derived BCC tumors are classified based on clinical presentation and histological growth pattern, and subtypes include nodular (nBCC, 50%), superficial (sBCC, 25%), infiltrative and micro-nodular lesions. Treatment options can be divided into surgical and non-surgical procedures, but management can be challenging as lesions commonly affect the face and are often multiple (2). Surgical removal can cause scarring and disfigurement, while less invasive pharmacological options typically prove unsatisfactory due to inadequate skin penetration of topical drugs, low efficacy using drug monotherapy, and risk of side effects associated with systemic treatment.

Synergy between the two anticancer agents, 5-fluorouracil (5-FU) and cisplatin is well-known in the oncological setting. Administered as a systemic treatment, cisplatin+5-FU provides improved outcomes for a range of epithelial cancers (3). In dermatology, the pyrimidine analog 5-FU is already a mainstay, approved for topical treatment of actinic keratosis and superficial BCC (4). However, efficacy is dependent on skin absorption and exposure dose; applied alone, the standard 5-FU regimen (5%, 50 mg/g) of twice daily application over 4 weeks shows limited penetration and high rates of treatment failure for deep-laying tumors (5, 6). Similarly, cisplatin is a widely used and highly potent anticancer agent with activity against BCC, but the compound's ability to penetrate intact skin is low (7, 8). At present therefore, the synergy demonstrated by cisplatin+5-FU has yet to be exploited as a topical strategy for BCC in the dermatological setting.

Ablative fractional laser (AFL) is a drug delivery modality that is steadily being implemented into dermatological practice (9). AFL induces localized tissue vaporization leaving microscopic thermal ablation zones (MAZs) surrounded by a rim of coagulated tissue in treated skin. By significantly reducing the skin's barrier function, these channels provide an effective and gentle route for topical drug delivery. Compared to systemic therapy, AFL enables the use of significantly lower drug doses, with the vast majority of the delivered dose being confined to the target skin (dose: 100 mg/m^2 vs 0.25 mg/cm^2 (cisplatin); 400-600 mg/m^2 vs 6.25 mg/cm^2 (5-FU)). As a result, side effects associated with systemic drug therapy can be avoided. Reasonable efficacy was recently reported in 28 sBCC patients after AFL-assisted delivery of topical 5-FU cream (left under 7-day

occlusion), with no adverse effects and transient local skin reactions (16). We hypothesize that therapeutic outcome can be further enhanced by combination with cisplatin.

In a recently concluded preclinical phase, we confirmed that AFL enhances penetration of cisplatin and 5-FU in *in vivo* and *in vitro* skin (10, 11). In an additional study in live pigs over 5 days, drug biodistribution and *in vivo* effects following the proposed combination treatment was assessed. In that study, using the same low dose topical AFL-assisted cisplatin+5-FU treatment, we found improved skin uptake of both drugs and enhanced, localized inflammatory reactions. Importantly, despite topical exposure over large skin areas (>100 cm^2), systemic cisplatin and 5-FU uptake remained undetectable measured hourly from 0-5 hours and at 48 and 120 hours using highly sensitive mass spectrometry techniques. Overall, treatment was well-tolerated and no systemic effects were observed. Supporting a synergistic mechanism, local effects were significantly greater when cisplatin and 5-FU were applied together (11). Already well-known as a systemic therapy in the oncology setting, the dual-drug, cisplatin+5-FU strategy may hold potential as a local treatment alternative for BCC lesions.

2. Aim

In an effort to develop a new topical treatment for BCC using synergistic chemotherapeutics, this study aims to investigate tolerability, tumor size and BCC clearance following AFL-assisted cisplatin+5-FU therapy in patients with BCC.

3. Study Design

3.1 Design

A prospective clinical, uncontrolled, open-label, explorative phase IIa trial* on patients with histologically- confirmed superficial and nodular BCC

3.2 Randomization and Blinding

Due to the uncontrolled open-label study design, no randomization/blinding is planned.

**Phase IIa trial: Small preliminary clinical trials to evaluate safety and/or efficacy in selected populations of patients with the disease or condition to be treated, diagnosed, or prevented. Objectives may focus on dose-response, type of patient, frequency of dosing, or numerous other characteristics of safety and efficacy.*

3.3 Intervention Summary

For a detailed description of interventions, please see Section 5.

Patients will receive AFL-assisted cisplatin+5-FU as a treatment for their BCC. In brief, treatment areas consisting of tumors and a 5 mm margin will undergo AFL exposure (Ultrapulse DeepFx CO_2 laser, Lumenis, Santa Clara, CA, USA) followed by 60 min topical application of a marketed and commercially available IV cisplatin solution (0.1% Accord Healthcare Limited A/S) at a dose of 0.25 ml per cm^2. After removal of cisplatin, a commercially distributed 5-FU cream (5% Efudix®, Meda Pharmaceuticals A/S) will be applied to the treatment area at a dose of 0.125 ml per cm^2 and left under occlusion. After skin evaluations on Day 1 and 5 after treatment, the same 5-FU dose will be applied, again left under occlusion. In total, 5-FU will remain on the skin for 7 days after AFL treatment whereafter it will be washed off.

An additional repeat AFL-cisplatin+5-FU treatment on Day 30 will be offered if tumors persist, based on clinical evaluation and imaging on Day 30.

3.4 Summary of Outcome Measures and Methods

Outcome measures and methods/techniques are summarized below. Detailed descriptions are presented in Section 6 and Table 1 (Appendix 1).

Primary outcome:

To investigate tolerability of topical AFL-assisted cisplatin+5-FU therapy for BCC by evaluating:

I. Severity and duration of clinical local skin reactions including erythema, edema, scabbing, flaking and pustulation assessed by a physician using an established 0-4 point scale (none, mild, moderate, severe) from 0-30 days post-treatment.

II. Occurrence of side effects (prolonged erythema/edema, hyper/hypopigmentation, scarring and infection) up to 3 months post-treatment.

Secondary outcome:

1) To monitor BCC tumor size and clearance based on clinical assessments and dermoscopy, supported by non-invasive imaging techniques including dynamic optical coherence tomography (D-OCT), reflectance confocal microscopy (RCM), high intensity focused ultrasound (HIFU) and histological analysis up to 3 months post-treatment.

3.4 Study Duration and Schedule

Patients will partake in 7 visits over a 3 month period at the dermatological department of Bispebjerg Hospital (time schedule in Section 5):

1 Screening visit
1 Treatment day
5 Control visits after treatment on Day 1, 5, 14, 30 and Month 3

The study is expected to run between March 2018 - March 2019

Recruitment start	1. March 2018
Treatment start	1. March 2018
Final patient treatment	1. December 2018
Final control visit	1. March 2019

3.5 Preliminary Study

In the beginning of the trial period, a preliminary study in 4-6 patients of the intended treatment will be conducted to confirm: a) uniform cisplatin and 5-FU uptake in treated BCC tumors and b) no clinically relevant systemic drug uptake. Thus, up to 6 patients will each provide a single 4 mm punch biopsy of a treated BCC and one 5 ml blood sample, both taken 24 hrs after AFL-assisted cisplatin+5-FU. Drug biodistribution imaging of tumor sections and drug quantification in blood will be performed at Copenhagen University using well-established analytical techniques. A biobank and contracts of collaboration pertaining to the exchange of anonymized data between institutions will be devised.

4. Participants

Up to 12 patients with superficial BCC and 12 patients with nodular BCC will be evaluated following AFL-assisted cisplatin+5-FU treatment. The unspecific inclusion of "up to" 12 patients reflects the explorative nature of this tolerability study. Should fewer patients per group provide adequate data on LRSs associated with the treatment (for example if all patients demonstrate severe LSRs), the inclusion of 12 patients will not be performed in order to spare them potential discomfort.

Recruitment will be performed by physicians at the dermatological department, Bispebjerg Hospital and by private practice dermatologists in the Copenhagen area. Procedures for informed consent are included in Bilag 2-4.

4.1 Inclusion criteria

Subjects who meet all of the following criteria are eligible to participate in this study:

1. Histologically-verified, previously untreated superficial or nodular BCCs on the scalp, face, extremities or trunk
2. >18 years of age at baseline
3. Legally competent, able to give verbal and written informed consent
4. Subject in good general health, is willing to participate and can comply with protocol requirements.
5. Fitzpatrick skin phototype I-III
6. Female subjects of childbearing potential[1] must be confirmed not pregnant by a negative urine pregnancy test prior to trial treatment.

[1]Female subjects are considered of childbearing potential unless they have been hysterectomized or have undergone tubal ligation or have been post-menopausal for at least one year prior to first visit.

4.2 Exclusion criteria

Subjects meeting any of the following criteria are not eligible to participate in this study:

1. High-risk BCC
    i. Tumors in the following anatomical locations: midface, orbital, ears
    ii. Size: >20 mm in facial/scalp areas or > 50 mm in non-facial/non-scalp areas
    iii. Subtype: morpheaform and micronodular BCC
    iv. History: Gorlin syndrome or immunosuppression
2. Previous treatment of the BCC lesion
3. Known allergy to cisplatin or Efudix®
4. Other skin diseases present in the treatment area
5. Tattoo in the treatment area which may interfere with or confound evaluation of the study
6. History of keloids which is deemed clinically relevant in the opinion of the investigator
7. Fitzpatrick skin phototype IV-VI
8. Lactating or pregnant women

4.3 Restrictions during Trial

Patients must not receive concurrent treatment of the tumor/treatment area during the study. Concomitant treatment for conditions other than the BCC may be continued throughout the trial without change in dosage.

4.4 Withdrawal Criteria

Subjects may withdraw from the trial for any of the following reasons:

1. Unacceptable treatment efficacy: the investigator is free to withdraw the subject at any time based on a medical judgment.
2. Unacceptable adverse events: any adverse event that the investigator or the subject considers unacceptable.
3. Exclusion criteria: any exclusion criteria which emerge/become apparent during the subject's participation in the clinical trial.
4. Voluntary withdrawal: subjects are free to withdraw from the clinical trial at any time and for any reason. If applicable, the subject's legal representative can withdraw the subject from the trial.

Subjects who discontinue treatment for any reason will be offered conventional BCC treatment and subsequent control according to national guidelines. Those who withdraw will be substituted. The research team will use already collected data after withdrawal unless the participant objects.

4.5 Patient Renumeration

Patients do not receive economic compensation for trial participation.

5. Treatment Procedures and Interventions

5.1 Treatment Procedure

5.2.1 Treatment Area Demarcation

At baseline (Day 0), BCC delineation will be performed clinically and by D-OCT, RCM and HIFU imaging. The treatment area will include the delineated tumor and a 5 mm margin of surrounding skin. After delineation, treatment areas will be photographed and mapped on a transparent template to document baseline tumor size and location.

5.2.2 Ablative Fractional Laser (AFL):

Before AFL exposure, hairs will be shaved and treatment areas numbed with standard local analgesia (lidocaine HCL 1% +epinephrine 1:100.000 injection). Depending on tumor thickness, treatment areas will then be exposed to 1-2 stacked pulses of maximum 50 mJ/microbeam (mb) and up to 10 % density. Specific laser settings are established based on results from a currently ongoing clinical trial at Bispebjerg Hospital (EudraCT-nr 2017-002843-14).

5.2.3 Topical Cisplatin

After AFL exposure, a well consisting of a customized hydroactive bandage (DuoDerm Hydroactive™, ConvaTec, Flintshire, UK) is applied to the test area, defined as the BCC and 5 mm margin, and sealed shut with transparent dermatological film (TegaDerm® film dressing, 3M Medical, MN, USA). Cisplatin solution 0.1% (Accord Healthcare Limited A/S) is applied through the transparent film using a blunt-tipped syringe, ensuring spatial containment of the solution with no loss to the environment. The amount of solution will fill the well to ensure coverage of the entire skin surface. Accordingly, up to 0.25 ml per cm^2 (0.25 mg) will be applied depending on tumor delineation. Cisplatin is left on the skin for 60 minutes, whereafter the solution is removed by light suction using a blunt-tipped syringe. After drug removal, all materials will be disposed of according to guidelines for materials contaminated with cytotoxic-agents.

5.2.4 Topical 5-Fluorouracil (5-FU)

After cisplatin removal, the well bandage is disposed of and a thick amount of Efudix® 5% 5-FU cream (Meda Pharmaceuticals A/S, UK) is applied to the entire test area (corresponding to up to 0.125 ml per cm^2 skin). After 5-FU application, transparent dermatological film (TegaDerm® film dressing, 3M Medical, MN, USA) is applied to the test area as an occlusive dressing. The cream is left on the skin until next control visit. Thus, on Day 1 and 5, Efudix cream is reapplied at the same dose. The cream is removed on Day 7 after AFL treatment.

STUDY SET UP	Visit 1 Screening	Visit 2 Treatment Day 0	Visit 3 Day 1	Visit 4 Day 5 +/- 2 days	Visit 5 Day 14 +/- 3 days	Visit 6 Treatment* Day 30 +/- 5 days	Visit 7 Month 3 +/- 5 days
Screening & enrolment							
Informed consent	✔						
Inclusion/exclusion criteria	✔						
Relevant medical history	✔						
Location of BCC	✔						
Pregnancy test when relevant		✔				✔	
Interventions							
AFL		✔				(✔)	
Apply topical cisplatin(60min)		✔				(✔)	
Apply 5-FU under occlusion		✔	✔	✔		(✔)	
Clinical LSR evaluation							
FDA-approved LSR scale		✔	✔	✔	✔	✔	
Clinical photos		✔	✔	✔	✔	✔	✔
Safety							
Clinical evaluation		✔	✔	✔	✔	✔	✔
Tumor size and clearance evaluation							
Clinical/dermoscopic photos		✔	✔	✔	✔	✔	✔
D-OCT		✔				(✔)	✔
RCM		✔					✔
HIFU		✔				(✔)	✔
Biopsy							✔

*Offered if BCC residual or recurrence; (✔): Treatment/technique can be relevantly applied if tumor residuals are identified clinically or by D-OCT/RCM and there is no crusting etc. LSR: Local skin reactions, RCM: Reflectance confocal microscopy, D-OCT: Dynamic optical coherence tomography

5.2 Intervention Details

- **Ablative Fractional Laser (AFL):** Laser irradiation with be performed using the UltraPulse® laser system with DeepFx handpiece (Lumenis, Santa Clara, USA) which is a 10,600 nm CO_2 laser CE-marked for dermatological use. The procedure is expected to take 15 min.

- **Cisplatin Solution**: The topically-applied cisplatin solution (Accord Healthcare Limited A/S) is approved for commercial use and is administered intravenously at significantly higher doses in the conventional oncological setting. The drug will be provided by the department of Dermatology at Bispebjerg Hospital. 1 ml contains 1 mg cisplatin (0.1%). Other ingredients are sodium chloride and sterile water. For the study, the drug will be supplied in labelled, 3 ml blunt tipped syringes (preprepared by the hospital pharmacy pending a

contractual agreement) and will be light protected and stored at room temperature. The solution will in this study be applied topically for 60 min.

- **Efudix® 5-FU Cream:** The cream (Meda Pharmaceuticals A/S, UK) is used commercially for topical use where standard regimens for BCC consist of twice daily application for 4-6 weeks. The cream sold under special license and is provided via the department of Dermatology at Bispebjerg Hospital. The drug is packaged in tubes of 20 grams and stored at 5 degrees. 1 mg contains 50 mg 5-FU (5%). Other ingredients are stearyl alcohol, vaseline, polysorbate 60, propylene glycol, methyl parahydroxybenzoate, propyl-parahydroxybenzoate and purified water. Topical application under occlusion for up to 7 days will be used on treated area.

5.3 Supplementary treatment

Management of pain will be 1 g paracetamol or 200 mg ibuprofen. No rescue medicine is necessary.

5.4 Compliance

All treatments are performed at the dermatological department, Bispeberg Hospital reducing the need for compliance-ensuring efforts. Securing adequate 5-FU occlusion will occur on control visits.

6. Outcome Measures & Methods

Outcome measures are evaluated in a non-blinded manner by physicians at the department of dermatology, Bispebjerg Hospital.

6.1 Primary Outcome

Tolerability measured as:

I) Local Skin Reactions (LSRs)

Non-blinded, clinical evaluation of erythema, edema, flaking, crusting/scabbing and pustulation in treated areas will be performed by a physician using a FDA-approved 0-4 point LSR scale at Days 1, 5, 14 and 30 after AFL exposure (Appendix 1). Standardized,

clinical photographs are taken to document LSRs at each visit.

II) Side Effects

Non-blinded, clinical evaluation of prolonged erythema or edema, hyper- or hypopigmentation, scarring and infection will be evaluated up to 3 months after initial AFL treatment.

6.2 Secondary Outcome

Tumor Response measured as:

I) Tumor size (mm) and clearance (yes or no) will be evaluated clinically and using non-invasive D-OCT, HIFU and RCM imaging at baseline, Day 30 and Month 3 (Section 5, Appendix 1). If residual tumor is identified at 3 months follow-up, patients will receive conventional treatment according to national guidelines. Imaging techniques are described in section 6.3.

II) Histological verification of tumor clearance will be performed 3 months after first treatment using tissue sections from a 4 mm punch biopsy. If residual tumor is identified at 3 months follow-up, patients will receive conventional treatment according to national guidelines.

6.3 Methods for Outcome Assessment

6.3.1 Clinical Evaluation and Digital Photography

Clinical evaluation of LSRs (erythema, edema, flaking, crusting/scabbing and pustulation) in treated areas will be performed by a non-blinded physician using a FDA-approved LSR scale at Days 1, 5, 14 and 30 after baseline. The scale assesses severity of individual LSR parameters on a 0-4 points scale: none, mild, moderate, severe. Tumor size (mm) and clearance (yes or no) will also be evaluated by standard clinical dermoscopy at Day 30 and Month 3 (Appendix 1).
Digital photography using a Canon Camera will be performed prior to, during, after treatment, and at control visits under standardized conditions. All images are assigned an ID-number and anonymized.

6.3.2 Imaging

All imaging devices have the size of a modern ultrasonography system and handheld, flexible probe. All systems are CE-marked and FDA approved.

High Intensity Focused Ultrasound (HIFU)

HIFU will be performed at baseline, day 30 (if imaging is not obstructed by overlying skin reactions) and month 3 after treatment. A GE LOGIQ P9 unit (General Electric Medical Systems, known as GE Healthcare, Little Chalfont, Buckinghamshire, UK) with a high frequency probe 9 - 15 MHz will be used to identify tumors in skin. The system is CE marked to council directive 93/42/EEC on medical devices (FDA K152309) with a 50-200 µm resolution and a penetration depth of 0.75-2 cm. HIFU allows volumetric images of tumors in real-time, using 4D transducers. BCC tumors are characterized as well-defined, black, round to elongated areas often with distinct internal white dots. The HIFU modality is sensitive to the generally slower blood flow in skin. During imaging, standard imaging gel is placed on the skin in order to avoid skin compression under examination. The acquired images are saved in a digital archiving computer system for subsequent evaluation.

Dynamic Optical Coherence Tomography (D-OCT)

D-OCT will be applied at baseline to delineate horizontal and vertical BCC tumor margins and subsequently customize settings for relevant AFL channel dimensions. D-OCT will also be used to assess tumor size and clearance at control visits on Day 30 (if imaging is not obstructed by overlying skin reactions) and Month 3 after treatment (Section 5, Appendix 1). A commercially available, CE-marked (CE 545451, FDA K153283) OCT system (Vivosight® DX, Michelson Diagnostics Ltd., Kent, UK) with a laser wavelength of 1305 nm will used.

OCT works similarly to ultrasound: instead of acoustical waves, the reflection of infrared light emitted from the skin is measured, and the signal strength is imaged as a function of position. The applied OCT system has a 5–7.5 µm resolution and a penetration depth of 1–2 mm. In this study, a multi-slice modality consisting of 250 B-scans will be used. The OCT skin images are presented in both cross-sectional and *en face* mode. BCCs appear as well-defined, dark, round to elongated islands with clefting, arborizing vessels and increased blood flow. Images which visualize microcirculation in the skin are acquired using the dynamic OCT-signals (D-OCT) also termed "speckle variance" using the Vivosight system and relies on the higher variation of speckles caused by motion i.e. blood cells. The D-OCT data is collected concurrently with structural OCT data, and

displayed as a red overlay on the conventional OCT images. D-OCT images can be analysed and vessel flow calculated using an integrated software tool (Michelson diagnostics Ltd. Kent, UK). Flow is represented by an arbitrary number since D-OCT does not measure flow in m/s.

Reflectance Confocal Microscopy (RCM):

In vivo RCM is an advanced bedside microscope that can be attached to the skin. It offers visualization of epidermis and upper dermis at almost histological resolution. In this study, a commercially available CE-marked (CE 0459, FDA K080788) confocal microscope (VivaScope Multilaser 1500®, Caliber ID Inc., Rochester, NY, USA) in 785 nm mode is used. Horizontal tumor margins are confirmed at baseline and Month 3 after treatment (Section 5, Appendix 1). RCM provides horizontal sections of the skin with a penetration depth of 200–300 µm and a lateral resolution of 0.5 µm. VivaBlocks® will be acquired from each test area in 3 depths: between 30–40µm, at dermo-epidermal junction (80-110 µm), and between 150–200 µm. Subsequently, series of image stacks, "VivaStacks", will be obtained from skin surface to papillary dermis. BCCs are indentifed as having palisading bright cells in characteristic BCC islands with clefting, inflammation and dilated vessels. RCM has previously been used to non-invasively identify BCC lesions and monitor topical 5-FU treatment (12-15).

Imaging Software and Analysis

For the evaluation of D-OCT, RCM and HIFU images, qualitative and quantitative measurements will be obtained. Skin morphology in D-OCT and RCM images is evaluated using the integrated VivoSight® software for the OCT and the integrated VivaScan® software for the RCM VivaScope®. Furthermore, OCT, RCM and HIFU images will be analyzed using ImageJ software (Java-based freeware, National Institutes of Health, Maryland, USA).

6.3.3 Histology

At the 3 month visit, a 4 mm punch biopsy will be taken centrally in treatment area for histological verification of tumor clearance. Biopsy tissue will undergo standard sectioning and histological evaluation by a board-certified pathologist at the Department of Pathology, Bispebjerg Hospital. If tumor residual/recurrence is confirmed, test areas will be treated according to clinical guidelines.

7. Potential Risks and Side Effects

In general, no serious or systemic side effects are assumed but will be reported to the relevant authorities if they occur. Topical 5-FU cream and AFL are used daily at Bispebjerg Hospital's dermatological clinic and are well-tolerated. Cisplatin has previously been examined in a live pig study where no systemic drug uptake or effects were observed. Any obtained information about adverse effects related to this study will be recorded systematically.

7.1 AFL

During AFL irradiation, protective safety goggles will be worn by investigators and patients. To alleviate pain, treatment areas will be numbed. Injection of local analgesic is associated with discomfort. AFL causes transient edema and redness lasting for 5-10 days. Clinical healing of AFL microwounds takes 4-7 days. Infection risk after AFL alone is <10%. Post inflammatory hypo/hyperpigmentation may occur, but usually resolves after 1-2 months. Scarring, most commonly after treatment on eyelids, neck or chest, is rarely reported after AFL.

7.2 Topical Cisplatin and 5-FU

Storage, handling, personal protective equipment and disposal of chemotherapeutics as outlined by hospital guidelines, will be followed. Neither cisplatin nor 5-FU will come in contract with mucus membranes.

The side effect profile associated with intravenous cisplatin+5-FU therapy is not anticipated, as neither cisplatin nor 5-FU has been detected in the blood of live pigs measured hourly from 0-5 hours, 48 and 120 hours after topical AFL-assisted cisplatin+5-FU exposure. In that study, treatment consisted of a similar schedule/dose applied over a significantly larger skin area (>100 cm^2). In this study, negligible systemic drug exposure is thus expected, an element that will be reassessed in the described preliminary study. On the other hand, transient local effects are common and expected after AFL-assisted cisplatin+5-FU on skin, likely to resolve within 30 days. When administered alone, AFL+cisplatin caused redness and edema peaking 48 hours after topical exposure, followed by a healing response. Using the combination of AFL-cisplatin+5-FU, the anticipated pattern is an early inflammatory phase characterized by erythema, a necrotic phase characterized by skin erosion, and finally healing (re-epithelialization). Local treatment effects can

be notable with blistering/ulceration. Estimated incidences after topical treatment with the two drugs are:

- Mild to moderate erythema (94-100%)
- Mild to moderate edema (33-99%)
- Scabbing (50-95%)
- Erosion (50-95%)
- Hypo/hyperpigmentation resolved after 1-3 months (30%)
- Pruritus (20%)
- Erythema lasting greater than 12 weeks (5%)
- Exudate, bleeding or crusting (1%)
- Infection (1%).
- Allergy (1%)
- Temporary hair loss in the treated area (<1%)

Dihydropyrimidine dehydrogenase (DPD) deficiency is an extremely rare genetic condition that renders 5-FU toxic to affected individuals. Toxicity typically presents within 96 hrs of administration. While extremely unlikely to occur due to the uncommonness of the mutation and the minimal levels of 5-FU anticipated to permeate into the systemic circulation, investigators will stay vigilant about this potential risk.

7.3 Non-invasive imaging

Cyanoacrylate oil used during imaging may lead to skin irritation and itching. There are no known adverse effects to HIFU, RCM or OCT. The systems are CE-marked and FDA-approved. Vivosight complies with FCC Class B and Vivascope with FCC Class A. All systems fulfill the technical specifications set by the Health Authorities in Denmark and are approved by the Medico Technical Department at Bispebjerg Hospital.

7.4 Punch Biopsy and Blood Sampling

Prior to 4 mm biopsy local analgesic will be used to reduce pain. The biopsy may leave a punctiform scar. Needle-pricks associated with injection and blood sampling (preliminary study) are associated with discomfort. The risk of infection is low (<1%), and will be managed with antibiotics provided by the investigator.

8. Safety Considerations

Patients are followed closely in the initial 30 days after treatment and particularly in the first 14 days. Complete tumor removal is ensured through histological analysis at Month 3. In the event of inadequate treatment response at 3 months, or in the event of withdrawal, patients will receive conventional BCC treatment and follow-up according to national guidelines.

8.1 Definitions

- Incident: any unwanted incident after treatment with a medical product occurring during the trial. Treatment and incident need not be related.
- Adverse Event (AE): Any unexpected medical event without known causal relation to the treatment will be reported as an AE. The event can be of any nature, an incident, a symptom or disease that is temporarily linked to the treatment of cisplatin/5-FU/AFL or the combination, whether or not it is considered related to exposure or not.
- Adverse Reaction (AR): A harmful or unforeseen reaction with a known or expected association to cisplatin/5-FU/AFL or the combination will be reported as an AR.
- Serious Adverse Event (SAE): An adverse event that results in death or leads to serious deterioration in the health of a subject, resulting in:
    - A life-threatening illness or injury, OR
    - A permanent impairment of a body structure or a body function, OR
    - Inpatient or prolonged hospitalization, OR
    - Medical or surgical intervention to prevent life-threatening illness or injury or permanent impairment to a body structure or a body function
    - an adverse event that leads to fetal distress, fetal death or a congenital anomaly or birth defect
- Unexpected Serious Adverse Effect (SUSAR): A serious adverse event that is unexpected according to the product summary, but suspected to be treatment-related (cisplatin/5-FU/AFL or the combination), will be reported as an SUSAR.

8.2 Documenting and Reporting Adverse Events (AE) and Adverse Reactions (AR)

The investigators are responsible for routine assessments of AEs and ARs. All clinical complaints, symptoms, or signs that meet AE or AR definitions will be documented in the study record and the patient's medical record. Erythema and/or edema are anticipated sequelae of treatment; LSRs included in the LSR Grading Scale are reported as LSRs and not as AEs in the CRF, even if they require treatment. All AEs and ARs are described according to duration (start and end date), severity, association with drugs, whether further treatment is required and whether the AE has led to a serious adverse event. The location of the event is recorded as either "treated area" or "non-treated area". AEs and ARs will be followed until they have resolved.

The severity of an AE/AR is graded as:

- Mild: AE is transient and does not affect daily activities
- Moderate: AE causes patient discomfort and affect daily activities
- Severe: AE affect the patient's daily activities to a significant degree and may mean invalidity/incapacity for work or else be life-threatening.

The outcome of an AE/AR is described as:

- Resolved
- Improvement without sequelae
- Improvement with sequelae
- Persistent, follow-up necessary
- Persistent, follow-up not necessary

The investigators assessment of whether an AE is associated with the trial medication is classified as follows:

- Yes: an AE which after careful evaluation has a temporal association with the treatment and is in all probability related to the trial medication.
- No: an AE which after careful evaluation is definitely judged to be attributable to an external cause (disease, environmental exposure etc.) treatment and is in all probability related to the trial medication.

8.3 Documenting and Reporting of Serious Adverse Events (SAE) and Unexpected Serious Adverse Reactions (SUSAR)

The investigator is responsible for reporting all SAEs after the patient has signed the consent form until the final control visit. SAEs are reported within 24 hours to sponsor-investigator Merete

Haedersdal. The investigator is obliged to undertake follow-up consultations until the SAE/SUSAR has been resolved or in the case of permanent disability, stabilized. The product summary for Cisplatin Accord and Efudix are used to determine whether or not a SAE is unexpected. All SAEs and SUSARs are recorded in the patient's medical record and on a separate sheet of the CFR. SUSARs with a fatal or life-threatening outcome are to be reported to the Danish Health and Medicine Authority within 7 days after the reveal and a report on the development of the SUSAR latest 8 days after being reported to the Danish Health and Medicine Authority, while other SUSARs are to be reported within 15 days. Reporting is performed using the e-form on the Danish Health and Medicine Authority website.

8.4 End of Trial Reporting

At the end of the trial, a final report with all AEs, ARs, SAEs, and SUSARs is handed to the Danish Health and Medicine Authority and the Regional Committee on Health Research Ethics, latest 90 days after conduction. If the trial ends ahead of schedule, a report will be submitted to the above-mentioned instances within 15 days. The results from the trial will also be sent to the Danish Health and Medicine Authority and the Regional Committee on Health Research Ethics latest one year after completion.

9. Statistics

9.1 Sample size

The primary objective of this explorative trial is to investigate tolerability of AFL-assisted cisplatin+5-FU to ensure that intolerable/severe or serious related adverse events remain minimal for the observed efficacy. While no formal statistical calculation has been performed, 12 superficial and 12 nodular BCCs in 24 subjects are considered sufficient to estimate benefit/risk of the treatment.

9.2 Study populations

Treated study participants are divided into the following populations:

<u>Per-protocol (PP) population:</u>

Includes only patients that have completed all control visits as planned. Larger deviations such as

breach of inclusion criteria, inadequate outcome assessment or larger differences in treatment, are assessed before statistical analysis is conducted.

Intention-to-treat (ITT) population:

Includes all patients that have received treatment. In the event of missing data, the population is analyzed using the "last observation carried forward" approach to minimize bias associated with loss to follow up.

9.3 Statistics

Non-parametric or parametric statistics are used for LSR-scores, tumor size assessment and clearance rates. Descriptive data will be presented with medians and interquartile ranges (IQR) or means and standard deviations. Wilcoxon matched-pairs test will be used to test differences between test areas at various time points. P-values will be corrected for multiple comparisons when relevant and a level of $p<0.05$ considered statistically significant. Analyses are performed by SPSS version 24 (IBM Corporation, Armonk, NY, USA).

10. Quality Control, Data Protection & Storage

10.1 Quality Control

The declaration of Helsinki II will be respected as well as the standards of good clinical research. Respect for privacy as well as physically and mentally integrity of the participants will be maintained. The study will be performed in accordance with ICH GCP Guidelines and Danish Health care authorities. It will be registered to The National Committee on Health Research Ethics, Danish Health and Medicine Authority, The Danish Data Protection Agency and www.clinicaltrials.gov. Study conduction and reports are in accordance with GCP CPMP/ICH/135/95 and European Medicines Agency directive 2001/83/EC.

10.2 Data Protection and Storage

Information on previous investigations and treatments pertaining to the BCC tumor (up to 4 months from inclusion) will be accessed through the patient medical records to ensure tumors are histologically-verified and have not previously been treated.

Research using the Capital Region of Denmark's data is considered to be public research. Use and distribution of data collected in this study including photographs and biopsies, will be discussed with patients during the consent process. The project will be reported to the Danish Data Protection Agency ("Datatilsynet"). Lists of screened and participating patients including names, study ID number and date of birth will be devised. All data collected will be anonymized and protected by the Danish law regarding management of personal information and "Sundhedsloven". Data will be registered and stored for 5 years (with the exception of the biobank) after study termination at the department of dermatology, Bispebjerg Hospital. Access, monitoring and inspection of source data and study records will be permitted to the Danish Health Ministries and the GCP unit at Bispebjerg Hospital.

10.3 Source Data

A list describing source data locations (i.e. relevant medical journal descriptions from the public/private sector, pathology results) will be devised prior to initation of the study.

10.4 Biobank

In the preliminary study, one 4 mm punch biopsy taken from the treated skin area and one 5ml blood sample per patient will confirm adequate local drug uptake and limited systemic permeation. Biopsies and blood samples are sent for analysis by collaborators at Copenhagen University. No extra material will be taken. Any remaining biological material will be returned to primary investigator Merete Haedersdal, Bispebjerg Hospital at the end of the study for potential use in future investigations. The material will be handled confidentially and will not be used in other scientific studies without Videnskabsetisk Komite's permission. Prior to storage, permission will be sought from Datatilsynet as well as patients. The biobank expires September 1, 2019 whereafter the material is kept for 10 years for future research and thereafter be destroyed.

11. Ethics

No previous trials have investigated AFL-assisted cisplatin+5-FU as a topical treatment in patients. Based on our preclinical results, we expect to successfully treat study participant's BCCs. During the trial, individual participants will be followed closely by treating phycisians. In the event of side

effects, these will be managed according to guidelines by investigating physicians. In the event of an unsuccessful treatment outcome, histological assessment should detect residual tumor tissue at 3 months control—a duration which is in accordance with general guidelines for established non-surgical BCC treatments. Patients will receive conventional therapy to ensure complete tumor removal. Beyond removal of the tumor, patients are not anticipated to benefit from the study.

While cisplatin and 5-FU are chemotherapeutics, side effect profiles associated with IV therapy (i.v. doses: 100 mg/m^2 (cisplatin); 400-600 mg/m^2 (5-FU)) cannot be transferred to the topical administration route used here. We do not anticipate systemic drug exposure due to the applied ultralow drug doses and small treatment surface areas. On the other hand, notable local erythema/edema, flaking/scaling, crusting, and erosion/ulceration are anticipated and represent, in addition to the inconvenience of multiple visits to the Hospital as well as invasive biological sampling, drawbacks of study participation. Still, as with standard 4 week topical 5-FU treatment, we expect local skin reactions to be tolerable and heal without sequelae.

On a boarder socioeconomic scale, the study may have an impact on future BCC treatment, a cancer form that each year affects more people than breast-, prostate-, lung- and colon cancer combined. Skin cancer represents a massive and ever-expanding health concern, and the demand for more gentle treatments particularly in aging, sun-damaged or immuno-suppressed populations is increasing. We expect that developing a new, minimally-invasive topical treatment would be beneficial to the tens of millions of patients who yearly receive a BCC diagnosis. These benefits include the option of forgoing surgery and associated complications, in favor of a more gentle and repeatable treatment that would ensure preservation of function and appearance of vital anatomical structures. Importantly, the laser technology used in this project is commonly available and has previously led to improved treatments for precancerous lesions that have now been implemented at our facility. Both investigated drugs are now produced cheaply and generically. The treatment is therefore not expected to pose an insurmountable healthcare cost should it be implemented. It is thus determined that the knowledge gained in this study outweigh the disadvantages and side effects that patients may experience as a result of their participation.

12. Financial Considerations and Insurance

Prof. Merete Hædersdal is initiator of the study and carries chief responsibility for the trial. Primary investigator is PhD student Emily Wenande. Internal funds at Dermatology Department Bispebjerg Hospital and research grants awarded to Merete Hædersdal and Emily Wenande will cover project-related costs, including reimbursement for study materials. All funding will be disbursed to relevant project accounts at Bispebjerg Hospital. No personal honoraria are awarded to investigators or participants. None of the collaborators have any personal relation or economic stake in the laser technology used in this trial. Treatments are performed at Dermatology Department Bispebjerg Hospital and patients are covered under the Hospital's patient insurance.

13. Information to Participants

After recruitment at the dermatological department, Bispebjerg Hospital or via private practice dermatologists, participants will be asked to provide informed consent at a prescheduled screening visit (Section 5). Investigators (Emily Wenande, Kristoffer Hendel, Katrine Togsverd Bo) are responsible for giving clear verbal and written information about aims, design and risks of the study, as stated in "*Information og samtykke til deltagelse i sundhedsvidenskabelige forsknings-projekter*" by "Sundheds- og Ældreministriet". In a private setting undisturbed and not in use by others, the participant will be made aware of their right to have an assessor present, that participation is voluntary, and that withdrawal is possible at any time during the study. Participants will be given adequate consideration time (minimum 24 hours). Participants will be asked to sign a consent form and two separate forms regarding use of clinical photos and biological material. Forms will all be stored together. A copy of the consent form will be given to participants and another copy will be stored in the study journal.

14. Publication of Results

The study will be sought published in an international dermatological journal and presented at scientific conferences, regardless of positive, negative or inconclusive results. No company has involvement or influence on publication of results or transmission of data. Authorships are given according to the Vancouver guidelines.

References:

1. Stern RS. Prevalence of a history of skin cancer in 2007: results of an incidence-based model. Arch Dermatol. 2010;146(3):279-82.

2. Neville JA, Welch E, Leffell DJ. Management of nonmelanoma skin cancer in 2007. Nat Clin Pract Oncol. 2007;4(8):462-9.

3. Chabner BA, Longo DL, Grem LJ, Ryan DP, Wadlow RC. Cancer Chemotherapy and Biotherapy: Principles and Practice. Philadelphia: Wolters Kluwer Health; 2015.

4. Arits AH, Mosterd K, Essers BA, Spoorenberg E, Sommer A, De Rooij MJ, et al. Photodynamic therapy versus topical imiquimod versus topical fluorouracil for treatment of superficial basal-cell carcinoma: a single blind, non-inferiority, randomised controlled trial. Lancet Oncol. 2013;14(7):647-54.

5. Micali G, Lacarrubba F, Nasca MR, Schwartz RA. Topical pharmacotherapy for skin cancer: part I. Pharmacology. Journal of the American Academy of Dermatology. 2014;70(6):965 e1-12; quiz 77-8.

6. Mohs FE, Jones DL, Bloom RF. Tendency of fluorouracil to conceal deep foci of invasive basal cell carcinoma. Arch Dermatol. 1978;114(7):1021-2.

7. Moeholt K, Aagaard H, Pfeiffer P, Hansen O. Platinum-based cytotoxic therapy in basal cell carcinoma--a review of the literature. Acta Oncol. 1996;35(6):677-82.

8. Simonetti LD, Gelfuso GM, Barbosa JC, Lopez RF. Assessment of the percutaneous penetration of cisplatin: the effect of monoolein and the drug skin penetration pathway. Eur J Pharm Biopharm. 2009;73(1):90-4.

9. Wenande E, Erlendsson AM, Haedersdal M. Opportunities for laser-assisted drug delivery in the treatment of cutaneous disorders. Semin Cutan Med Surg. 2017;36(4):192-201.

10. Wenande E, Olesen UH, Nielsen MM, Janfelt C, Hansen SH, Anderson RR, et al. Fractional laser-assisted topical delivery leads to enhanced, accelerated and deeper cutaneous 5-fluorouracil uptake. Expert opinion on drug delivery. 2017;14(3):307-17.

11. Wenande E, Olesen UH, Boesen M, Sturup S, Gammelgaard B, Haedersdal M. Fractional laser-assisted topical delivery of the anticancer agent cisplatin [abstract 92]. Lasers Surg Med. 2017;49(S28):22.

12. Ulrich M, Lange-Asschenfeldt S, Gonzalez S. Clinical applicability of in vivo reflectance confocal microscopy in dermatology. G Ital Dermatol Venereol. 2012;147(2):171-8.

13. Nori S, Rius-Diaz F, Cuevas J, Goldgeier M, Jaen P, Torres A, et al. Sensitivity and specificity of reflectance-mode confocal microscopy for in vivo diagnosis of basal cell carcinoma: a multicenter study. Journal of the American Academy of Dermatology. 2004;51(6):923-30.

14. Ulrich M, Lange-Asschenfeldt S, Gonzalez S. The use of reflectance confocal microscopy for monitoring response to therapy of skin malignancies. Dermatol Pract Concept. 2012;2(2):202a10.

15. Ulrich M, Reinhold U, Falques M, Rodriguez Azeredo R, Stockfleth E. Use of reflectance confocal microscopy to evaluate 5-fluorouracil 0.5%/salicylic acid 10% in the field-directed treatment of subclinical lesions of actinic keratosis: subanalysis of a Phase III, randomized, double-blind, vehicle-controlled trial. J Eur Acad Dermatol Venereol. 2017.

16. Hsu SH, Gan SD, Nguyen BT, Konnikov N, Liang CA. Ablative Fractional Laser-Assisted Topical Fluorouracil for the Treatment of Superficial Basal Cell Carcinoma and Squamous Cell Carcinoma In Situ: A Follow-Up Study. Dermatol Surg. 2016;42:1050-3.

APPENDIX 1- OUTCOME MEASURES AND TECHNIQUES

Outcome measures	Techniques
LSR (Primary outcome) Days 1-30	CLINICAL ASSESSMENT • FDA-accepted 0-4 point LSR scale
Side Effects (Primary outcome) Up to 3 months	CLINICAL ASSESSMENT • Prolonged erythema • Prolonged edema • Infection • Hyperpigmentation • Hypopigmentation • Scarring
Tumor size (mm) and clearance (Secondary outcome) Baseline, Day 30 (not RCM) and at Month 3	• Clinical evaluation • Dermocscopy • OCT (Vivosight) • HIFU (Logiq P9) • RCM (Vivascope) • Histology