Optical coherence tomography-assisted 1064nm Nd:YAG laser therapy of superficial and nodular basal cell carcinomas with ≤1mm tumor thickness

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Preface

„A laser is a solution seeking a problem“

- Dr. Theodore H. Maiman

Light Amplification by Stimulated Emission of Radiation. In other words, LASER. Coined as a milestone in the field of Physics, those five letters gave name to a complex device that has notoriously revolutionized the field of medicine. From laser-assisted in situ keratomileusis for refractive-error correction in ophthalmology to fractional laser resurfacing in the treatment of acne scarring in dermatology, the use of several kinds of laser as surgical tools has become a benchmark in daily medical practice.

When referring to laser dermatology, the term usually embraces aesthetic procedures regarding ablation of benign-lesions, treatment of scars, antiaging, depigmentation and removal of hair, tattoos and non-malignant vascular lesions.

Since a long time in Germany, laser ablation of pre-cancerous skin lesions has been considered a valid therapy. Conversely, ablative and non-ablative laser treatment of malignant skin cancers is restricted to consideration in low-risk basal cell carcinomas with existing contraindication for surgery or topical therapy. Initial research data in the treatment of those lesions show promising results, but the clear lack of further investigations in this topic leaves the door open to lasers being a potential alternative for low-risk non-melanoma skin cancer.

So, are we already at the gates of a new leading-edge chapter in laser dermatology that could finally merge laser with dermato-oncology? In other words, could dermato-oncology use Maiman’s solution to a pre-existing problem?

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List of abbreviations

ALA	Aminolevulinic acid
BCC	Basal cell carcinoma, basalioma
CI	Confidence interval
5-FU	5-Fluorouracil
FWHM	Full width at half maximum
LR+	Positive likelihood ratio
LR-	Negative likelihood ratio
MAL	Methyl aminolevulinate
Nd	Neodymium
Nd:YAG	Neodymium-doped Yttrium Aluminum Garnet
NMSC	Non-melanoma skin cancer
NPV	Negative predictive value
OCT	Optical coherence tomography
PDT	Photodynamic therapy
PPV	Positive predictive value
UV	Ultraviolet radiation
WHO	World Health Organization

1. Introduction

1.1. Basal cell carcinomas: epidemiology of a growing threat

Basal cell carcinomas (BCC) are the most prevalent malignant skin neoplasm and the most common kind of cancer worldwide. The incidence of these tumors in Germany has reportedly reached almost 250 new cases per 100.000 inhabitants per year.

Also being the most frequent form of non-melanoma skin cancer (NMSC), basaliomas develop in the basal layer of the epidermis, resulting from a malignant shift of bulge stem cells located in the hair follicles. Despite less than 1% of BCC originate metastasis, they tend to show a strong locally-invasive growth. Without a prompt detection and appropriate treatment of the lesions, BCC can derive in tissue destruction and sometimes mutilation.

BCC generally affect individuals in their mid-to-late adulthood (> 50 years of age) and show great predilection for the face and neck (> 80% of the cases), as they usually appear in sun-damaged skin areas after long-term ultraviolet radiation (UV) cumulative exposure. Other risk factors embrace chemical carcinogenics, genetic predisposition, immunosuppression and other types of ionising radiation. Male subjects with light skin-type (Fitzpatrick phototype I-II) are in higher risk of developing this skin cancer.

1.2. Classification: a clinico-histological outlook

According to the World Health Organisation (WHO), BCC can be classified into several subtypes, mainly attending to histological characteristics, but additionally contemplating clinical traits of the lesion. Those BCC variants are: superficial, nodular, micronodular, infiltrating, sclerosing/morpheaform, basosquamous/metatypical, pigmented, sarcomatoid, fibroepithelial and BCC with adnexal differentiation. Nodular BCC comprise three subvarieties (keratotic, nodulocystic/cystic and adenoid), whereas BCC with adnexal differentiation include infundibulocystic BCC.

Likewise, these tumors can be stratified by risk of recurrence in low-risk and high-risk BCC. Superficial and nodular BCC pertain to the first category.

Around 1 to 30% of all BCC belong to the superficial subtype, showing ≤ 1mm of tumor thickness. Yet 60 to 80% of all BCC cases refer to the nodular form, independently of their skin depth invasion. Combinations of more than one subtype pattern affect under 40% of all BCC, mostly merging nodular and micronodular fractions. In those cases, it is advisable to orientate the treatment based on the most aggressive subtype.

1.3. Diagnosis: a multidisciplinary approach

1.3.1. Clinical findings and dermatoscopy

Clinically, BCC can easily be recognised, even at early stages, due to a combination of their distinctive appearance (identifiable with the naked eye or using a magnifying glass) and dermatoscopic features. Those classically comprise a smooth-surfaced/desquamative erythematous maple-leaf-like macula or border-defined pearly nodule, linear and arborising telangiectasias, blue-grey ovoid nests and/or globules, spoke wheel areas and, in some of the cases, central ulceration.

- Superficial BCC present as erythematous maculae or plaques with well-circumscribed delimitation. Superficial scaling or central clearing might also be identified. They show predilection for the trunk.
- Nodular BCC are typically pearly papules or nodules with a smooth shiny surface and arborizing telangiectasias. The head and neck are their preferred locations.

1.3.2. Histopathology

As a complement to the clinical-dermatoscopic diagnostic approach, excisional or incisional biopsies can be performed. Generally, 2-3mm punch-biopsies are preferred. Despite histology being the definitive method in BCC diagnosis and the gold standard in BCC-suspected diagnosis verification, complications of a skin biopsy may occur. Those include: type I hypersensitivity reaction to preoperative local anaesthesia, bleeding,

oedema, local pain, biopsy-site hyposensitivity and long-term tissue-scarring, compromising aesthetic results.

This technique facilitates an identification of BCC subtype and also measures tumor thickness, that being ≤ 1mm if locally-superficial or > 1mm if invasive.

Histologically not being true carcinomas, BCC are accordingly considered semi malignant tumors. They show a basaloid epithelial tissue component of ectodermal provenance, which is essentially a formation of tumor parenchyma and mesodermal-origin connective tissue stroma embedding the former.

- Superficial BCC display multiple lobular foci of basaloid cells that emerge from the epidermis or from the sides of follicles or eccrine ducts, surrounded by mixoid stroma and band-like lichenoid infiltrates. Those cells project into the papillary dermis and superficially attach to it. Tumor thickness achieves a maximum of 1mm.
- Nodular BCC exhibit discrete nests of malignant basaloid cells with peripheral palisading that extend into the dermis and mucoid stroma containing plump spindle cells.

Angiogenesis of BCC allegedly derives from the vascular plexus, its horizontal-layered components and vertically-oriented connecting vessels. The superficial vascular plexus delimitates the junction between papillar and reticular dermis and comprises anastomosing small-caliber arterioles that branch into capillaries and supply the overlying epidermis and adnexal structures. Besides, the deep dermal vascular plexus sets the boundaries of the reticular dermis and the subcutaneous tissue. Its structure embodies medium-caliber vessels arising from larger vessels from the adipose septae of the subcutis.

Grunt et al. (1985) recognized teleangiectatic widened thin-walled venous vessels squeezed between tumor beds of BCC and the thin atrophic overlying epidermis. The lumen of those presumed venous vessels was large, but the walls were endothelial lining with barely any supporting tissue layers, which made them resemble capillaries. On that ground, the authors concluded BCC tumor cell beds were enveloped by basket-like capillary plexus.

1.3.3. Optical coherence tomography

In order to avoid undesirable side-effects of invasive diagnostic techniques, optical coherence tomography (OCT) stands as a clinically-proven non-invasive imaging technique, providing cross-sectioned defined-field portrays of the skin in real time.

OCT combines principles of sonography and optical interferometry, using near-to-infrared wave-length light. The *VivoSight*® scanning system (VivoSight Dx; Michelson Diagnostics Ltd., Maidstone, UK) operates with 1305nm light emission into the evaluated skin area (6x6mm), in direct contact with the machine. The device records the reflected light beams coming from the skin and displays a two-dimensional image in a black-grey-white scale. The image depth ranges from 1 to 2mm, whereas its lateral resolution is under 5.5μm and its axial resolution under 7.5μm.

A complete overview of the epidermis, dermis and dermo-epidermal junction can be recognized, as well as some skin adnexa and vascularization.

In a healthy skin coronal OCT image, a high-intensity signal line (white to light grey) defines the input of light contacting the *stratum corneum*. This is axially followed by a lower-intensity signal layer (darker grey) corresponding to the epidermis. Following this layer appears a higher-intensity one (light grey, darker than the input signal) delimiting the dermis. Within the dermis, blood vessels appear as long transversal low-intensity signal structures and hair follicles emerge as vertical also low-intensity signal formations, with enlarged shadowing.

With strong consensus, OCT is an accepted BCC-diagnostic method recommended in German medical practice guidelines, also credited to evaluate treatment response in those lesions.

The visualisation of altered skin architecture in cross-sectional OCT images of BCC leads to a better discrimination and delineation of those lesions, reaching 87% of reliability in multicentre studies, when combined with clinical eye and dermatoscopy.

Among the most common morphological criteria in superficial BCC OCT imaging are low-signal long subepidermal blood vessels. Also, low-signal half-full-C moniliform

epidermal structures, with darker basal edges. The epidermis tends to be enlarged and the dermo-epidermal junction slightly vanishes.

Conversely, nodular BCC usually display a thinned epidermis, cysts and low-signal ovoid structures with darker delimitation. The latter are suspected to be the result of peritumoral mucinosis and often appear surrounded by high-signal stroma.

Moreover, the OCT methodology provides exact measurements of the penetration depth of the tumor lesion. Dynamic OCT softwares provide a complete analysis of the vascular plexus, including calculation of the density, diameter and depth of the blood vessels nurturing the tumor. Diameters > 20μm and depths < 500μm can be detected by the device. If the skin take is not focused within the precision margins of the OCT and the skin layers appear too high or too low on screen, the parameters might not be recognised. In addition, if the blood flow is too slow, dynamic OCT cannot identify the blood-vessel structures.

1.4. Treatment of basal cell carcinomas with ≤ 1mm thickness

1.4.1. Surgical excision: the gold-standard

Complete surgical-excision therapy remains the gold standard, especially in tumors with > 1mm thickness, but also under this tumor depth. Safety margins of 3-5mm are preferred. Recurrence rates after five years reach 2-8%.

In some cases, prior histopathological confirmation of the BCC is favoured. Then, incisional biopsies would be performed before the complete removal of the lesion. Despite that, it is known that spontaneous regression after incisional biopsies occurs in 20% of the cases.

1.4.2. Other treatment options

If contraindications to undergo surgical treatment are present or patients refuse this option, second-line therapies must be contemplated. In BCC with ≤ 1mm tumor

thickness, topical treatments are of choice. Laser therapy is relegated to a third-line, when surgery and topical treatments cannot be considered or failed, as well as in case of expressed decision of the patient.

1.4.2.1. Topical treatments: second-line therapy

Topical treatments with local immunotherapy (imiquimod 5%), local chemotherapy (5-FU) or photodynamic therapy are alternatives restricted to superficial basaliomas (superficial subtype, Breslow ≤ 1mm), when surgery is contraindicated or due to preference of the patient.

- Imiquimod 5% is an immune-response modifier that promotes the secretion of interferon-α, interleukin-6 and tumoral necrosis factor-α via activation of toll-like receptor-7 in immune cells. Through the release of these cytokines and chemokines, a cell-mediated immune response generates an anti-tumoral effect on the skin. Imiquimod 5% is a topical treatment applied on superficial BCC for five days weekly during a total of six weeks. Moderate-to-severe local adverse events include erythema, oedema, erosion, scabbing, pruritus and paresthesia. According to randomized controlled trials, clearance rates vary between 60 and 80% over 3-12 months after treatment of superficial BCC. Clearance rates of nodular BCC are restricted to limited evidence and oscillate between 42 and 81%.

- 5-fluorouracil or 5-FU is an inhibitor of thymidylate synthase, an enzyme involved in the synthesis of pyrimidine thymidylate and, hence, DNA replication. Topical 5-FU in the treatment of superficial BCC should be applied twice daily during six weeks. Moderate-to-severe local adverse events are similar to those seen in imiquimod 5% and include erythema, oedema, erosion, scabbing, pruritus, paresthesia, ulcering and eschar-building. The incidence of at least one adverse event reaches 97-100% in published studies. Limited data on efficacy and long-term response are available, but 16-week clearance rates of 50 to 90% have been reported.

- Photodynamic therapy consists in the application of exogenous photosensitizers, 5-aminolevulinic acid (ALA-PDT) or methyl aminolevulinate (MAL-PDT), on superficial BCC. Penetration capacity of photosensitizers is limited to 1-2mm, which restricts the effectivity of this procedure to superficial tumors. Malignant and premalignant cells selectively intake photosensitizers and convert them into protoporphyrin IX (PpIX). After three hours, the area is illuminated during ten to twenty minutes using 635nm red light, which activates PpIX. This induces the release of reactive oxygen species and cytotoxicity of tumoral cells. Regarding treatment response, clearance rates differ between studies and BCC-subtype:
  - A preliminary controlled clinical trial compared to placebo was performed by Tope et al. (2004). The authors reached histologically-confirmed clearance rates of 79% after six months of ALA-PDT for nodular BCC.
  - In a randomised controlled trial compared to surgery, Rhodes et al. (2004) reached 91% clearance rates of nodular BCC with 85% with good-excellent cosmetic response.
  - Foley et al. (2009) achieved histologically-confirmed clearance rates of 60-73% after six months of ALA-PDT for nodular BCC in another small randomised controlled clinical trial. Cosmetic outcomes were graded good to excellent in 98% of the treated lesions.
  - In a prospective cohort study, Fantini et al. (2011) reported complete clearance in 33% of nodular BCC and 82% of superficial BCC after two treatments of MAL-PDT and mean follow-up of 23.5 months. The authors described location of the lesions on extremities as a negative response predictor.
  - Zou et al. (2016) conducted a meta-analysis of five randomised controlled trials, presenting clearance rates of 79% five years after MAL-PDT for nodular BCC.

In a recent randomised clinical trial, Arits et al. achieved clearance rates after 12 months of 83.4 % for topical imiquimod, 80.1% for topical 5-FU and 72.8% for MAL-PDT. After 36 months, clearance rates decreased to 79.7 % for topical imiquimod, 68.2 % for topical 5-FU and 58 % for MAL-PDT. It was demonstrated that topical 5-FU is inferior to topical imiquimod 5% and non-inferior to MAL-PDT, whereas imiquimod 5% is superior

to both topical 5-FU and MAL-PDT in the treatment of superficial BCC after 3-5 years of follow-up.

1.4.2.2. Laser therapy: third-line rescue treatment

According to European guidelines, ablative and non-ablative laser therapy can be considered a treatment option for low-risk BCC, when surgical or topical treatments are contraindicated.

- Ablative CO2 and Erbium:YAG lasers showed so far relapses in the depth of the treated area. Therefore close control after treatment is indispensable.
- Non-ablative lasers have been subject of investigation in the past years.
  - Pulsed-dye lasers (PDL) achieve selective thrombosis of afferent vessels, preventing epidermal and dermal damage. Results in BCC-clearance are miscellaneous: Allison et al. (2013) reported clearance in one out of seven tumors, Shah et al. (2009) achieved 92% regression rate after four treatments administered every two weeks, Konnikov et al. (2011) obtained 90% regression at 12-21 months after treatment, Ballard et al. (2011) recorded 56% clearance after one PDL session, whereas Karsai et al. (2015) revealed 78% clearance rates of low-risk BCC. Recurrence is suspected to rely on the limited penetration of the laser, which is unable to outreach the superficial dermis.
  - Since 1975, Nd:YAG lasers have been issue of research. Despite that, scarce data of small patient samples are available on the topic and yet no clinical trials have been conducted.

1.5. 1064nm Nd:YAG laser: an open door in BCC treatment

1.5.1. Laser physics: understanding the principles

1.5.1.1. Light Amplification by Stimulated Emission of Radiation

In 1960, physicist Theodore Maiman developed the first functioning optical laser using a synthetic ruby crystal. He defined the process as Light Amplification by Stimulated Emission of Radiation, conceiving the word LASER, which would give name to his newly created machine.

A laser is a device that stimulates orbital electrons to emit light at particular wavelengths. This light is amplified and results in a narrow beam of radiation.

Every laser incorporates a fully reflective mirror on one side, a partly reflective mirror on the other side and a solid, liquid or gaseous medium in between. Both mirrors constitute the laser resonator.

Photons deriving from an electrical energy source excite the atoms present in the laser's medium. As stable electrons jump from their lowest to their highest energy level, when the amount of excited atoms exceeds the number of atoms in ground state, population inversion occurs. Once those excited electrons interact with incoming photons of equal energy, they release their energy and experience an optical decay, transitioning through dropping energy levels.

Every time an electron falls to a lower energy level, a new photon is emitted. The energy of that photon corresponds to the energy difference of the electron levels. This photon is identical to the photon which originally stimulated the emission: it has the same energy, same wavelength and same direction. Both original and newly emitted photons travel from that point on together, causing their wave amplitudes to amplify.

This production of new photons is known as stimulated emission and generates the laser beam, which is released through the partly reflective laser output. The resulting laser beam is narrow-bundled monochromatic light with a specific wavelength.

1.5.1.2. Wavelength, absorption and scatter

Light travels in waves. The wavelength of a laser is the spatial distance between two successive peaks in every wave. This parameter is predetermined by the kind of laser and cannot be modified.

Endogenous chromophores, such as melanin, oxyhaemoglobin, haemoglobin and water, selectively absorb different wavelengths in the light spectrum. The way light beams

interact with tissue is determined by absorption and scatter. When applying a laser on the skin, the longer its wavelength (for example, 1064nm), the lower the laser absorption in the uppermost skin layers. This leads to a deeper penetration of the laser into the skin and a greater scatter of the beam. Thereby, more energy will be needed to heat the target and acquire the resultant effect.

Long laser wavelengths originate low energy photons. This is justified by the inversely proportional relation between the energy level of photons produced by the laser's medium and the wavelength of the light generated by the device. The wavelength of a laser, together with other factors like pulsed laser emission, determine the power of the laser.

1.5.1.3. Laser parameters: configuring the device

Lasers must be programmed to attain a certain effect. To do so, the following parameters must be adjusted:

- Pulse: flash of light or emission of a laser beam, which can either be short or long.
- Pulse width: duration of one pulse (msec). Laser pulses follow a Gaussian time distribution, determined by intensity and time. Full width at half maximum (FWHM) is usually denoted as pulse width and actually refers to the duration of the pulse at its half maximum intensity.
- Pulse rate or pulse frequency: number of pulses per second (Hz).
- Fluence or pulse density: energy of a laser pulse or optical energy delivered per unit area (J/cm^2).
- Power: energy of the laser delivered to the desired target per second (J/sec or W).
- Power density: energy of the laser delivered to the desired target per second per unit area (W/cm^2).
- Spot size: diameter of the laser beam (mm).

1.5.2. 1064nm Nd:YAG laser and selective photothermolysis

Nd:YAG is a solid-state laser that uses neodymium-doped yttrium aluminum garnet crystal as medium. A triply ionised neodymium replaces small fractions of yttrium ions in the crystal and provides the laser activity. At a wavelength of 1064nm, this laser reaches deeper structures in the skin, targeting red pigment from oxyhaemoglobin and, therefore, blood vessels. At 1.5-3msec pulse width, 1064nm Nd:YAG laser shows significant selectivity for arterial blood.

In 1983, Anderson et al. stated that inherent optical and thermal properties of brief pulses of visible light could provide target selectivity to cutaneous microvessels. During laser emission, the absorption of photons from pigmented structures in the skin triggers photochemical reactions and significant heating. Rapid localized heating generates shock waves that propagate through the skin layers, causing mechanical damage or vaporisation. Likewise, mammalian tissues heated to 70-100 ºC may suffer protein denaturation, which leads to coagulation necrosis. This process develops into closing of blood vessels through hemostasis, achieved via denaturation of plasma proteins. Thermal diffusion or scattering occurs when targeted tissues transfer their heat to their cooler circumambient. At the end of laser exposure, targets may have surpassed temperatures required for thermal denaturation, while surroundings remain below it. For this reason, only thermal damage without denaturation affects the latter.

By specifically targeting blood vessels and sparing overlying or immediately neighbouring tissues, selective photothermolysis can be successfully achieved. This technique depends on selective absorption of brief optical radiation pulses to exclusively generate heat at certain pigmented targets. Those targets must have greater optical absorption at a precise wavelength than their surrounding tissues.

- The wavelength of a laser determines the depth of its effect, where selective photothermolysis can happen. Microvessels, like those found in the vascular plexus of BCC, need longer wavelengths, as major tissue chromophores in their surroundings tend to have greater absorption at shorter wavelengths.

- Long laser exposures heat tissues uniformly and cause nonspecific coagulation necrosis. An instantaneous laser pulse shows extreme temperature differences between the target and its surroundings, which can lead to shock wave damage. On that account, the transition from nonspecific to specific thermal damage succeeds if laser exposure duration (pulse width) equals and next exceeds thermal relaxation time (t_r) for targets. Anderson et al. targeted oxyhaemoglobin contained in 10-50μm-diameter microvessels from the superficial plexus and concluded that t_r was around 50μsec for 20μm-diameter vessels. The authors concluded selective thermolysis requires short pulses or millisecond-domain pulse width for non-capillary vessels.

- Higher laser fluences enhance selective photothermolysis by earning an immediate brown decoloration of blood due to haemoglobin denaturation, a sudden decrease in blood flow as a result of plasma denaturation, permanent hemostasis in view of tissue protein denaturation and vessel rupture plus hemorrage thanks to vaporisation or shock wave damage.

All in all, selective photothermolyisis by means of laser exposure is optimized selecting a wavelength that penetrates the skin appropriately, minimizes the absorption by overlying epidermis and maximizes the absorption by blood vessels.

1.5.3. Current scope of 1064nm Nd:YAG laser in BCC-treatment

Non-ablative long-pulsed 1064nm Neodymium-doped Yttrium Aluminum Garnet laser (Nd:YAG) therapy of superficial basal cell carcinomas with Breslow thickness ≤ 1mm is a treatment option contemplated in European medical practice guidelines.

The success data of this technique is restricted to eight publications, conducted in Russia, Germany, Egypt and the United States, in chronological order:

- The first publication on this topic dates back to 1975. Wagner et al. conducted a prospective cohort study with 76 BCC distributed on the head, neck, thorax, abdomen and extremities. Tumor size was not restricted and histologic-variant of the BCC were not specified. Single-session treatments were performed using a pulsed

Nd laser (Pulsar-1000) with a solid neodymium-doped glass optical generator. The authors highlighted the importance of selecting the correct magnitude of pulse density in order to achieve the appropriate depth and extent of tumor destruction. Pulse density oscillated between 400 and 600J/cm^2, using the lower range in pigmented or vascularized lesions. Higher ranges were applied to light-coloured keratotic tumours. The process was described as slightly painful, but no numeric data were provided. Pulse duration was 1 msec. Coagulation necrosis was proposed as mechanism of action. After 2 to 30 months follow-up, no BCC-recurrence occurred. Thus, no confirmatory biopsies were taken. Cosmetic effects were defined as quite satisfactory, except in lesions exceeding 2-2.5cm, in which rough scarring might develop. Yet again no detailed data were specified.

- In 1985, Brunner et al. published a prospective cohort study, in which 200 BCC were treated in one session with Nd:YAG laser under local anaesthesia. Primary and recurrent BCC were included. Most of the BCC were located on the head and neck and had no size restrictions. In this case, pulse density was 800-4.000 J/cm^2 and pulse duration 1-3 seconds. Biopsies were taken after 48 hours and after 6 weeks, with proceeding follow-up of 18 months. No tumor cells were identified in endpoint-biopsies. Clinical and histological results were accounted as promising, but subsequent studies with long-term follow-up were advised for further conclusions. The necrosis resulting from a coagulating effect up to a depth of 5mm was suggested as mechanism of action of the Nd:YAG laser.

- Published in 2004, El-Tonsy et al. conducted a prospective cohort study with 37 biopsy-confirmed BCC (superficial, nodulo-ulcerative, morpheaform and pigmented), located on the head and neck. The lesions, primary or relapses after other treatments, were had also no size restrictions. Nd:YAG laser sessions took place every six weeks until biopsy-confirmed clearance of the lesion. Laser parameters were 10-15W and 8mm spot size. Afterwards, patients were followed every six months during 3-5 years. In total, 36 patients experienced complete clearance (97.3%). Coagulation was the suggested mechanism of action of the procedure.

- Later in 2010, Moskalik et al. disclosed a retrospective cohort-study in which 1060nm Nd:YAG or pulsed Nd laser (Pulsar-1000 and Pulsar-1000M) were used to treat in single sessions 3346 histologically-confirmed primary facial BCC (2719 pulsed Nd laser, 627 Nd:YAG) and 188 recurrent facial BCC (all pulsed Nd laser). The parameters for the Nd laser were: 1060nm wavelength, pulse duration of 1-4.5msec, pulse density of 700-1000J, spot diameters of 0.5 and 1.5cm. The configuration of the Nd:YAG laser was: 1060 or 1320nm wavelength, pulse duration of 1msec, frequency of 10-40Hz, maximum pulse energy of 0.6J and spot diameter 1mm. A total of 1-5 impulses at 40- to 60-second intervals were performed, achieving a total energy of 118-3520J. After a follow-up from 3 months to 5 years, recurrence rate was 1.8% for primary BCC treated with pulsed Nd laser, 2.5% for primary BCC treated with Nd:YAG and 3.7% in for the recurrent BCC treated with pulsed Nd laser. Also, 72.3% of all BCC recurrences appeared within the first year. In 78% of recurrent BCC, the initial laser session was repeated.

- Ortiz et al. released a prospective cohort study in 2015 including 13 superficial, nodular and micronodular primary BCC on the trunk and extremities, confirmed with an initial shave-biopsy. All BCC had a diameter < 1.5cm. The lesions were treated with 3 passes of 1064nm Nd:YAG laser in one-session schemata. Spot size was 5mm, fluence 80-120J/cm^2 and pulse duration 10msec. No local anaesthetic was used. One month later, the complete excision of all lesions was performed and a 92% clearance was histologically proven (12/13 BCC), ascending to 100% at high fluences of 120J/cm^2 (10/10 BCC). The suggested mechanism of action was selective photothermolysis. No patients under anticoagulant therapy were included in the study.

- The same team published a second prospective cohort study in 2018, this time comprising 31 superficial, nodular and pigmented primary BCC on the trunk and extremities. The lesions were biopsy-proven and showed diameters < 2.1cm. Again, no local anaesthetic was used. One-session treatments with 1064nm Nd:YAG laser were completed with the following configuration: 5-6mm spot, fluence of 125-140J/cm^2 and pulse duration of 7-10msec. After 1 month, all BCC were surgically

removed and histologically analysed, achieving a 90.3% clearance (28/31 BCC). The 3 BCC that remained present after treatment were located on the shoulder, back and abdomen, and belonged to superficial and nodular subtypes. Patients undergoing anticoagulant therapy were excluded from the study, except patients taking acetylsalicylic acid or non-steroidal anti-inflammatory drugs.

- Ahluwalia et al. (including Ortiz) authored a publication in 2019 based in a retrospective cohort-study with 16 patients with < 1.5cm-diameter superficial and nodular BCC on the head neck, trunk and extremities. All tumors were biopsy-confirmed, but only one BCC was additionally examined with OCT prior to and after treatment. A single session of 1064nm Nd:YAG laser (5mm spot size, 140J/cm^2 and 8msec pulse duration) was accomplished, after applying 3ml of 5mg/ml lidocaine hydrochloride 0.5% as local anaesthetic. Patients were followed during 6-15 months. If a suspected relapse occurred, a biopsy was taken. In this study, a clearance of 100% of all BCC was achieved.

- The last reported publication belongs to Markowitz et al. and dates back to 2019. A total of 17 BCC in several locations, including the face, were retrospectively reviewed. No prior biopsy was taken, only clinical and dermatoscopic criteria were taken into account in the diagnosis, as well as reflectance confocal microscopy (RCM) as confirmatory method. One to three treatments in intervals of 1 to 2 months with 1064nm Nd:YAG laser with 5-6mm spot, 125-140J/cm^2 fluence and 7-10msec pulse duration. Two months after the last laser session, clearance reached 100%, as confirmed clinically and via OCT. Out of all BCC, 82.4% cleared after one session, 5.8% were located on the back and cleared after two sessions, whereas 11.8% were found on the face and back and cleared after three laser treatments.

The present experience in Europe relies on Prof. Dr. Julia Welzel at the Uniklinik Augsburg in Germany, also with positive outcomes using 1064nm *Sciton*® Nd:YAG laser (120-130J/cm^2, 8msec, 0.6Hz). No current data provided by treating centres in Europe have been so far published.

1.5.3. Loose ends in 1064nm Nd:YAG laser: a chance for further research

Despite all published data on the treatment of BCC with 1064nm Nd:YAG laser, numerous questions remain open:

- Which lesions respond better to therapy?
  Location and tumor thickness of the BCC have been so far presented as descriptive parameters in all published studies. Furthermore, depth has not been reported in any study. But, could all mentioned characteristics be used as response predictors?

- Which histological features achieve better response to treatment?
  A lack of clarity in the potentially Nd:YAG-targetable BCC-subtypes is evidenced. In accordance with European guidelines, Ortiz et al. targeted BCC with Breslow thickness ≤ 1mm, but included several subtypes in the study (superficial, nodular, micronodular and pigmented). All other publications broadened the subtype-variant or did not specify it.

- Which laser settings obtain better outcomes?
  Nd:YAG laser configuration also remains ambiguous. According to the latest available literature, fluence should vary between 80 and 140J/m^2, pulse duration should last 7-10msec and spot size should oscillate between 5 and 6mm. Pulse rate is not specified in the publications from the past 6 years.

- Which is the mechanism of action?
  Regardless of the optimistic, yet scarce, published results in the use of Nd:YAG to treat BCC in the uppermost skin layers, the mechanism how these tumors are erased from the skin still remains unknown. Considering the coagulating power of the laser, some publications suggest tumor clearance could be accomplished due to selective photothermolysis, a blood vessel-shrinking effect. This should trigger a thrombosis or complete sclerosis of the small blood vessels nurturing the tumor, resulting in an interruption of its vascularisation and hence causing tumoral cell death.

2. Initial hypothesis and objectives

The aim of this study is to conduct preliminary research in order to evaluate the use of OCT-assisted 1064nm Nd:YAG laser in the treatment of superficial and nodular basal cell carcinomas with ≤ 1mm tumor thickness.

The main objectives of this thesis are:

- To determine if OCT is an accurate and reproducible complementary method to clinical-dermatoscopic diagnosis of superficial and nodular BCC with ≤ 1mm tumor thickness by:
  - Evaluating the reliability of OCT compared to histopathology in the diagnosis of those BCC.
  - Calculating the interpersonal variability of OCT diagnoses between observers.

- To ascertain if OCT could replace skin biopsies as a confirmatory method of clinical-dermatoscopic diagnosis and allow same-day direct diagnosis-treatment schemata of superficial and nodular BCC with ≤ 1mm tumor thickness.

- To prove if OCT is a reliable clearance-confirmation method, comparable to skin biopsy in follow-up phases after 1064nm Nd:YAG laser treatment.

- To evaluate if laser therapy of superficial and nodular BCC with ≤ 1mm tumor thickness using 1064nm Nd:YAG is an effective treatment method with successful short-term clearance rates.

- To ascertain if 1064nm Nd:YAG could become the first go-to option in second-line treatments of superficial and nodular BCC with ≤ 1mm tumor thickness.

- To verify if treatment of superficial and nodular BCC with ≤ 1mm tumor thickness using 1064nm Nd:YAG laser has satisfactory cosmetic outcomes, better than those achieved after surgery or second-line therapies.

- To illustrate if 1064nm Nd:YAG laser achieves high patient tolerability and satisfaction in the treatment of superficial and nodular BCC with ≤ 1mm tumor thickness.

- To define targetable lesions and outcome predictors by describing tumor features and histological characteristics associated to better or worse response to 1064nm Nd:YAG treatment.

- To determine an effective 1064nm Nd:YAG laser configuration to achieve complete clearance of superficial and nodular BCC with ≤ 1mm tumor thickness.

- To examine if selective photothermolysis is a plausible explanation for BCC clearance after 1064nm Nd:YAG laser, according to dynamic-OCT findings.

- To conduct a preliminary study in order to justify whether a randomised controlled trial would be reasonable to validate 1064nm Nd:YAG as a second-line treatment option of superficial and nodular BCC with ≤ 1mm tumor thickness.

As an initial hypothesis it is suggested that OCT-assisted Nd:YAG laser treatment of superficial and nodular BCC with ≤ 1mm tumor thickness is a safe and effective diagnostic and treatment method with comparable clearance rates to second-line therapies. Improved patient satisfaction, tolerability, practicability and cosmetic end result compared to surgery and second-line therapies are proposed.

3. Material and methods

3.1. Study design

This thesis was designed as an observational retrospective cohort study, based on the experience in the use of 1064nm Nd:YAG laser therapy in superficial basal cell carcinomas at MVZ Dermatologisches Zentrum Bonn GmbH (Friedensplatz 16, 53111 Bonn).

The author of the study was Ariana Palacio Giral, resident doctor in Dermatology and Venerology at MVZ Dermatologisches Zentrum Bonn GmbH. The academic author designed and performed the study, conducted the data analysis and wrote the manuscript. The OCT statistical analyses were completed at the Statistics and Bioinformatics Unit (UEB) from Vall d'Hebron Hospital Research Institute (VHIR).

The clinical supervisor of the study was Prof. Dr. Uwe Reinhold, director of the centre mentioned above. The histology supervisor and thesis tutor was Prof. Dr. Erhard Bierhoff, director of Heinz-Werner-Seifert Insitut für Dermatopathologie (Trierer Str. 70, 5315 Bonn). Both centres belong to CORIUS Gruppe®.

Data were retrospectively collected from December 2019 until February 2022.

3.2. Study participants

A total of 39 patients with altogether 50 BCC were enrolled in the study.

Inclusion criteria were: female, male or non-binary patients presenting histologically-confirmed ≥1 superficial or nodular basal cell carcinomas with ≤1mm thickness, originally to be treated with 1064nm Nd:YAG laser.

Exclusion criteria were defined as follows:

- A lack of informed consent or a revoked participation in the study.

- A lack of compliance, described as ≥2 documented missed appointments within both therapy and follow-up phases.
- BCC treated with therapies other than Nd:YAG laser.
- Non-histologically verified BCC.
- Histologically-verified non-superficial non-nodular BCC.
- Histologically-verified basal cell carcinomas with Breslow >1mm.
- Patients with primary or secondary immunodeficiency. The presence of other concomitant illnesses and/or the intake of medication this effect did not exclude the participation in the study.

No restrictions were made regarding age, BCC location or number of lesions.

Relapsing BCC were not excluded, understanding those as newly diagnosed basal cell carcinomas on a previously BCC-affected area after a ≥6-month complete clearance of the lesion, confirmed with histology.

3.3. Ethical considerations

All patients were properly informed that their results and photographic documentation might be used in a doctoral thesis and further medical publications for research purposes, always in anonymous format. No patient was included in the study without their informed consent.

All patients included in the study were planned to undergo a treatment with 1064nm Nd:YAG laser from a start and were therefore offered to participate in the study. The treatment decision was *de facto* independent from the study and the plan to use this technique was only an inclusion criteria. The only contemplated justifications of treatment with Nd:YAG were an informed decision of the patient, with medical approval, along with a treatment indication/acceptance according to the German medical practice guidelines (e.g. patients with contraindication for surgery).

3.4. Study protocol

This was an observational retrospective cohort study including 50 BCC. Inclusion criteria were: adult patients (>18 years of age) presenting one or more histologically confirmed superficial or nodular BCC with tumor thickness ≤1mm on any corporal area. Informed consent was obtained from all patients enrolled in the study. Patients' demographic characteristics were initially recorded (age and gender). All pre-treatment biopsies were evaluated by a single expert dermatopathologist. Tumoral characteristics according to pathology were documented: localization, tumor thickness and histological subtype.

In addition, all tumors were imaged and mapped prior to treatment and at 3 months after the first treatment with dynamic Optical Coherence Tomography (D-OCT) (VivoSight Dx; Michelson Diagnostics Lt., Maidstone, UK). OCT imaging provided a vertical section view, 6mm wide, with depth penetration of 1mm at a resolution of 7.5µm lateral by 5µm vertical. This technique provided an en-face field of view of 6mm x 6mm. OCT was used for the diagnosis of BCC and for evaluating tumor thickness and lateral extent. In addition, D-OCT was utilized to systematically evaluate the tumor-associated vascular plexus, providing estimates of depth of the plexus and the density of blood vessels in tumor sites.

All lesions were treated with 1064nm Nd:YAG laser (ClearScan™ 1064nm; Sciton Inc., Palo Alto, CA) 15 minutes after local anesthesia with mepivacaine hydrochloride solution at room temperature (10mg/ml). Laser treatment was performed using a scanning handpiece with a fluence of 120J/cm^2, pulse width of 8msec and pulse delivery rate of 0.6Hz. The scanning handpiece delivered successive pulses of 4.8mm diameter in an hexagonal pattern, to rapidly and evenly treat the whole lesion area, including a 5mm treatment margin around the mapped tumor border. Tumoral margins were identified clinically and also via D-OCT scans of the tumor-feeding vasculature. The laser software allowed selection of three different hexagonal pattern sizes of approximately 5mm, 15mm or 25mm diameter, to suit the desired treatment area. Immediately after laser pulse delivery, the tumor tissue was clinically observed for change to gray color. If it did

not, a second pass was performed after a delay of 3 minutes to allow the lesion to cool. A third and last pass took place 3 minutes after, in case of absence of gray coloration.

As seen in Figure 1, a first follow-up was scheduled at one month after treatment, where all lesions were exclusively clinically evaluated. If previously treated areas showed clinical or dermatoscopic signs of remaining BCC, a second laser session was performed, following the same protocol from the previous session.

At 3 months follow-up, local adverse effects were recorded and clinically classified as mild, moderate or severe, along with patient tolerability and satisfaction. The latter were ranked using a 5-point Likert scale (1=very satisfied, 2=somewhat satisfied, 3=neither satisfied nor dissatisfied, 4=a little dissatisfied, 5=very dissatisfied). All treated lesions were assessed by clinical evaluation and dynamic OCT imaging. At this point, a final control biopsy was taken. The biopsy was performed in areas of suspicious foci, identified clinically or via OCT.

Clinical remission was evaluated one year after confirmed clearance via endpoint biopsy.

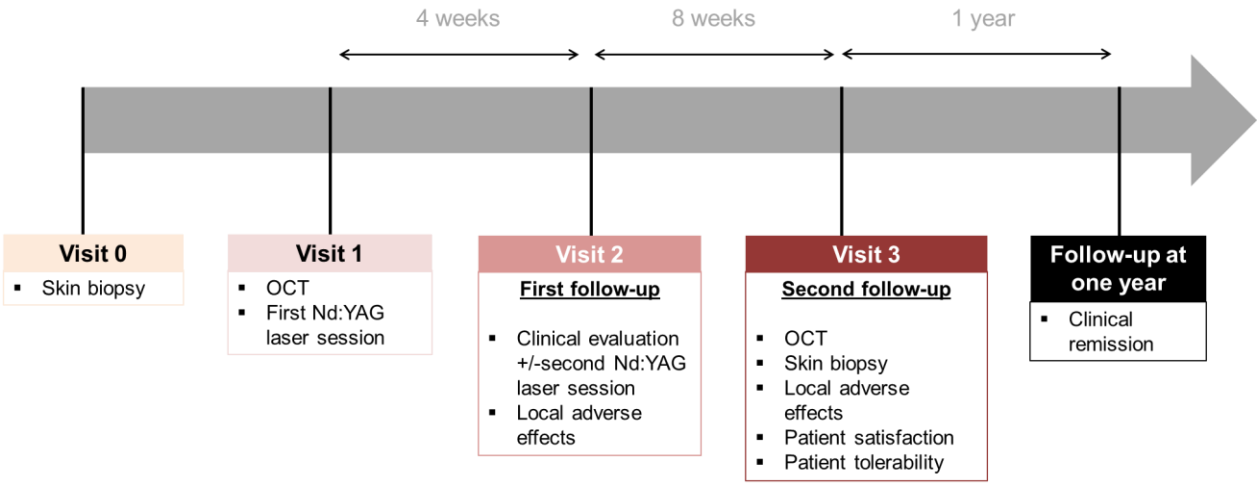

Figure 1. Timeline of study development with treatment and follow-up visits. After taking a skin biopsy at Visit 0, patients undergone OCT and a first Nd:YAG laser session at Visit 1. A total of 4 weeks later took place a first follow-up (Visit 2) and 8 weeks after that a second follow-up (Visit 3). A last follow-up took place one year after Visit 3.

Lastly, as some of the procedures took place during the COVID-19 pandemic, protective FPP-2 or surgical masks were worn by all patients and FPP-2 masks by doctors and staff present. The appropriate hand and material-disinfection procedures were also accomplished. Any skin-contact with all patients from doctors and other staff members were conducted using medical disposable plastic hand gloves.

3.5. Statistical analysis

All analyses were performed with Microsoft Excel (Microsoft Corporation, 2010. Microsoft Excel). Confidence intervals were calculated using a Wilson score binomial interval. P-values were calculated with Fisher’s exact test for an alpha level of 0.05.

4. Results

4.1. Treatment of BCC with 1064nm Nd:YAG

4.1.1. Descriptive analysis

39 patients were included in the study. There were a total of 23 female and 27 male subjects. Mean age was 68 years, ranging 36 to 88. As described in Figure 2, of all 50 BCC, 9 were nodular BCC (18%), whereas 41 were superficial BCC (82%).

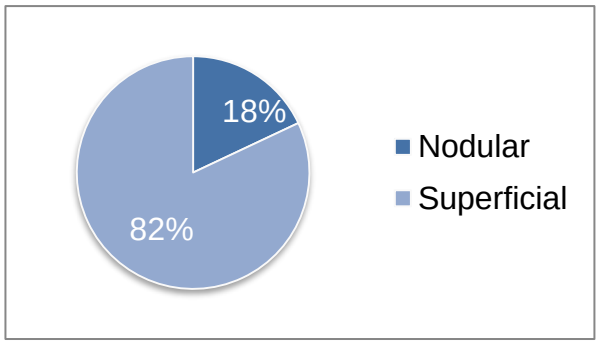

Figure 2. Total BCC according to subtype. From a sample size of 50 tumors, 18% were histologically classified as nodular and 82% as superficial.

Regarding the location of the lesions, as shown in Figure 3: a total of 13 BCC were located on the face (26%), 14 on the back (28%), 6 on the chest (12%), 3 on the abdomen (6%) and 14 on extremities (28%). Within facial lesions, 2 BCC were found on the forehead (15.4%), 4 on the cheek (30.8%), 4 on the nose (30.8%) and 3 on the ear (23.1%).

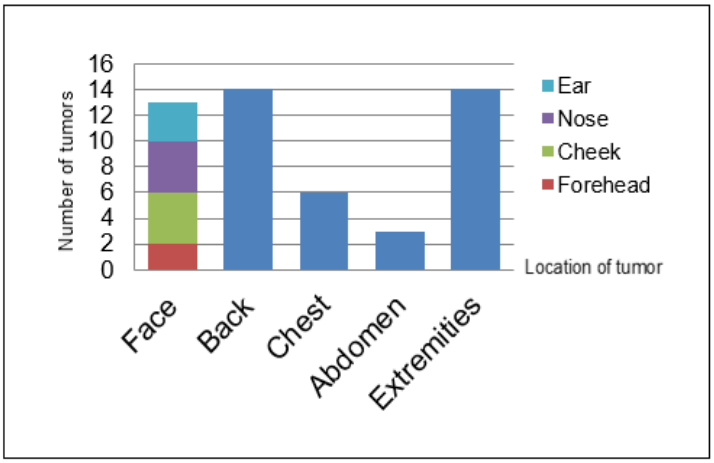

Figure 3. Total BCC according to location. Out of a total of 50 tumors, 13 BCC were found on the face. Within those, 2 tumors were on the forehead, 4 on the cheek, 4 on the nose and 3 on the ear. From the total 50 tumors, 14 were located on the back, 6 on the chest, 3 on the abdomen and 14 on the extremities.

According to tumor thickness, as depicted in Figure 4: a total of 27 BCC had a tumor thickness ≤ 0.5mm (54%), 17 BCC had a tumor thickness from 0.6 to 0.9mm (34%) and 6 BCC showed a tumor thickness of 1mm (12%).

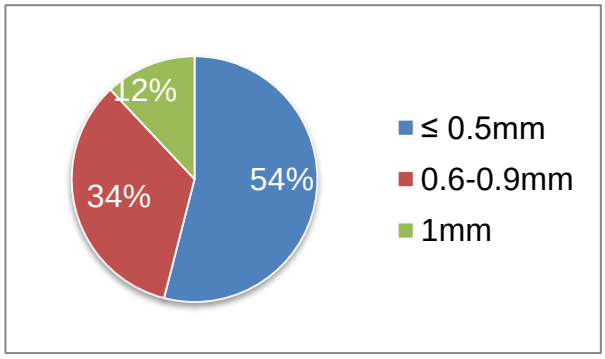

Figure 4. Total BCC according to tumor thickness. A total amount of 27 tumors presented a tumor thickness ≤ 0.5mm, 17 tumors showed a tumor thickness from 0.6mm to 0.9mm and only 6 lesions had a tumor thickness of 1mm.

4.1.2. Analysis of clearance rates after treatment

Overall, 39 BCC cleared after the treatment with 1064nm Nd:YAG (78%).

- BCC-subtype: as seen in Figure 5, of all cleared BCC, 8 were nodular (20.5%) and 31 superficial (79.5%).

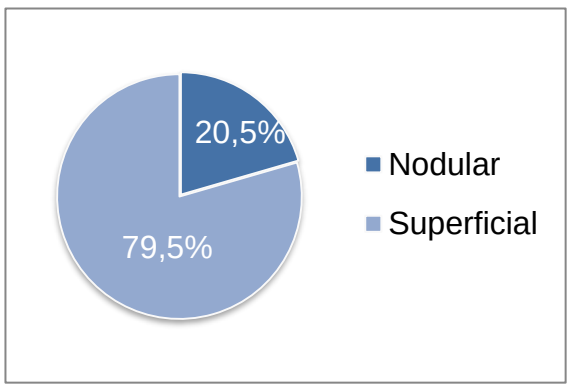

Figure 5. BCC-subtype within cleared BCC. Of all total 39 BCC that cleared after treatment, 20.5% were of nodular subtype and 79.5% superficial.

As portrayed in Table 1, when calculating clearance rates within every subgroup of BCC-subtype, it was established that:

- 8 out of all 9 nodular BCC achieved complete clearance (88.9%).
- 31 out of all 41 superficial BCC cleared (75.6%).

BCC-subtype	Treated	Cleared	Clearance rate
Nodular	9	8	88.9%
Superficial	41	31	75.6%
TOTAL	50	39	78%

Table 1. Clearance rate according to BCC-subtype. Nodular BCC achieved a clearance rate of 88.9%, with a total of 8 out of 9 BCC cleared at endpoint. Likewise, superficial BCC reached a clearance rate of 75.6%, with 31 out of 41 BCC cleared after treatment. The total clearance rate was 78%, with 39 out of 50 BCC cleared.

Of the 11/50 BCC that failed to clear, 10 (91%) were superficial and 1 (9%) was nodular. Compared with the distribution of BCC subtypes in the whole population (82% superficial BCC, 18% nodular BCC), proportionally slightly more failures occurred with superficial tumors and slightly fewer with nodular.

- Tumor thickness: as displayed in Figure 6, of all cleared BCC, 19 presented tumor thickness ≤ 0.5mm (48.7%), 14 showed tumor thickness 0.6-0.9mm (35.9%) and 6 had tumor thickness of 1mm (15.4%).

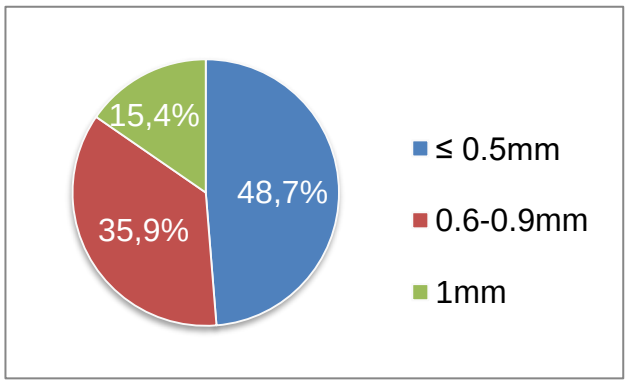

Figure 6. Tumor thickness within cleared BCC. Out of the total 39 BCC that cleared after treatment, 48.7% had a tumor thickness ≤ 0.5mm, 35.9% presented a tumor thickness between 0.6 and 0.9mm and 15.4% revealed a tumor thickness of 1mm.

As exhibited in Table 2, when analysing clearance rates within every subgroup of tumor thickness, the following results were obtained:

- Of all 27 BCC with tumor thickness ≤0.5mm, 19 cleared (70.37%).
- Of all 17 BCC with tumor thickness 0.6-0.9mm, 14 cleared (82.35%).
- All 6 BCC with tumor thickness of 1mm cleared (100%).

Tumor thickness	Treated	Cleared	Clearance rate
≤ 0.5mm	27	19	70.37%
0.6-0.9mm	17	14	82.35%
1mm	6	6	100%
TOTAL	50	39	78%

Table 2. Clearance rate according to tumor thickness. Within the 27 total tumors portraying a tumor thickness ≤ 0.5mm, 19 tumors cleared, achieving a clearance rate of 70.37% in this subgroup. Of the 17 BCC with a tumor thickness between 0.6 and 0.9mm, 14 tumors cleared, reaching a clearance rate of 82.35%. All 6 treated BCC with a tumor thickness of 1mm cleared. Overall, 39 out of 50 BCC cleared, attaining a global clearance rate of 78% for all treated tumors.

Of the 11/50 BCC that failed to clear, 8 (73%) were ≤0.5mm thick, 3 (27%) were 0.6-0.9mm thick and none (0%) were 1.0mm thick. This compares with the proportion of BCC subtypes in the whole population of 54% that were ≤0.5mm thick, 34% that were 0.6-0.9mm thick and 12% that were 1.0mm thick. Therefore, proportionally slightly more failures were identified with thin BCC (≤0.5mm) and slightly fewer with 1.0mm thick lesions. Overall clearance rate was 78% (39/50 BCC) compared to 75% (33/44) for BCC <1mm thick and to 100% (7/7) for BCC 1.0mm thick.

- Location: as seen in Figure 7, of all cleared BCC, 10 were facial (25.6%). Of those, 3 were located on the cheek (30%), 4 on the nose (40%) and 3 on the ear (30%). None of the BCC on the forehead cleared.
  Of all cleared BCC, 8 were located on the back (20.5%), 6 on the chest (15.4%), 2 on the abdomen (5.1%) and 13 on extremities (33.3%).

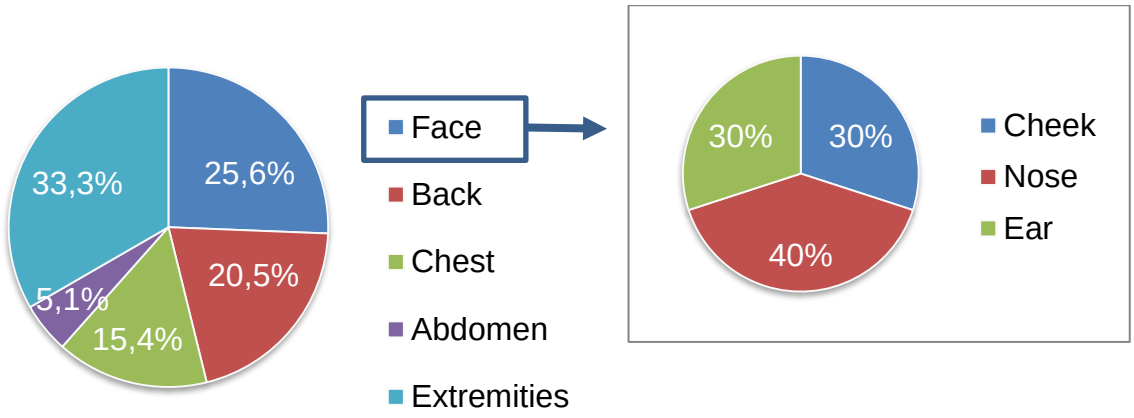

Figure 7. Location within cleared BCC. A total of 25.6% cleared BCC were located on the face. Within this category, 30% of all facial BCC were found on the cheek, 40% on the nose and 30% on the ear. From all 39 cleared BCC, 20.5% were located on the back, 15.4% on the chest, 5.1% on the abdomen and 33.3% on the extremities.

When evaluating clearance rates within every subgroup of location, as performed in Table 3, the following outcomes were achieved:

- Of all 13 facial BCC, 10 cleared (76.9%). Within the face, 3 out of all 4 BCC on the cheek achieved clearance (75%), as well as all BCC on the nose and ear (100%, respectively). None of the forehead BCC responded to therapy.
- Of all 14 BCC located on the back, 8 cleared (57.14%).
- All 6 BCC on the chest cleared (100%).
- Of all 3 BCC on the abdomen, 2 cleared (66.67%).
- Of all 14 BCC on extremities, 13 cleared (92.86%).

Of the 11/50 BCC that failed to clear, 7 (64%) were on the trunk, 3 on the face (27%) and 1 on extremities (9%). According to BCC locations in the whole population (46% on the trunk, 26% on the face, 28% on the extremities), proportionally slightly more failures appeared on the trunk and slightly fewer on the extremities. Regarding sub-regions of the face or trunk, the number of assessed BCC was too small to compare results.

Location	Treated	Cleared	Clearance rate
Face	13	10	76.9%
Forehead	2	0	0%
Cheek	4	3	75%
Nose	4	4	100%
Ear	3	3	100%
Back	14	8	57.14%
Chest	6	6	100%
Abdomen	3	2	66.67%
Extremities	14	13	92.8%
TOTAL	50	39	78%

Table 3. Clearance rate according to location. Facial BCC achieved a clearance rate of 76.9%, with 10 out of 13 tumors cleared after treatment. Within this category, no BCC on the forehead cleared, 75% of the BCC located on the cheek cleared (3 out of 4 tumors cleared) and all BCC on the nose and ear cleared (respectively, 4 out of 4 and 3 out of 3 tumors). BCC positioned on the back reached a clearance rate of 57.14% with 8 out of 14 tumors cleared, whereas all BCC located on the chest cleared. Abdominal BCC presented a clearance rate of 92.8%, with only one of all treated tumors in the area not cleared at endpoint. BCC found on the extremities had a clearance rate of 92.8%, also with one single tumor not cleared after treatment.

Overall, treatment failures were slightly more likely to correspond to superficial BCC, of thickness ≤0.5mm, located on the trunk. Nevertheless, failures did occur in all subtypes, thicknesses and locations.

- Clinical remission at one year: of the 39 BCC that showed clearance at 3-month follow-up, a total of 4 lesions were lost to one-year follow-up due to non-medical reasons. Remission at one year occurred in 100% of those initially cleared BCC (35/35).

4.1.3. Analysis of adverse effects and cosmetic end results

Adverse effects were recorded at visit 2 and visit 3, both post laser. Analyses were completed per treated area.

- As seen in Figure 9, at one-month follow-up (visit 2), 56% of the lesions showed mild local erythema (28/50) and 28% experienced central scabbing with mild peripheral erythema (14/50).

- At three-month follow-up (visit 3), none of the treated BCC presented scarring. However, 4% of the lesions displayed mild hypopigmentation (2/50), 22% mild erythema (11/50) and 16% moderate erythema (8/50). Of the 21/50 lesions that exhibited some local adverse effects at endpoint, 38% (8/21) were found on biopsy to have remaining BCC. All of the 8 cases of moderate erythema were found to be non-responsive BCC. Of the other 29 lesions that showed no signs of local adverse effects at 3 months after treatment, 90% (26/29) were clear according to histology and 10% (3/29) were not clear.

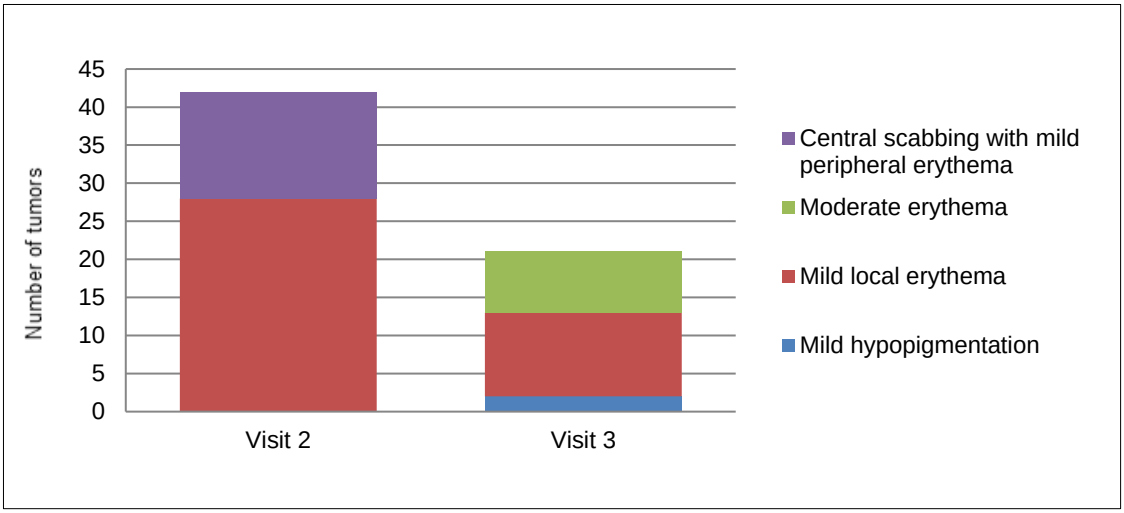

Figure 9. Evolution of local adverse effects. At Visit 2 (one-month follow-up), 28 tumors showed mild local erythema and 14 lesions displayed central scabbing with mild peripheral erythema. At Visit 3 (three-month follow-up), 2 lesions had a mild hypopigmentation, 11 lesions presented a mild local erythema and 8 a moderate erythema.

As a matter of example, photographic evolution of two BCC treated with 1064nm Nd:YAG, as well as their cosmetic end results, are shown below:

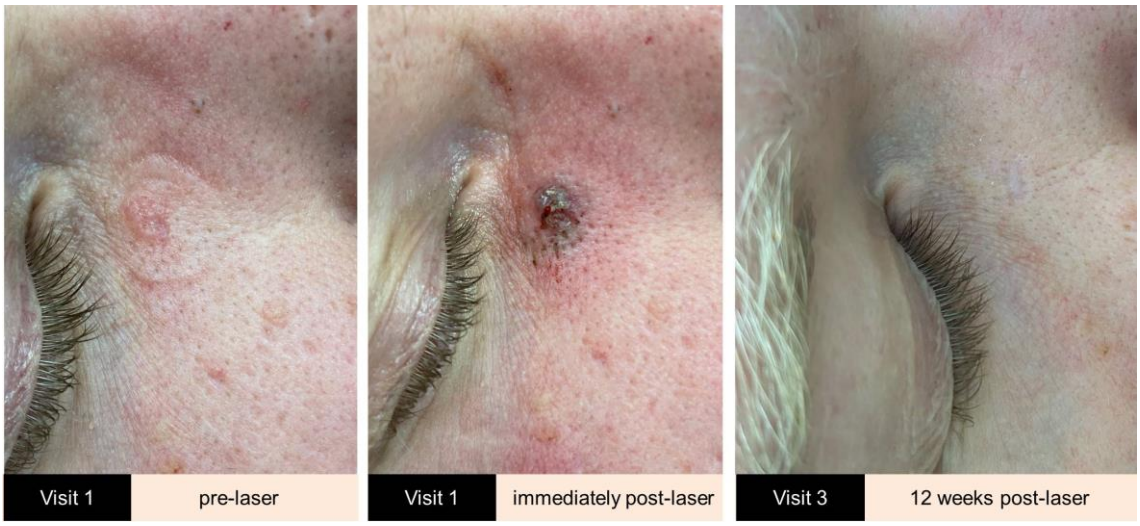

Figure 10. Evolution of facial superficial BCC with good cosmetic end result due to remaining mild hypopigmentation.

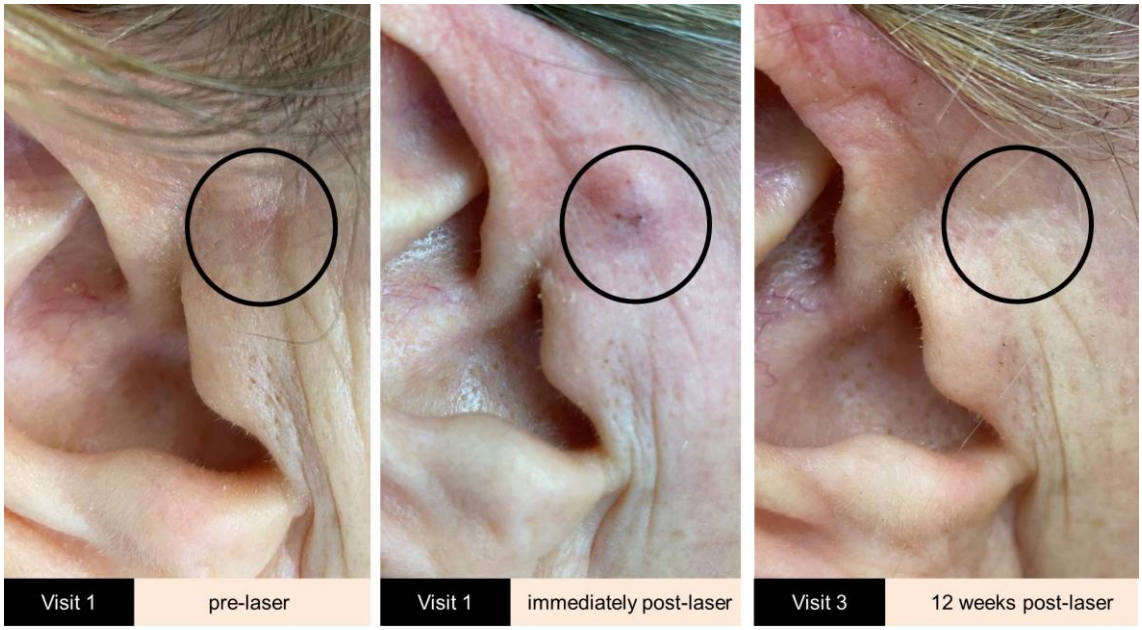

Figure 11. Evolution of facial superficial BCC with excellent cosmetic end result due to resulting intact skin.

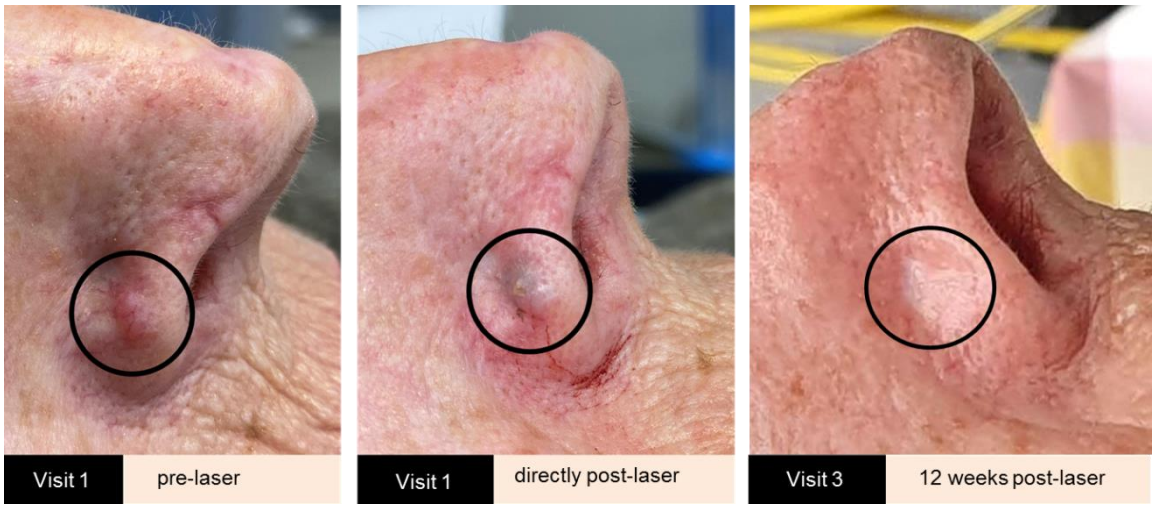

Figure 12. Evolution of facial nodular BCC with excellent cosmetic end result due to resulting intact skin.

4.1.4. Analysis of patient satisfaction and tolerability

Patient satisfaction and tolerability showed excellent results. Mean values of 1.30 in patient satisfaction and 1.08 in tolerability of the treatment were obtained. All scores ranged between “very” and “somewhat satisfied”, showing an overall highly positive evaluation from the perspective of patients. Patients who still had presence of BCC after treatment were also included in this evaluation.

As shown in table 13, 86% of all participants described the procedure as excellent, whereas 2% reported good satisfaction, 8% acceptable and 4% sufficient. No participants described the procedure as insufficient in terms of satisfaction.

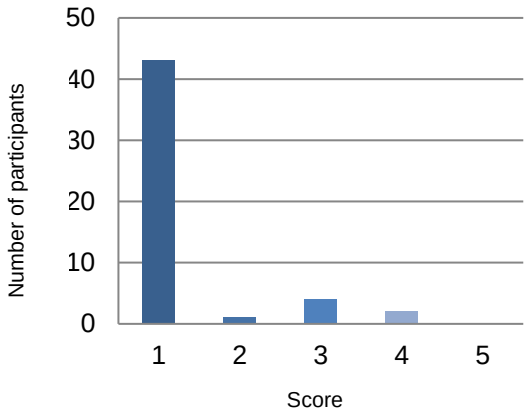

Figure 13. Distribution of patient satisfaction. Results based on a 5-point scale (1=excellent, 2=good, 3=acceptable, 4=sufficient, 5= insufficient). Results were recorded per patient and tumor individually, with a total of 50 participants. 43 participants rated the procedure as excellent (86%), 1 as good (2%), 4 as acceptable (8%) and 2 as sufficient (4%).

With reference to patient tolerability, 92% of the patients recalled the procedure to have excellent tolerability. The other 8% described the laser treatment as good (see Figure 14).

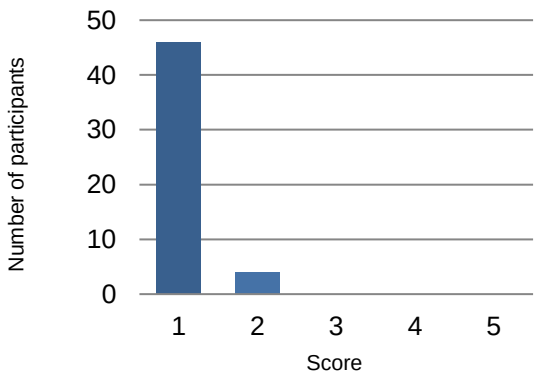

Figure 14. Distribution of tolerability according to patients. Results based on a 5-point scale (1=excellent, 2=good, 3=acceptable, 4=sufficient, 5= insufficient). Results were recorded per patient and tumor individually, with a total of 50 participants. 46 participants rated the treatment as excellent in terms of tolerability and the remaining 4 participants described it as good.

4.2. OCT in the diagnosis of BCC

4.2.1. Pre-laser

Three selected observers analysed the OCT taken prior to skin biopsy and laser treatment. Their results were compared to those of histology (gold-standard) and the following matching diagnoses were achieved:

- As seen in Table 4, Observer 1 correctly diagnosed BCC in 41 out of 50 OCT (82%) and incorrectly missed BCC in 5 out of 50 OCT (10%). That observer correctly diagnosed 7 nodular BCC (77.8%) and 34 superficial BCC (82.9%), incorrectly missing 1 nodular BCC (11.1%) and 4 superficial BCC (9.8%).
- Observer 2 and 3 correctly diagnosed BCC in 40 out of 50 OCT (80%) and incorrectly missed BCC in 6 out of 50 OCT (12%). Those observers correctly diagnosed 7 out of 9 nodular BCC (77.8%) and 33 out of 41 superficial BCC (80.5%), incorrectly missing 1 nodular BCC (11.1%) and 5 superficial BCC (12.2%).

All observers considered the same 4 OCT non-evaluable.

Observer	OCT	Histology		ALL	N
		Nodular BCC	Superficial BCC		
1	Non-evaluable	1 (11.1%) CI[0.3; 48.2]	3 (7.3%) CI[1.5; 19.9]	**4 (8%) [2.2; 19.2]**	50
	No diagnosis of BCC	1 (11.1%) CI[0.3; 48.2]	4 (9.8%) CI[2.7; 23.1]	**5 (10%) [3.3; 21.8]**	
	BCC diagnosed	7 (77.8%) CI[40; 97.2]	34 (82.9%) CI[67.9; 92.8]	**41 (82%) [68.6; 91.4]**	
2	Non-evaluable	1 (11.1%) CI[0.3; 48.2]	3 (7.3%) CI[1.5; 19.9]	**4 (8%) [2.2; 19.2]**	50
	No diagnosis of BCC	1 (11.1%) CI[0.3; 48.2]	5 (12.2%) CI[4.1; 26.2]	**6 (12%) [4.5; 24.3]**	
	BCC diagnosed	7 (77.8%) CI[40; 97.2]	33 (80.5%) CI[65.1; 91.2]	**40 (80%) [66.3; 90]**	
3	Non-evaluable	1 (11.1%) CI[0.3; 48.2]	3 (7.3%) CI[1.5; 19.9]	**4 (8%) [2.2; 19.2]**	50
	No diagnosis of BCC	1 (11.1%) CI[0.3; 48.2]	5 (12.2%) CI[4.1; 26.2]	**6 (12%) [4.5; 24.3]**	

	BCC diagnosed	7 (77.8%) CI[40; 97.2]	33 (80.5%) CI[65.1; 91.2]	**40 (80%) [66.3; 90]**	

Table 4. Diagnosis of BCC with OCT according to observer and compared to histology. Confidence intervals (CI) were defined as the mean of the estimate +/- the variation in that estimate, calculated with a T-test. Observer 1 correctly diagnosed 82% of all BCC (77.8% of all nodular BCC and 82.9% of all superficial BCC) and incorrectly missed 10% of the BCC (11.1% of nodular BCC and 9.8% of superficial BCC). Observers 2 and 3 correctly diagnosed 80% of all BCC (77.8% of all nodular BCC and 80.5% of all superficial BCC) and incorrectly missed 12% of all BCC (11.1% of nodular BCC and 12.2% of superficial BCC).

The reliability of agreement between observers when diagnosing BCC with OCT pre-laser was evaluated using Fleiss-Kappa. Fleiss-Kappa is a statistical measure determining the level of agreement between a group of observers when rating categorical items. It was established that a Fleiss-Kappa above 0.75 showed a substantial agreement, unlikely expected by chance. Values between 0.40 and 0.75 were considered a moderate agreement and values under 0.40 a poor agreement.

A Kappa of 0.758 was obtained (see Table 5). This value correlates with a substantial inter-observer agreement. A Kappa of 1 (almost perfect agreement) was achieved for diagnosis of non-evaluable BCC, a Kappa of 0.602 (moderate agreement) for no diagnosis of BCC and a Kappa of 0.744 (substantial agreement) for diagnosis of BCC.

Subjects	50
Raters	3
Kappa	0.758
z	12
p-value	0

	Kappa	z	p-value
Non-evaluable BCC	1.000	12.247	0.000
No diagnosis of BCC	0.602	7.372	0.000
BCC diagnosed	0.744	9.106	0.000

Table 5. Reliability of agreements between observers when diagnosing BCC with OCT pre-laser. Standard scores (z, number of standard deviations from the mean value) were calculated and significance test (p-value, likelihood of the obtained results being by chance, understanding $p \leq 0.05$ as statistically significant) performed. A total of 50 subjects (total number of BCC) were analyzed by 3 raters obtaining a Fleiss-Kappa of 0.758, correlating with a substantial inter-observer agreement with a z-score of 12 (12 standard deviations above mean value) and a p-value of 0 (statistically significant). In the identification of non-evaluable BCC, a Fleiss-Kappa of 1 was achieved, whereas Kappas of 0.602 and 0.744 were respectively obtained by negative and positive diagnosis of BCC, both values being statistically significant and >7 standard deviations above the mean value.

The same three initial observers analysed the OCT taken after laser treatment and prior to last skin biopsy. As shown in Table 6, their results were again compared to those of histology (gold-standard) and the following matching diagnoses were achieved:

- Observer 1 correctly identified clearance in 35 BCC (89.7%) and incorrectly identified 4 BCC as cleared, when they were not (10.3%). That observer correctly identified recurrence in 6 OCT (54.5%) and incorrectly defined 5 lesions as recurrent, when they were indeed cleared (45.5%).
- Observer 2 correctly identified clearance in 37 BCC (94.9%) and incorrectly identified 2 BCC as cleared, when they were not (5.1%). That observer correctly identified recurrence in 6 OCT (54.5%) and incorrectly defined 5 lesions as recurrent, when they were indeed cleared (45.5%).
- Observer 2 correctly identified clearance in 33 BCC (84.6%) and incorrectly identified 6 BCC as cleared, when they were not (15.4%). That observer correctly identified recurrence in 5 OCT (45.5%) and incorrectly defined 6 lesions as recurrent, when they were indeed cleared (54.5%).

Observer	OCT	Histology		ALL	N
		Clearance	Recurrence		
1	Clearance	35 (89.7%) CI[75.8; 97.1]	5 (45.5%) CI[16.7; 76.6]	**40 (80%) [66.3; 90]**	50
	Recurrence	4 (10.3%) CI[2.9; 24.2]	6 (54.5%) CI[23.4; 83.3]	**10 (20%) [10; 33.7]**	
2	Clearance	37 (94.9%) CI[82.7; 99.4]	5 (45.5%) CI[16.7; 76.6]	**42 (84%) [70.9; 92.8]**	50
	Recurrence	2 (5.1%) CI[0.6; 17.3]	6 (54.5%) CI[23.4; 83.3]	**8 (16%) [7.2; 29.1]**	
3	Clearance	33 (84.6%) CI[69.5; 94.1]	6 (54.5%) CI[23.4; 83.3]	**39 (78%) [64; 88.5]**	50
	Recurrence	6 (15.4%) CI[5.9; 30.5]	5 (45.5%) CI[16.7; 76.6]	**11 (22%) [11.5; 36]**	

Table 6. Diagnosis of clearance or recurrence of BCC with OCT according to observer and compared to histology. Confidence intervals (CI) were defined as the mean of the estimate +/- the variation in that estimate, calculated with a T-test. Observer 1 correctly diagnosed clearance in 89.7% of the tumors and recurrence in 54.5% of real recurrent cases. Observer 2 correctly identified clearance in 94.9% of actually cleared tumors and recurrence in 54.5% of relapses. Observer 3 correctly pointed clearance in 84.6% of all cleared BCC and recurrence in 45.5% of relapsing BCC.

The reliability of agreement between observers when diagnosing BCC with OCT post-laser was again calculated using Fleiss-Kappa. A Kappa of 0.53 (moderate agreement) was obtained. Also a Kappa of 0.53 was achieved for diagnosis of clearance and recurrence individually (see Table 7).

Subjects	50
Raters	3
Kappa	0.53
z	6.49
p-value	8.67^{-11}

	Kappa	z	p-value
Clearance	0.530	6.488	0.000
Recurrence	0.530	6.488	0.000

Table 7. Reliability of agreements between observers when diagnosing clearance or recurrence of BCC with OCT post-laser. Standard scores (z, number of standard deviations from the mean value) were calculated and significance test (p-value, likelihood of the obtained results being by chance, understanding $p \leq 0.05$ as statistically significant) performed. A total of 50 subjects (total number of BCC) were analyzed by 3 raters obtaining a Fleiss-Kappa of 0.53, correlating with a substantial inter-observer agreement with a z-score of 6.49 (total standard deviations above mean value) and a p-value of 8.67^{-11} (statistically significant). In the identification of clearance and recurrence, a Fleiss-Kappa of 0.530 was achieved, both values being statistically significant and >6 standard deviations above the mean value.

Additionally, an evaluation of the accuracy in the diagnosis of BCC with OCT post-laser was carried out (see Table 8). The parameters used were defined as:

- Accuracy: closeness between results and true values.
- Sensitivity: ability of the observer to diagnose true recurrent BCC with OCT. A diagnosis of true recurrent BCC was determined according to histology (gold-standard).
- Specificity: ability of the observer to diagnose true cleared BCC with OCT. A diagnosis of true cleared BCC was determined according to histology (gold-standard).
- Positive predictive value (PPV): probability that recurrent BCC diagnosed with OCT are truly recurrent.

- Negative predictive value (NPV): probability that cleared BCC diagnosed with OCT are truly cleared.
- Positive likelihood ratio (LR+): increase in the odds to have recurrent BCC, when the observer diagnosed recurrent BCC with OCT. LR+ ranges from 1 to infinity, where values > 1 increment the probability of disease when test results are positive.
- Negative likelihood ratio (LR-): decrease in the odds to have cleared BCC, when the observer diagnosed cleared BCC with OCT. LR- ranges from 0 to 1, where 0 implies the lowest probability of disease if the test results are negative.

It was assumed that total diagnoses of recurrent and cleared BCC would occur when all three observers agreed in that diagnosis. With that premise, the parameters stated above were calculated and the following results obtained:

	Observer 1	Observer 2	Observer 3	Total
Accuracy	82% (68.56; 91.42)	86% (73.26; 94.18)	76% (61.83; 86.94)	**86%** (73.26; 94.18)
Sensitivity	54.55% (23.38; 83.25)	54.55% (23.38; 83.25)	45.45% (16.75; 76.62)	**36.36%** (10.93; 69.21)
Specificity	89.74% (75.78; 97.13)	94.87% (82.68; 99.37)	84.62% (69.47; 94.14)	**100%** (90.97; 100)
PPV	60% (26.24; 87.84)	75% (34.91; 96.81)	45.45% (16.75; 76.62)	**100%** (39.76; 100)
NPV	87.5% (73.2; 95.81)	88.1% (74.37; 96.02)	84.62% (69.47; 94.14)	**84.78%** (71.13; 93.66)
LR+	5.32 (1.82; 15.56)	10.64 (2.49; 45.51)	2.95 (1.11; 7.87)	Inf (NaN; Inf)
LR-	0.51 (0.26; 0.98)	0.48 (0.25; 0.92)	0.64 (0.37; 1.12)	**0.64** (0.41; 0.99)

**Inf= Infinity; NaN = Not a Number*

Table 8. Evaluation of accuracy in the diagnosis of cleared or recurrent BCC with OCT post-laser. Overall, OCT showed a high specificity (100%) and positive predictive value (100%), as well as a high negative predictive value (84.78%). However, sensitivity was low (36.36%). The positive likelihood ratio was favourable (infinity), whereas the negative likelihood ratio was again low (0.64).

Globally, OCT achieved specificity and positive predictive value of 100%. On these grounds, OCT was fully reliable in diagnosing true cleared BCC. When diagnosing clearance with OCT, the probability that BCC were truly cleared was maximal.

Accuracy and negative predictive value were high. This suggests that results and true values were extremely close. Besides, if a diagnosis of clearance was made with OCT, the probability that BCC were truly cleared was high.

In contrast, sensitivity was low, which denotes that OCT failed in diagnosing true recurrent BCC. Positive likelihood ratio was favourable (infinity), as it supported the true presence of BCC when lesions were diagnosed with OCT. Negative likelihood ratio was poor (0.64), implying a slight decrease in the probability of clearance compared to pre-test probability, when OCT diagnosed clearance.

4.2.3. Results of dynamic OCT

Dynamic OCT provides information on the characteristics of the vascular plexus irrigating the evaluated area. It enables practitioners to measure the depth of the plexus, the diameter of the blood vessels within this structure and the density of this vascular network. In this study, the evaluated area corresponded to the location of BCC lesions. Therefore, measurements of the vascular plexus belonged to the blood vessels irrigating the tumor.

Vessel diameter and density of the plexus are calculated at the depth of the plexus, if the latter can be identified. If depth is non-evaluable, no complementary data can be provided. This situation occurs when:

- The skin lies too low or too high compared to high-resolution margins of the OCT caption
- The depth of the plexus is > 500μm
- The diameter of the vessels is < 20μm
- Blood flow is too slow, for example in patients with diabetes mellitus

As presented in Table 9, the D-OCT images showed a very wide variability of the vessels in the lesion in terms of plexus depth, vessel diameter and density at both baseline and follow-up. No clear trends were identified in the extracted estimates of vessel diameter or density.

In 36% of the cases (18/50) the device did not detect enough vessels pre-treatment to provide a measurement. Of the other 32 lesions, the average measured plexus depth was 226µm. Many of the lesions exhibited fine arborizing vessels very close to the skin surface.

At 3-month follow-up, plexus depth mostly increased, to average 320µm in cleared lesions and to average 263µm in non-cleared lesions, perhaps indicating that the surface vessels had been destroyed by the treatment in all cases. The proportion of lesions for which the device did not detect enough vessels to provide a measurement was higher for cleared lesions (58%, 23/39) than for non-cleared (36%, 4/11).

Patient Number	Pre-Laser			Post-Laser		
	Depth of the plexus (µm)	Diameter of blood vessels (µm)	Density of blood vessels (%)	Depth of the plexus (µm)	Diameter of blood vessels (µm)	Density of blood vessels (%)
1	NE	NE	NE	687	114	4,7
2	51	49	1,6	228	100	18
3	NE	NE	NE	NE	NE	NE
4	131	37	2,2	NE	NE	NE
5	NE	NE	NE	NE	NE	NE
6	61	0	3,2	335	90	17
7	125	0	1,5	149	33	9,6
8	272	190	41	NE	NE	NE
9	149	0	0,9	370	104	20
10	NE	NE	NE	342	113	25
11	NE	NE	NE	375	77	15
12	112	28	4,1	NE	NE	NE
13	96	14	4,5	513	119	44
14	NE	NE	NE	NE	NE	NE
15	158	25	1,5	NE	NE	NE
16	NE	NE	NE	92	42	2,3
17	163	71	7,8	NE	NE	NE
18	113	28	2,8	339	79	12
19	NE	NE	NE	NE	NE	NE
20	148	22	1,4	NE	NE	NE
21	NE	NE	NE	NE	NE	NE
22	104	29	3,9	179	55	17
23	179	121	1,2	NE	NE	NE
24	NE	NE	NE	241	0	3,6
25	393	127	9,8	NE	NE	NE

26	57	122	13	NE	NE	NE
27	NE	NE	NE	198	87	22
28	291	186	22	189	47	7,6
29	295	103	24	NE	NE	NE
30	NE	NE	NE	366	24	8
31	351	67	16	NE	NE	NE
32	NE	NE	NE	191	46	0,9
33	503	49	2,4	NE	NE	NE
34	369	101	5,8	NE	NE	NE
35	92	108	2,9	75	0	0,1
36	381	80	9,3	NE	NE	NE
37	399	43	10	NE	NE	NE
38	180	50	8	NE	NE	NE
39	522	126	37	1250	45	2
40	188	57	4	NE	NE	NE
41	NE	NE	NE	NE	NE	NE
42	478	104	9,3	NE	NE	NE
43	137	124	9,8	179	63	7,1
44	192	117	25	95	49	19
45	129	12	4,9	277	0	1,3
46	NE	NE	NE	NE	NE	NE
47	421	73	1,7	NE	NE	NE
48	NE	NE	NE	NE	NE	NE
49	NE	NE	NE	111	0	0,8
50	NE	NE	NE	180	69	6,8
Mean value	144,8	45,26	5,85	139,22	27,12	5,28

**NE=non-evaluable*

Table 9. Evolution of dynamic OCT parameters throughout the treatment. A wide variability in the results regarding the evaluation of the diameter of the blood vessels and density of the blood vessels was obtained. On this account, no firm conclusions can be drawn, apart from a single trend concerning an increase in the plexus depth post-laser.

5. Discussion

Laser treatment of BCC is relegated to a third-line alternative, if surgery and topical therapies have failed or are contraindicated. Despite the application of lasers in Dermato-Oncology has been broadly evaluated, limited research on 1064nm Nd:YAG has been conducted.

Due to its capacity to penetrate deep into the dermis and its ability to target vascular structures, 1064nm Nd:YAG is a promising treatment option of BCC. High clearance rates, successful cosmetic end results and excellent patient satisfaction and tolerability may justify the ascension of this technique to a second-line choice.

5.1. 1064nm Nd:YAG laser

Currently, laser treatment of BCC is classed as a third-line alternative if surgery and topical therapies have failed or are contraindicated[14]. However, due to its capacity to penetrate deep into the dermis and its ability to target vascular structures, 1064nm Nd:YAG is a promising treatment option of BCC.

5.1.1. Comparison to previous study designs

This is the ninth study evaluating the use of Neodymium lasers for non-melanoma skin cancer[8-17] and the fifth specifically for BCC treatment using 1064nm Nd:YAG at high fluences[12-15]. Previous studies differ in scope (<31 lesions) and the use of laser parameters specified in fluence ranges. In the present study, a larger cohort size of 50 lesions was evaluated, using fixed laser treatment parameters and a scanning handpiece to deliver a highly controlled even thermal dose to every lesion. In addition, tumor clearance was histologically verified. BCC subtype and thickness were systematically analyzed to investigate potential trends that might guide further research.

As in this thesis, all previous studies included superficial and nodular BCC. Globally, only two other studies included facial lesions and none compared effectivity attending nor to location of the lesion, neither tumor thickness.

5.1.2. Comparison of results with previous data and new hypothesis

This study provides new data for further discussion:

- Greater tumor thickness achieved better response to treatment, as all BCC with tumor thickness equal to 1mm cleared (p=0.09). No previous studies evaluated response according to this parameter. This could be explained by greater vascularisation of greater size tumors, as more tumoral tissue requires increased number of blood vessels nurturing the additional mass. In that sense, the more ramifications of blood vessels irrigating the tumor, the more target chromophores the laser would find and the more precise its effect should be.

- Nodular BCC showed higher clearance rates (p=0.27). In previous research, it was suggested that superficial BCC have less robust vascularisation in comparison to other subtypes and therefore experience poorer results in laser therapy. The results in this study support those premises.

Despite our findings suggest that thicker tumors and nodular histological subtype respond better to therapy, our results did not reach statistical significance ($p<0.05$). Therefore, further studies including a larger number of lesions would be needed to confirm our hypothesis.

- A global clearance rate of 78% was achieved. After one year, all cleared BCC remained cleared. Ortiz et al. published clearance rates of 92% and 90.3% after one month, whereas Ahluwalia et al. and Markowitz et al. of 100%.

    - On one hand, Markowitz did not include an endpoint biopsy or excision of the treated lesions. In this study, not all clinically suspected clearances matched histological results in the endpoint sample. Therefore, clearance was determined

as per histology. Results may differ between both studies due to a lack of histological confirmation and a plausible misdiagnosis of clearance.

- On the other hand, Ortiz and in some occasions Ahluwalia included histological endpoint clearance of the lesions and still obtained higher clearance rates. In 2015, Ortiz et al. described better results at higher fluences of 120J/cm^2 and all following publications used fluences of 125-140J/cm^2. As only fluences of 120J/cm^2 were used in this study, it would be possible that fluences over this value were more effective in treating BCC in some corporal areas. Despite that, local adverse effects and cosmetic end results might then be poorer.

- Finally, the difference in clearance results might be explained by the notoriously larger lesion sample in this study.

- Clearance rates varied according to location of the lesion:

  - All BCC located on the chest cleared, accomplishing excellent results. Lesions on extremities and face showed very good clearance rates, whereas abdominal BCC reported good clearance rates. BCC located on the back displayed poor clearance rates (p=0.03). Ortiz et al. also described lower clearance rates on the back and abdomen in both of their studies.

  - Within facial BCC, all lesions located on the nose and ear cleared, whilst good clearance rates characterized BCC on the cheek. None of the two BCC on the forehead cleared.

    - In previous literature, pulse dye laser was evaluated in the treatment of facial BCC and achieved clinical clearance rates of 75% at a mean follow-up of 11 months. This study suggests that 1064nm Nd:YAG could be more effective in the treatment of BCC on this location.

- Only two of the four previous studies on 1064nm Nd:YAG included facial BCC. No further details of location within facial areas were mentioned. On that account, this is the first study analysing treatment response in specific facial regions.

- Differences in clearance according to location might be justified by the vascularisation of the area and the thickness of both epidermis and dermis.
  - Southwood (1955), Artz et al. (1979) and Lee et al. (2002) studied the thickness of the epidermis and dermis of the skin on histological samples. According to their findings, the epidermis was thicker on the cheek (98.2μm), followed by the forehead (93.6μm), extremities (values ranging from 42μm to 102μm), back (62-76μm), chest (39-98μm) and abdomen (40-79μm). Analogously, the dermis was thicker on the back (1805- 1945μm), then on the abdomen (1640-1492μm), chest (1400-1337μm), extremities (943-1357μm), cheek (1076.6μm) and forehead (788.2μm). Global skin thickness, including epidermis and dermis, was maximal on the back.
    Our lack of clearance on the back could respond to a greater thickness of the skin in this area. Modifications to reach major penetration and hence target lesions at greater skin depths seem plausible to increase response in this area. A larger spot size might be helpful to achieve this goal.

  - Another study by Yokoshiki et al. (2017) evaluating epidermal and dermal thickness used a 3-dimensional ultrasound microscope to measure those values. Results indicated thicker epidermis on the forehead compared to the cheek, but thinner dermis on the forehead again compared to the cheek. Equivalent results between ultrasound microscope and histology were obtained on dermal measurements, but epidermal thickness varied between both methods. Due to a lack of consensus, no further considerations can be made regarding skin thickness on the forehead and treatment response.

- All facial locations corresponded to highly vascularised areas, but results varied between sublocations. On these grounds, vascularisation cannot be considered a response-defining factor for facial lesions. Additionally, 1064nm Nd:YAG targeted blood vessels in the vascular plexus irrigating the tumor and not the major vessels on the area. Furthermore, in all cleared and non-cleared lesions, dynamic OCT parameters (depth of the plexus, diameter of blood vessels and density of plexus) decreased after treatment. This might indicate that non-cleared lesions might have had partial response and would need further treatment sessions to achieve clearance.

- Another possible explanation could be the phenomenon described by Mehrabi et al., according to which higher maximum temperatures and rates of cooling of the skin achieve better response to 1064nm Nd:YAG. Those characteristics are found in areas such as the cheek, where in this study good clearance rates were obtained. Mehrabi identified lower maximum temperatures and rates of cooling on the back and on the chest. In this case, our lack of response on the back matches Mehrabi's theory, whereas our 100% clearance on the chest differs from it.
  One concern could be that Mehrabi's study was performed on healthy skin. In order to determine if maximum temperature and cooling rates of the skin play a role in resulting clearance of BCC, the above mentioned parameters should be investigated on BCC-affected skin.

- When contrasting our clearance rates with those of second-line therapies, results are comparable:
  - Topical imiquimod showed clearance rates of 60-80%, 83.4% and 79.7% for superficial BCC and 42-81% for nodular BCC.
  - Topical 5-FU achieved 50-90%, 80.1% and 68.2% clearance of superficial BCC.
  - ALA-PDT reported 60-91% clearance rates for nodular BCC.
  - MAL-PDT recorded 82%, 19% and 72.8% clearance rates for superficial BCC and 33-79% for nodular BCC.

Results from all above mentioned studies vary noticeably within and also between publications. However, they tend to be similar or even worse to clearance rates achieved in our study with 1064nm Nd:YAG, as overall clearance rate was 78%, 75.6% for superficial BCC and 88.9% for nodular BCC. In consequence, the fact that 1064nm Nd:YAG laser therapy of BCC is only accepted as a third-line treatment should be revised and its efficacy in comparison to second-line therapies evaluated with further studies.

- Minor local adverse effects were common among participants, those being local mild erythema or central scabbing with peripheral erythema 4-6 weeks after the first laser session. So far, local adverse effects have only been assessed by Ortiz et al. in their two studies, where mild erythema and scabbing after one month were described, but no quantification of events was made. Since this is the first study evaluating local adverse effects and their frequency, further research would be advisory. Furthermore, from the point of view of patients, those local adverse effects had no impact on patient satisfaction with the treatment, as excellent results were gathered.

- Selective photothermolysis was suggested in previous literature as a possible mechanism of action of 1064nm Nd:YAG in BCC treatment. Our D-OCT results were not conclusive regarding the effect of Nd:YAG laser on the vascular plexus, but hinted that it may have resulted in destruction of some larger, shallower vessels. The thermal relaxation time of thinner dermal capillaries, including those feeding the tumor, is likely to be in a lower msec range[18] than the 8msec pulse width used in this study. These findings support a long-pulsed laser bulk heating[18] effect rather than selective thermolysis as the main mechanism of action.

5.2. OCT

5.1.1. Comparison to previous study designs

The application of OCT in the diagnosis of BCC was studied in four previous publications. Morgensen et al. (2009) reported 79-94% sensitivity, 85-96% specificity

and moderate inter-observer agreement between a total of six observers. Ulrich et al. (2015) obtained 87.4% accuracy, 95.7% sensitivity, 75.7% specificity and added an 85.2% positive predictive value and 92.1% negative predictive value. Cunha et al. (2011) achieved 19% sensitivity, 56% specificity and 18% positive predictive value, whereas Maier et al. (2014) reported 75% sensitivity, 64% specificity and 61% positive predictive value. In another trial conducted by Olmedo et al. (2006), 35% of lesions were accounted non-interpretable.

Attuned to Cunha et al., this study showed limited sensitivity of OCT. In both studies, the reason is suspected to be a limited sample size.

All other OCT outcomes in this study align with those from Morgensen, Ulrich and Maier, and even show improved inter-observer agreement, that being substantial pre-laser and also moderate post-laser. This strengthens the idea that, despite being observer-dependent, the recognition of standardised signal patterns through regular OCT training could lead to accurate diagnosis of BCC and prevent unnecessary skin biopsies. In patients with multiple BCC, the use of OCT could also evade multiple skin biopsies and a subsequent higher side-effect risk, including scarring.

In that event, OCT proved to be an effective, reliable and reproducible diagnostic method of BCC. Its strong diagnostic power both pre- and post-treatment defines OCT as a validated diagnostic and clearance-confirmation technique.

Overall, OCT facilitates fast and painless diagnosis of BCC lesions, enabling subtype distinction of the lesion and delimitation of tumor margins, both vertically and horizontally. It optimises the use of skin biopsy, reducing undesirable side effects. Ultimately, OCT reduces the time-span between diagnosis and treatment of BCC and therefore avoids delays between steps, enhancing patient-compliance and potentially allowing a same-day diagnosis-treatment concept.

5.3. Limitations

This study has the limitations of being open-label and lacking randomisation to placebo or gold-standard treatment. Despite including a larger sample of lesions compared to prior publications, the study is still considered small and should therefore be considered a pilot study for a randomised clinical trial with a larger tumor sample.

Due to a lack of permission from patients, photographic records of lesions were limited. Photographic follow-up of more lesions would be advisory for further publications, in order to illustrate skin changes within time.

This study has a maximum follow-up of 6 months after confirmation of lesion clearance with histology. To determine long-term remission of BCC, yearly follow-up visits up to 5 years would be advisable. If lesions do not relapse within those 5 years, a complete curation could be accepted, according to oncology guidelines.

Finally, the study was conducted during the COVID-19 pandemic. For that reason, some elder patients postponed their follow-up sessions and their lesions were accounted as lost.

6. Conclusions

OCT-assisted 1064nm Nd:YAG laser treatment of superficial and nodular BCC with ≤1mm tumor thickness is a safe and effective diagnostic and treatment method with comparable clearance rates to second-line therapies. This technique shows improved patient satisfaction, tolerability, practicability and cosmetic end result compared to surgery and second-line therapies.

With the completion of this study, it can be concluded that:

- OCT is an accurate and reproducible complementary method to clinical-dermatoscopic diagnosis of superficial and nodular BCC with ≤1mm tumor thickness. Despite being observer-dependent, this procedure shows high intra- and inter-observer reliability compared to histopathology.

- In the future of Dermato-Oncology, OCT could replace skin biopsies as a confirmatory method of clinical-dermatoscopic diagnosis and allow same-day direct diagnosis-treatment schemata of superficial and nodular BCC with ≤1mm tumor thickness. In order to achieve this goal and given a positive predictive value of 100%, higher sensitivity should be achieved. Further studies with greater BCC sample would be needed to confirm this hypothesis. For the purpose of defining the interpersonal variability and thus reliability of OCT as a diagnostic method for BCC, more evaluators should be compared. It would also be preferable to conduct a study comparing diagnostic OCT-results according to experienced OCT-users and less experienced observers, so that the impact of OCT-training could be better designated.

- With 100% specificity and high negative predictive value, OCT is a reliable clearance-confirmation method, comparable to skin biopsy in follow-up phases after 1064nm Nd:YAG laser treatment.

- Laser therapy of superficial and nodular BCC with ≤ 1mm tumor thickness using 1064nm Nd:YAG is an effective treatment method with successful short-term clearance rates. Clearance of BCC tends to remain stable up to one year after treatment. Therefore, 1064nm Nd:YAG could become the first go-to option in second-line treatments of superficial and nodular BCC with ≤1mm tumor thickness.

- Considering that spontaneous clearance of BCC after biopsy has been defined as 20%, this study discarded the hypothesis that the laser has no effect.

- Treatment of superficial and nodular BCC with ≤ 1mm tumor thickness using 1064nm Nd:YAG laser has satisfactory cosmetic outcomes, ranging good to excellent. Aesthetic results are better than those achieved after surgery or second-line therapies. Moreover, this laser therapy obtains high patient tolerability and satisfaction.

- This study succeeded in defining targetable lesions and outcome predictors associated to better or worse response to 1064nm Nd:YAG treatment. Greater tumor thickness correlated to better response to treatment, as did location on specific skin areas (nose, ear, chest and extremities).

- The laser configuration of 1064nm Nd:YAG used in this study (120J/cm^2, 8msec, 0.6Hz) is an effective option to achieve complete clearance of superficial and nodular BCC with ≤1mm tumor thickness. Higher fluences could be more effective in the treatment of BCC located on the forehead and back.

- Selective photothermolysis is a plausible explanation for BCC clearance after 1064nm Nd:YAG laser, according to dynamic-OCT findings.

- Current results of OCT-assisted 1064nm Nd:YAG laser treatment of BCC support a potential application of the laser on same-day diagnosis-treatment protocols. Incoming patients with clinically suspected BCC would undergo OCT tumoral confirmation and directly receive laser treatment of those lesions. These same-day

schemata would reduce unnecessary delays in treatment and the subsequent prolonged psychological consequences on patients, including the development of so-called cancerophobia.

All things considered, a randomised controlled trial would be reasonable to validate 1064nm Nd:YAG as second-line treatment of superficial and nodular BCC with ≤1mm tumor thickness. This further study could also determine if 1064nm Nd:YAG could even be a first-line therapy for BCC located on specific body areas, such as the nose, ear or chest. In this trial, laser treatment should be compared to gold standard (surgical excision), including diagnostic and clearance-confirmation biopsies and a long-term follow-up (≥5 years), which would offer precise data on the efficacy of the laser and its clearance rates within time. This would allow to define if 1064nm Nd:YAG laser could become an accepted second- or even first-line therapy for BCC with ≤1mm tumor thickness.

7. List of figures

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9. Bibliography

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