# Bispebjerg Hospital, Department of Dermato-Venereology: Current and Future Research Activities

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## Overview of research activities

The Department of Dermato-Venereology and the associated Wound Healing Centre at Bispebjerg Hospital in Copenhagen published a total of 116 scientific papers, books and book chapters in 2016, corresponding to approximately 15% of all publications originating from the hospital that year. The number of scientific publications has grown during the past 5 years, as has the number of scientific personnel, which, at present, constitute 6 full professors, two affiliate professors, 6 associate professors, and approximately 10 post-doctoral and 16 PhD students, as well as several master's and bachelor students. The department has molecular diagnostic, fungal and immunofluorescence laboratories, a histopathology facility in relation to Mohs' micrographic surgery, and rodent staples for experiments on mice.

The research activities are of remarkable breadth and encompass several topics within clinical, epidemiological, pharmacological, as well as basic and molecular dermato-venereology and wound healing (Table I). Particularly strong areas of research are photodermatology, cutaneous lymphomas and skin oncology, laser dermatology and dermatological imaging techniques, eczema and contact allergy, tattoos and tattoo complications, as well as clinical databases on chronic urticaria, hidradenitis suppurativa, atopic dermatitis and psoriasis. A summary of these areas of research is presented here.

## Photodermatology

The department's research into the effects of UV radiation and sunlight deals with sun behaviour, damaging effects of UV radiation, positive effects of UV radiation, as well as prevention and treatment of UV-induced damage. The effect of UV radiation depends on skin sensitivity and exposure dose. The acute effect is sunburn, and the chronic effects are lentigines, skin ageing, cutaneous malignant melanoma, actinic keratosis, and keratinocyte skin cancer. Knowledge about sun exposure has long built on sun behaviour questionnaires. We have developed UV dosimeters to obtain objective data on UV exposure (1). Dosimeter data show that up to 40% of the annual UV dose is received during just one week of summer vacation in the Mediterranean, and very high doses are received on very sunny and hot days in Denmark, whereas the day-to-day UV dose is of little importance. This also means that protection against UV radiation should focus on high-dose situations. Sun protection is important in order to avoid skin damage and we have developed a new strategy for increased protection by applying sunscreen twice before sun exposure. This is much more efficient than the advice to use one application every 2 hours, or to use a handful of sunscreen.

*Table I. Overview of noticeable research topics at the department*

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| Skin cancer |
| Photodermatology |
| Molecular skin-oncology |
| Non-melanoma skin cancer |
| Malignant melanoma |
| Cutaneous lymphoma |
| Organ transplant recipients |
| Mohs' micrographic surgery |
| Lasers and imaging techniques |
| Experimental laser treatment |
| Laser-assisted drug delivery |
| Optical coherence tomography and skin ultrasound |
| Confocal microscopy |
| Inflammatory skin diseases |
| Atopic dermatitis |
| Contact dermatitis and contact allergy |
| Work-related dermatitis |
| Hidradenitis suppurativa |
| Chronic urticaria |
| Psoriasis |
| Acne |
| Tattoos and tattoo complications |
| Tattoo allergy and inflammatory reactions |
| Nomenclature of clinical tattoo complications |
| Tattoo pigment biokinetics |
| Evidence-based treatments |
| Venereology |
| HPV infection |
| Mycoplasma and gonorrhoea |

We perform studies on hairless mice to find possible prophylactic substances against UV-induced cutaneous squamous cell carcinoma. The formation of vitamin D is a beneficial effect of UVB exposure (2). Pigmentation is thought to play an important role in reduced formation of vitamin D. However, we have shown that the effect on formation of vitamin D is primarily related to pigment genes, whereas pigment itself plays a minor role. Furthermore, the use of sunscreen has proven to be a minor problem related to the formation of vitamin D.

Establishing skin type has previously relied on questionnaires, but research carried out at the department has led to the development of a fast, objective measuring method. This, alongside objective measurements of sun exposure, has opened new research possibilities. Overdosing leads to lentigines and we have established the relation between the formation of lentigines, skin phototype and risk of skin cancer (3). Furthermore, we have developed a new skin cancer phototype questionnaire, which divides people into 4 groups, with a difference in odds ratio of approximately 5.4 for risk of skin cancer due to skin sensitivity alone.

We have developed methods for measuring eumelanin and pheomelanin pigments from skin biopsies, and current projects focus on relating this to skin phototype and risk of cutaneous malignant melanoma. A method is under development for rapid detection of cutaneous malignant melanoma by gene analysis on material lifted by tape from the stratum corneum above the suspected lesion.

Our research into the treatment of skin cancer and actinic keratosis has focused on photodynamic therapy (PDT). We have transformed the conventional PDT method into daylight PDT (Fig. 1). The photoactive component protoporphyrin IX (PpIX) is formed and activated continuously during daylight illumination, as opposed to conventional PDT, in which PpIX accumulates before illumination. This procedure makes the treatment much more agreeable and virtually painless for the patients. This development has meant a worldwide introduction of daylight PDT.

## Cutaneous lymphomas and skin oncology

The department has a long-standing interest in skin oncology, with a particular focus on primary cutaneous lymphomas and non-melanoma skin cancer. Over the last 15 years we have developed and maintained a multidisciplinary cutaneous lymphoma clinic integrating expertise in dermatology, medical and radiation oncology and dermatopathology. All new patients are reviewed during multidisciplinary rounds, where the diagnosis can be established on the basis of clinico-pathological correlation. Our clinic provides care to more than 300 patients with different form of cutaneous lymphomas of all stages. Examples of our contributions to the field are development of the low-dose total skin electron beam radiation for advanced mycosis fungoides and introduction of positron emission tomography–computed tomography (PET-CT) scanning as a routine staging procedure in advanced disease (4, 5). The low-dose protocol has been adopted internationally and is currently standard of care in many leading centres.

*Fig. 1.* Gazebo greenhouse designed for daylight photodynamic therapy (PDT) in the hospital gardens.

Our laboratory studies focus on defining new treatment targets and understanding the mechanisms of action of known therapies, such as psoralen plus ultraviolet A (PUVA), interferons and proteasome inhibitors. We have discovered that receptor NOTCH1 is aberrantly expressed in cutaneous lymphoma and that this molecule provides a promising treatment target (6). Our current interests focus on genetic heterogeneity and clonal evolution of cutaneous lymphomas and employ techniques of next-generation sequencing and bioinformatics pipelines developed in house specifically for this purpose.

## Laser dermatology

Lasers and light-based therapies are gaining increasing impact in dermatology with new, effective and selective treatments to large groups of patients. Experimental use of lasers in dermatology is a cornerstone of the department's research and is consolidated in an international, multidisciplinary research setting, pursuing to develop new treatments for patients with dermatological diseases, such as non-melanoma skin cancer, inflammatory acne, scars, and vascular lesions.

*Laser-assisted drug delivery* is a dedicated research area that was developed in collaboration with Harvard Medical School. The concept takes advantage of fractional laser channels to deliver topically applied drugs and provides the unique opportunity to directly target diseased tissue (7). By combining pharmacological and energy-based research, intensified topical treatments have been developed and translated into clinical benefit for high-risk patients, including organ transplant patients, with severe field cancerization and premalignant lesions. Further research initiatives aim at delivering anti-cancerous agents directly into tumour tissue, while monitoring tumour response with imaging techniques; thus having the perspective to develop a new, non-surgical treatment concept to patients with non-melanoma skin cancer.

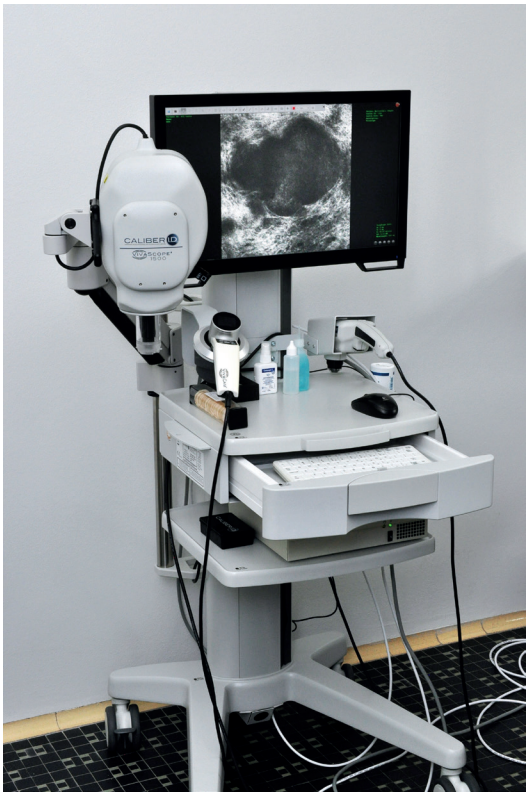


*Fig. 2.* Confocal microscope.

Our research activities cover a wide field from experimental laboratory experiments, *in vivo* murine and pig trials, early phase, proof of concept clinical trials, to larger, multicentre clinical trials. Scientific collaborators include national and international experts in dermatological laser techniques, skin cancer, pharmacy, non-invasive fluorescence analyses, optical coherence tomography (OCT) and re-scan confocal microscopy (RCM) imaging techniques (Fig. 2), chemical imaging with mass spectroscopy and analytical chemistry.

## Skin barrier function, contact dermatitis and atopic dermatitis

Research on skin barrier function has comprised experimental studies with focus on contact dermatitis, including standardized irritation models and assessment with bioengineering methods. Results from these have led to clinical studies, and intervention trials focusing on patients with hand eczema, and results have had direct clinical impact with respect to patient education and prevention of hand eczema. We have proposed a new classification of hand eczema (8), and a future goal is that all patients with hand eczema should be guided and treated depending on the specific sub-diagnosis of the eczema; a step forward in the direction of personalized medicine.

Another disease related to skin barrier function is atopic dermatitis, where colonization with *Staphylococcus aureus* is known as a major factor for severity and initiation of flares. Our studies investigating bacteria at the strain level (primarily *S. aureus*) in lesional and non-lesional atopic dermatitis skin have indicated a relationship between bacterial clonal lineage, genetic biomarkers and skin barrier function (9). This association between specific bacteria and skin barrier impairment is new and interesting, and more studies directed at the skin microbiome in atopic dermatitis are ongoing. The antibiotic resistance pattern for *S. aureus* in patients with atopic dermatitis is being followed, since this is important for the individual patient as well as for society. A new minimal invasive method, using tape-strips for sampling of stratum corneum has proven

useful for measurements of biomarkers/proteins in the skin (10). The method has the advantage to skin biopsies in that the sampling can be repeated over time, and leaves no injury or scar on the skin.

At a clinical level our studies have focused on patient education directed at patients with eczema. All patients with hand eczema are given an individual guided talk about prevention of hand eczema, also comprising practical demonstrations regarding gloves, hand-washing and disinfection. Patients with atopic dermatitis receive individual guidance, and are also invited to participate in the eczema school, which is a 3-hour group education with shifts between one-way teaching, group discussions and workshops. Patient education offered to patients with eczema has proven effective to reduce eczema severity in the patients; however, it is also immediately rewarding for the healthcare workers in the eczema clinic.

### Tattoos and tattoo complications

Tattoos and tattoo complications are an expanding research field in dermatology and toxicology (11, 12). With over 70 million Europeans and approximately 500 million world citizens being tattooed, the issue is highly relevant. The tattoo clinic at Bispebjerg Hospital was set up in 2008. Our clinical research, presently based on a material of 700 tattoo complications, have resulted in the delineation of new diagnostic entities, including patterns of plaque-elevation, excessive hyperkeratosis, ulcero-necrosis, papulo-nodular, neuro-sensitive and other distinct patterns of reaction, each with a specific causation leading to treatment. It is a novel discovery that the association between papulo-nodular reactions in black tattoos and sarcoidosis is 500-fold higher compared with non-tattooed persons. The new classification system for tattoo complications is reported to the 11$^{th}$ revision of the WHO International Classification of Diseases.

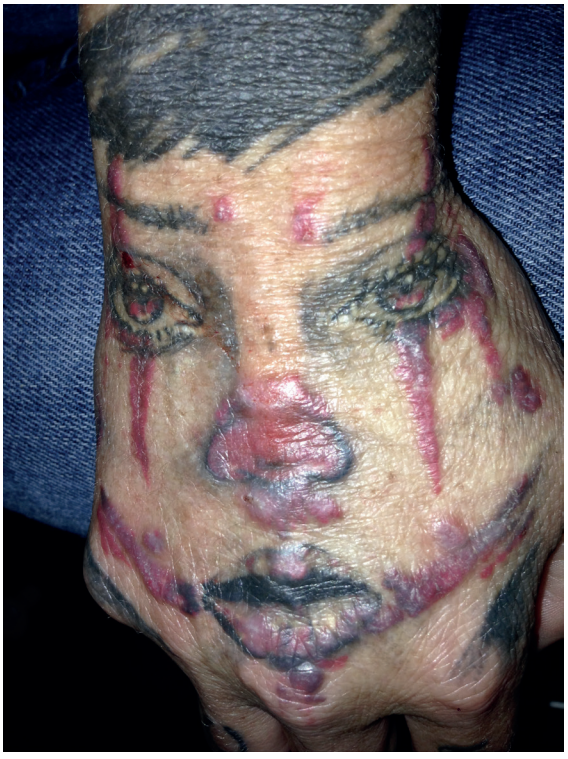

*Fig. 3.* Allergic reaction in a red tattoo with worsening following laser removal.

Our studies of mice have addressed the biokinetics of tattoo pigments and the risk of cancer. It is known that tattoo pigment is often found in regional lymph nodes. Studies of mice by histology and electron microscopy have demonstrated that tattoo pigments are distributed from the skin to the liver, and are found in the Kupffer cells, but not traced in other organs. Kupffer cells are considered part of the clearing system of particles reaching the blood. Our studies of mice showed that, even though particles reach the bloodstream, no cancer of internal organs occurred after one year. Also, the tattooed skin showed no relevant increase in skin cancer occurring in tattoos, and no photocarcinogenesis after one year of UVB exposure. The studies in mice were performed using inks banned on the Danish market due to containing potential carcinogens according to register data. The studies supported recent observations that tattoos and skin cancer are not associated, except by coincidence.

Our biokinetic studies of the local breakdown of organic tattoo pigments in human skin have attempted to identify the allergen(s) behind sensitivity towards red tattoo inks. Preliminary results have indicated that a limited number of red azo pigments are responsible, with one chemical being candidate as the epitope. Further studies including patch tests of patients with allergic tattoo reactions are ongoing. Light exposures can boost chemical breakdown of tattoo pigment and laser removal of allergic tattoo reactions is contraindicated (Fig. 3). Research activities also include a number of dedicated studies exemplified by evaluation of so-called magnetic resonance imaging (MRI)-burn in tattoos, with emphasis on the role, or not, of iron oxide and other pigments in the sensation of burn, which is not driven by measureable temperature increase of MRI-exposed pigments.

The tattoo clinic puts emphasis on social networking among clinicians and researchers. Recently, tattoo clinics were established at university hospitals in Amsterdam and New York, and a consultation was settled in Paris. The tattoo clinic is initiator of the European Society of Tattoo and Pigment Research; www.estpresearch.org.

## Clinical databases

The department has initiated and maintains several clinical and pharmacotherapeutic research databases containing patients with various skin diseases, particularly chronic urticaria, hidradenitis suppurativa, atopic dermatitis, psoriasis, systemic sclerosis, and chronic wounds.

As of October 2017, approximately 400 patients have been treated with omalizumab (anti-IgE) at our department. Of these, 325 have chronic spontaneous urticaria and 35 have chronic inducible urticaria, whereas another 40 patients have been treated off-label for various other itching skin disorders, notably atopic dermatitis. Results from this database have shown that omalizumab leads to complete resolution of symptoms in 70% of patients with chronic spontaneous urticaria and in 50% with chronic inducible urticaria, but to resolution in only 25% with detectable autoimmunity towards IgE or to the IgE receptor on mast cells and basophils (13). Our studies originating from the database have also investigated the effectiveness and safety of omalizumab in selected patients with related diseases, e.g. atopic dermatitis and systemic mastocytosis and in pregnant woman with chronic spontaneous urticaria. Ongoing studies focus on response patterns to omalizumab based on extensive biomarker profiling.

The department is a tertiary referral centre for patients with hidradenitis suppurativa and keeps databases on treatment outcomes and clinical and paraclinical characteristics of these, approximately 125 newly referred, patients per year. Studies on these patients focus on comorbidities, sub-phenotypes and quality of life. A recent study from the database population showed that patients with hidradenitis suppurativa have a thus-far unrecognized high occurrence of undiagnosed and undertreated cardiovascular risk factors, notably dyslipidaemia, hypertension, hyperglycaemia, and elevated levels of systemic inflammatory markers, independently of age; and moreover, the systemic inflammatory load seemed to be associated with increased risk of dyslipidaemia, independently of obesity (14). These findings signal that screening and treatment of cardiovascular risk factors are warranted in patients with hidradenitis suppurativa. Ongoing studies of the database population are focused on therapeutic response to biologic medications, deep phenotyping and disease burden.

From 2012 onwards all outpatients with atopic dermatitis referred to the department have been included in a clinical research database, allowing studies of the aetiology, natural history, management, and long-term follow-up of atopic dermatitis. With a yearly referral rate of around 150 new patients the database, at present, contains clinical and paraclinical data on ~700 patients with atopic dermatitis covering all ages. Studies originating from the database have so far shown that atopic dermatitis has a strong negative impact on the quality of life proportionally to the severity of the disease, and that presence of facial eczema and female sex are associated with low quality of life in patients with atopic dermatitis independently of eczema severity (15). Current studies concern genetic and serological biomarkers of atopic dermatitis.

We actively participate in the national database for biological treatments in psoriasis, which is internationally recognized and one of the largest registries of its kind in the world. We have helped to develop the concept of biological drug survival as an endpoint for the real-life assessment of the efficacy of biological therapy (16).

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