

Global identification of biomarkers in NASH development

Applicant name: GUBRA ApS
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1. Application

TITEL OG VIRKEMIDDEL

ANSØGNINGSOPLYSNINGER

Ansøgningens titel: Global identification of biomarkers in NASH development

Der søges om:: ErhvervsPhD i den private sektor

ERHVERVSPHD-PROJEKT

OPLYSNINGER OM PROJEKTET

Projektresumé (højst 500 anslag): The project will investigate the gene regulatory events associated with nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH) development with the aim of identifying novel biomarker candidates. A combination of laser capture microdissection, RNAseq and bioinformatic analyses will provide unique scientific insights into the cellular and biological pathways afflicted during the development of NAFLD/NASH disease and thereby identify novel biomarkers.

Startdato: 01-06-2016

Slutdato: 01-06-2019

Er dette en genansøgning?: Ja

Beskriv hvilke ændringer der er foretaget siden sidst her. Forklar gerne, hvordan de enkelte afslagsbegrundelser er imødekommet (højst 2.000 anslag) : Projektets metoder og hypotese er beskrevet mere præcist, ligesom fokus er blevet rettet mod opdagelsen af biomarkører i NASH udviklingen ved brug af LCM og efterfølgende next generation sequencing og bioinformatiske analyser. Der er foretaget omfattende ændringer i projektforslaget, hvorfor der ikke i projektbeskrivelsen er markeret ændringer, da hovedparten af projektbeskrivelsen er ændret.

Uderligere har PhD-kandidaten førsteforfattet en artikel der i øjeblikket er i revision ved World Journal of Gastroenterology: Kristiansen MNB, Veidal SS, Rigbolt KTG, Tølbøl KS, Roth J, Jelsing J, Vrang N and Feigh M. Obese mouse models of diet-induced nonalcoholic steatohepatitis – Tracking disease progression by liver biopsy. Manuskriptet har gennemgået peer-review og forventes accepteret for publikation snarligt.

Søges der med eller uden ErhvervsPhD-kandidat?: Med ErhvervsPhD-kandidat

Projektets forskningsfaglige hovedområde:: Sundhedsvidenskab

Projektets emneområde:: Bioteknologi, medicoteknik og sundhed

BUDGET

VIRKSOMHEDENS PROJEKTUDGIFTER

Bruttolønudgifter til ErhvervsPhD-kandidaten:	kr. 1.200.000,00
Udgifter til konferencer, kurser og udlandsophold:	kr. 70.000,00
Udgifter til rejser og ophold ved udenlandsk værtsuniversitet:	kr. 20.000,00
- Udstyr / faciliteter:	kr. 500.000,00
- Assisterende personale:	kr. 15.000,00
- Virksomhedsvejleder:	kr. 150.000,00
- Medvejleder(e):	kr. 20.000,00
- Administration / kontorhold:	kr. 120.000,00
- Andet:	kr. 10.000,00
Virksomhedens samlede forventede projektudgifter:	kr. 2.105.000,00
Kan og vil virksomheden selv finansiere de ovenstående udgifter, som ikke dækkes af det ansøgte tilskud?:	[X]

VIRKSOMHED

OPLYSNINGER OM VIRKSOMHEDEN, DER SKAL ANSÆTTE ERHVERVSPHD-KANDIDATEN

Virksomhedens cvr-nummer:	30514041
Virksomhedens navn:	GUBRA ApS
Adresselinje 1:	Agern Alle 1
Postnr.:	2970
By:	Hørsholm
Evt. hjemmeside:	www.gubra.dk
Evt. e-mail:	info@gubra.dk
Antal ansatte omregnet til fuldtidsstillinger:	65
Virksomhedens branchekode:	721100

ØKONOMISKE NØGLETAL FOR SIDSTE TRE ÅR

Omsætning 2014:	kr. 47.000.000,00
Omsætning 2013:	kr. 25.000.000,00
Omsætning 2012:	kr. 21.000.000,00
Resultat 2014:	kr. 10.000.000,00
Resultat 2013:	kr. 2.000.000,00
Resultat 2012:	kr. 3.000.000,00
Aktiver 2014:	kr. 23.700.000,00
Aktiver 2013:	kr. 13.700.000,00
Aktiver 2012:	kr. 9.000.000,00
Egenkapital 2014:	kr. 11.200.000.000,00
Egenkapital 2013:	kr. 3.500.000,00
Egenkapital 2012:	kr. 3.000.000,00

UNIVERSITET

OPLYSNINGER OM UNIVERSITETET, DER SKAL INDSKRIVE ERHVERVSPHD-KANDIDATEN

Universitet:	Københavns Universitet
Center / institut:	Department of Biomedical Sciences
Adresselinje 1:	Blegdamsvej 3
Postnr.:	2200
By:	København N
Ph.d.-skole, hvor ErhvervsPhD-kandidaten skal indskrives:	Graduate School of Health and Medical Sciences

VIRKSOMHEDSVEJLEDER

OPLYSNINGER OM VIRKSOMHEDSVEJLEDER OG EVT. MEDVEJLEDERE

Fornavn: Kristoffer
 Efternavn: Rigbolt
 Stilling: Bioinformatic Research Scientist
 Telefon: 31522650
 Email: kri@gubra.dk

1. MEDVEJLEDER

Fornavn: Sanne
 Efternavn: Møller Knudsen
 Stilling: Head of Chemistry and Biology Research

OPLYSNINGER OM UNIVERSITETSVEJLEDER OG EVT. MEDVEJLEDERE

Fornavn: Ben
 Efternavn: Vainer
 Stilling: Klinisk Professor, Overlæge
 Telefon: 35455396
 Email: ben.vainer@regionh.dk

OPLYSNINGER OM ERHVERVSPHD-KANDIDATEN

OPLYSNINGER OM ERHVERVSPHD-KANDIDATEN

Fornavn: Maria
 Efternavn: Kristiansen
 Cpr-nummer: 0107872388
 Privat email: mbaandrup@gmail.com
 Privat telefon: 26855131
 Nationalitet: Dansk
 Køn: Kvinde
 Samlet vægtet gennemsnit for hele uddannelsen (bachelor- og kandidatdel) inkl. speciale: 7,5
 Samlet vægtet gennemsnit for bachelordelen: 7,0
 Samlet vægtet gennemsnit for kandidatdelen: 8,2
 Karakter for speciale / afslutningsprojekt: 10
 Anvendt karakterskala:: 7-trinsskala

SUPPLERENDE OPLYSNINGER

SUPPLERENDE OPLYSNINGER

Supplerende oplysninger til
ansøgningen kan tilføjes her. :

Vi er bekendt med at kandidaten ikke opfylder kravene til karaktergennemsnit, kandidaten har dog opnået den krævede karakter for sit specialeprojekt.

Ydermere henleder vi udvalgets opmærksomhed på at kandidaten har:

- Førsteforfattet en artikel, der er i revision ved World Journal of Gastroenterology
 - Publiceret peer-reviewed artikel
 - Forskningserfaring fra ansættelse som forskningsassistent
 - Erhvervserfaring med projektemnet fra sit specialeprojekt
 - Erhvervserfaring med projektemnet fra ansættelse ved Gubra
- Detaljeret information om ovenstående fremgår af kandidatens CV.

Endvidere er ansøgningen vedhæftet anbefalinger fra tidligere vejleder og arbejdsgiver som supplerende bilag.

Vi er overbevist om at kandidaten er fuldt ud kvalificeret til at løfte projektet til største tilfredshed for både Gubra og universitetspartneren og håber I vil tage ovenstående i betragtning i jeres vurdering af kandidaten.

2. 01. Underskrifter

ErhvervsPhD-ansøgning

Skema til bilag med underskrifter

Ved underskrift tiltræder undertegnede at stå inde for ansøgningens oplysninger, og at de vil igangsætte og deltage i det ansøgte ErhvervsPhD-projekt, hvis det godkendes, senest et halvt år efter tilsagn er modtaget.

Det er ikke nødvendigt, at alle underskrifter er på samme ark – de kan vedhæftes i flere underskriftsbilag.

DATO

ErhvervsPhD-kandidat Maria Baandrup Kristiansen

DATO

Virksomhedsvejleder

DATO

Økonomiansvarlig i virksomheden

DATO

Universitetsvejleder



DATO 4/3-2016

Rektor, dekan eller institutleder Bente Merete Stallknecht

DATO

Ph.d.-økonomiansvarlig på universitetet (hvis anden end ovenstående)

DATO

Vejleder, første tredjepart

DATO

Vejleder, anden tredjepart

3. 01. Underskrifter

ErhvervsPhD-ansøgning

Skema til bilag med underskrifter

Ved underskrift tiltræder undertegnede at stå inde for ansøgningens oplysninger, og at de vil igangsætte og deltage i det ansøgte ErhvervsPhD-projekt, hvis det godkendes, senest et halvt år efter tilsagn er modtaget.

Det er ikke nødvendigt, at alle underskrifter er på samme ark – de kan vedhæftes i flere underskriftsbilag.


ErhvervsPhD-kandidat _____ DATO _____

Virksomhedsvejleder _____ DATO _____

Økonomiansvarlig i virksomheden _____ DATO _____

Universitetsvejleder _____ DATO _____

Rektor, dekan eller institutleder _____ DATO _____

 *TINA LEWIS* _____ DATO *2/3-2016*
Ph.d.-økonomiansvarlig på universitetet (hvis anden end ovenstående)
Afdelingschef forskning & innovation, KU-SUND

Vejleder, første tredjepart _____ DATO _____

Vejleder, anden tredjepart _____ DATO _____

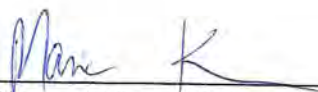
4. 01. Underskrifter

ErhvervsPhD-ansøgning


Skema til bilag med underskrifter

Ved underskrift tiltræder undertegnede at stå inde for ansøgningens oplysninger, og at de vil igangsætte og deltage i det ansøgte ErhvervsPhD-projekt, hvis det godkendes, senest et halvt år efter tilsagn er modtaget.

Det er ikke nødvendigt, at alle underskrifter er på samme ark – de kan vedhæftes i flere underskriftsbilag.


ErhvervsPhD-kandidat
DATO 04.03.2016


Virksomhedsvejleder
DATO 04.03.2016


Økonomiansvarlig i virksomheden
NIELS VRANG, CEO
DATO 04.03.2016

Universitetsvejleder
DATO

Rektor, dekan eller institutleder
DATO

Ph.d.-økonomiansvarlig på universitetet (hvis anden end ovenstående)
DATO

Vejleder, første tredjepart
DATO

Vejleder, anden tredjepart
DATO

5. 01. Underskrifter

ErhvervsPhD-ansøgning

Skema til bilag med underskrifter

Ved underskrift tiltræder undertegnede at stå inde for ansøgningens oplysninger, og at de vil igangsætte og deltage i det ansøgte ErhvervsPhD-projekt, hvis det godkendes, senest et halvt år efter tilsagn er modtaget.

Det er ikke nødvendigt, at alle underskrifter er på samme ark – de kan vedhæftes i flere underskriftsbilag.

DATO

ErhvervsPhD-kandidat

DATO

Virksomhedsvejleder

DATO

Økonomiansvarlig i virksomheden

DATO 2.3.2016

Universitetsvejleder

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Direkte: +45 35455316

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4/3-2016

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DATO

Ph.d.-økonomiansvarlig på universitetet (hvis anden end ovenstående)

DATO

Vejleder, første tredjepart

DATO

Vejleder, anden tredjepart

6. 02. Projektbeskrivelse

Industrial PhD project description

Basic information

Project title	Global identification of biomarkers in NASH development
Industrial PhD candidate	Maria Nicoline Baandrup Kristiansen
Company	Gubra ApS, Agern Alle 1, Hørsholm
University, center/institute	University of Copenhagen, Department of Biomedical Sciences, Blegdamsvej 3, 2200 København N, Afd. for Molekylær Patologi, Teilm-bygning, Frederik V's Vej 11, Patologiafdeling, Rigsh, 2200 København N
Any first third party	
Any second third party	

A. Objectives and success criteria (max. ½ page)

- The project's objectives

The aim of the project is to investigate the gene regulatory events associated with nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH) development with the aim of identifying novel biomarker candidates. The core of the project is the combination of laser capture microdissection (LCM) with next-generation sequencing (NGS) based analysis of the transcriptome. The use of LCM enables the isolation and characterization distinct hepatic cell types affected differentially by NASH, e.g. hepatocytes and hepatic stellate cells. This is in contrast to the conventional approach where complete biopsies are analyzed and average across all cells types are measured. To ensure that the identified biomarker candidates have translational value, samples from both rodent models and liver biopsies from human patients will be investigated in the project.

- The project's success criteria

The project will provide new knowledge of the complicated pathophysiology of NASH and hopefully lead to the discovery of potential biomarkers for NASH diagnosis/prognosis. To date, histological staging and grading via liver biopsy is the gold standard method for differentiating simple steatosis from NASH and for assessing the severity of liver fibrosis in patients with NASH. However, given the disease burden of NASH patients, the use of circulating biomarkers could be an attractive addition to the available approaches for NASH diagnostics. Thus, there is an unmet need for detecting noninvasive surrogate markers to be measured in peripheral blood. Identifying subtle gene expression changes in distinct cell types at different stages of NASH progression in animal models and humans will constitute a novel approach to bring forward important scientific knowledge about NASH progression and reveal new markers for diagnostic or prognostic purposes.

B. Commercial potential (max. 1 page)

- The project's commercial potential for the company

For the past 2 years, Gubra has worked intensively in gaining expert insight into animal models and histological changes related to NASH development and have successfully engaged in testing the efficacy of potential therapeutics in collaboration with several companies worldwide. Currently, no effective pharmacological treatment for NASH exist, whereas the underlying causes of NASH (obesity and diabetes) are spreading at alarming rates. As a consequence, NASH has become a highly prioritized area for a large group of pharmaceutical companies. It is clear that a deeper understanding of NASH progression combined with a biomarker detection platform, will be highly valuable within Gubra's contract research branch. Furthermore, the refinement of existing- and new methodologies within Gubra's core expertise area (LCM) is considered of high commercial value. Ultimately identification of new potential NASH biomarkers would enable Gubra to potentially partner such a discovery program with a pharmaceutical company (in a similar fashion as Gubra previously partnered a gut target discovery project).

C. State-of-the-art and theoretical background (max. 1 page excl. references)

- State-of-the-art and, if relevant, theoretical background for the Industrial PhD project's field of research

Thirty years ago, NAFLD did not even have a medical name, as physicians and researchers presumed the buildup of fat in the liver to be essentially benign. With the advent of drugs to better treat hepatitis C, NAFLD is now the face of liver disease in the U.S. with a library of research documenting that fat accumulation in the liver can lead to NASH, cirrhosis of the liver, eventually hepatocellular carcinoma and/or liver failure and death. Cases of NAFLD have doubled in the past two decades, while prevalence of other liver diseases has remained stable or even decreased, positioning NAFLD as the expected leading cause of liver transplants by 2020 (Lazo et al., 2013; Ratzliff V, Bellentani S, Cortez-Pinto H, Day C, 2010). Consequently, NAFLD is now acknowledged as a complex public health issue strongly associated to obesity and type 2 diabetes, with an estimated prevalence of 30% in adults and 10% in children (Browning et al., 2004; Carter-Kent et al., 2009; Schwimmer et al., 2006). Of these, approximately 25% progresses to NASH (Williams et al., 2011).

The pathogenesis of NASH was originally conceptualized as a disease of consecutive hits: the accumulation of fat in the liver cells (steatosis) that sensitized the liver to a second metabolic insult triggering a cascade of tissue damage (inflammation) resulting in fibrosis (Day & James, 1998). It is now appreciated that a more complex process involving multiple parallel metabolic dysregulations is responsible for tissue injury and disease progression (Alkhouri & McCullough, 2012; Larter & Farrell, 2006; Tilg & Moschen, 2010). Central to an understanding of the pathogenesis of NASH is the concept of lipotoxicity and the contributions of insulin resistance and oxidative stress to hepatocyte damage (Neuschwander-Tetri, 2010). Moreover, hepatocyte death and an ensuing inflammatory cascade likely represent the nexus between inflammation and fibrosis (Peverill, Powell, & Skoien, 2014). Recognition of the importance of the interactions between cells within the liver is pivotal for our understanding of liver pathogenesis.

Consequently, a high level of scientific activity is directed towards understanding the physiological and pathophysiological implications of NASH in the hope that novel diagnostics or treatments can be derived. Nevertheless, the various aforementioned aspects of NASH development have not previously been subject to an in-depth study on the cellular level. Recent advances in the "-omics" technologies, such as genomics, transcriptomics, proteomics, and metabolomics, have offered dramatically improved opportunities for investigation of changes in metabolic and signaling pathways and their interactions in complex diseases such as NASH (Robinson, Fernandes, & Husi, 2014). However, most of these analyses have been based on

whole liver biopsy homogenates and not analyses of the specific cellular sub-compartments (Moylan et al., 2014). Identifying subtle gene expression changes in isolated homogenous samples of specific cells (e.g. hepatocytes and hepatic stellate cells) in animal models and human patients are considered a highly novel approach deemed to bring forward important knowledge about the disease progression of NASH, and potentially reveal new diagnostic or prognostic markers.

D. Project description (max. 4 pages)

- Project description

The experimental part of the PhD will be divided into five phases. 1) The histological analysis and characterization of NASH in rodent and human material, 2) LCM based isolation of specific cells populations in rodent models and in human liver resections, followed by 3) NGS-based analysis gene regulation in the samples (Wang, Gerstein, & Snyder, 2009), 4) bioinformatics analyses data filtering and biomarker nomination (Su et al., 2014) and 5) validation and evaluation of potential novel biomarkers, see Figure 1. Validation of biomarkers will be carried out in rodent and patient material. Collectively, these approaches will generate data shedding new light on the hepatocellular events involved in the progression of NASH and provide an innovative combination of methods to identify novel biomarkers.

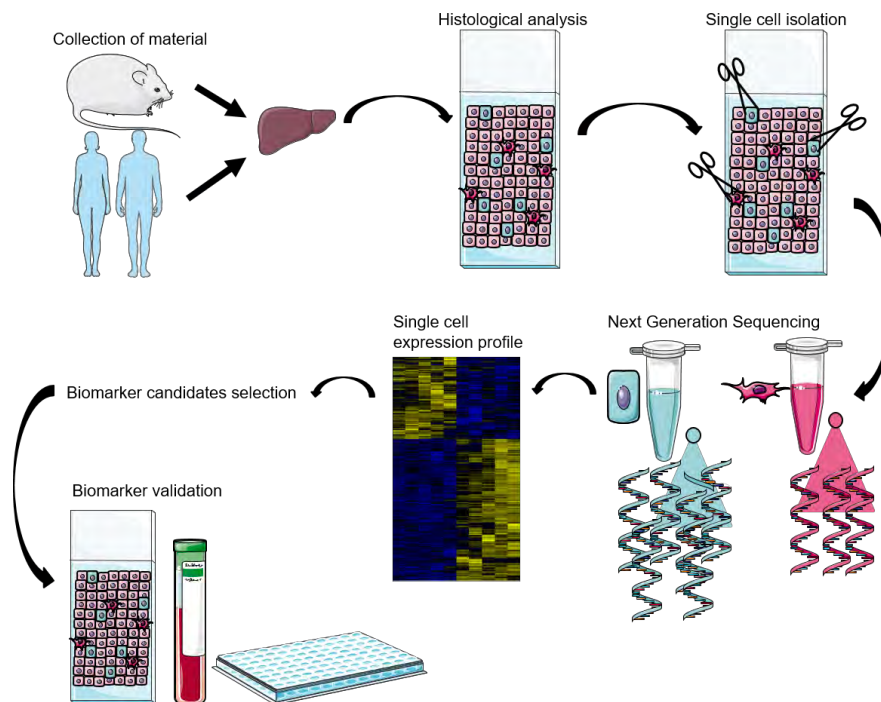


Figure 1. PhD project outline. Samples from rodent models and patient material will be collected, analyzed histologically, isolation of specific cells of interest for further gene expression profiling, stringent bioinformatic analysis resulting in selection of novel biomarker candidates to be further validated using rodent model and patient material, tissue for e.g. in situ hybridization and tissue homogenates and plasma for validation of biomarker candidates in circulation.

Animal model and histopathological characterization

Gubra has already established a diet induced model of NASH. This model is based on the Amylin NASH diet (AMLN diet) high in trans-fat, fructose and cholesterol. The model has been demonstrated as appropriate for pharmacological intervention (Trevaskis et al., 2012) and is currently the NASH model of choice at Gubra. The model is useful for the project as it reflect many features of the underlying factors in NASH associated with metabolic syndrome (hyperinsulinemia, insulin resistance, glucose intolerance, and

hypercholesterolemia) and display pronounced degrees of liver steatosis, fibrosis, inflammation and ballooning degeneration (Clapper et al., 2013 and Gubra submitted data) (figure 2).

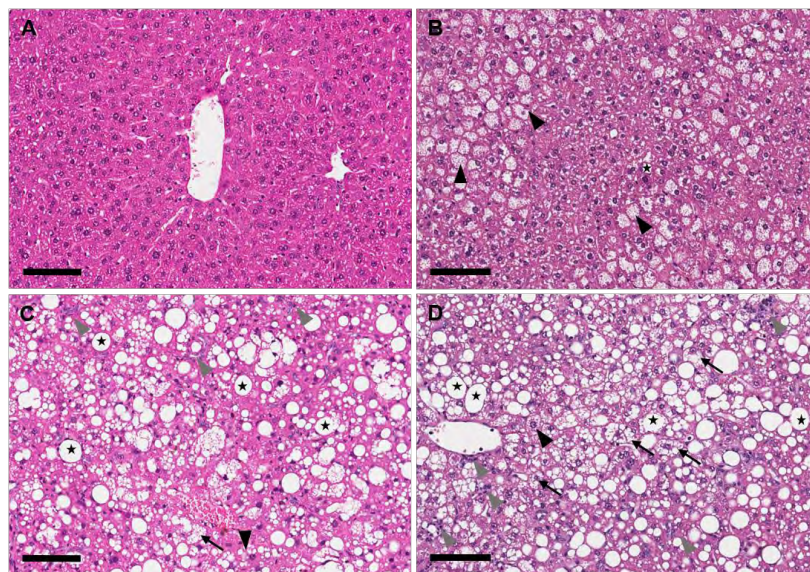


Figure 2 Histological assessment of steatosis, inflammation and ballooning degeneration as reflected by NAFLD Activity Score. Representative H&E stained sections from; C57BL/6J chow (A), Lep^{ob}/Lep^{ob} chow (B), C57BL/6J AMLN diet (C) and Lep^{ob}/Lep^{ob} AMLN diet (D). Macrovesicular steatosis indicated by stars, microvesicular steatosis indicated by black arrowheads, inflammation indicated by grey arrowheads, ballooning degeneration indicated by black arrows. Scale bar = 100μM

In order to identify rare cells involved in the development of NASH or affected by NASH development, further histological characterization will be performed. Different histological techniques will be performed and optimized for NASH characterization in rodent models and human specimens. Hematoxylin and eosin can be used for identification of micro- and macrovesicular steatosis and ballooning degeneration. Formation of fibrosis can be confirmed using Masson's trichrome, Sirius Red and using antibodies targeting e.g. type I and/or type III collagen formation. Additionally, antibodies will be employed for the histological characterization, such as anti- α smooth muscle actin (α -SMA) for the identification of activated hepatic stellate cells and immunostaining of keratin 8 or 18 for further confirmation of ballooning degeneration. This characterization and identification of rare cells will be performed in close collaboration with the University partner, that have years of histopathological experience with liver tissue markers in humans. To enable subsequent gene expression analysis, the staining protocols will be optimized to preserve RNA quality using Gubra's expertise within modification of immunohistochemical stains for RNA isolation and expression analysis.

Target cell extraction

LCM is a precise method for isolation of specific cells of interest from heterogeneous tissues. When LCM is combined with RNA analysis it is a powerful tool to examine global gene expression in diverse cell types in the same organ (Figure 3). LCM rests on microscopic identification of cells of interest (using standard histochemical or specific immunohistochemical labelling) in combination with infrared (IR) laser capturing or high resolution ultraviolet (UV) laser cutting, resulting in an enriched cell sample without tissue damage. LCM samples will be used for total mRNA expression profiling using RNA sequencing (Illumina next generation sequencing platform will be used). The single cell capture methodology will enable a cell-specific molecular profiling of diseased and disease-free tissue. Gubra has already established a LCM facility with expert knowledge within the isolation of single cells and has applied the techniques to different tissues from both rodents and humans.

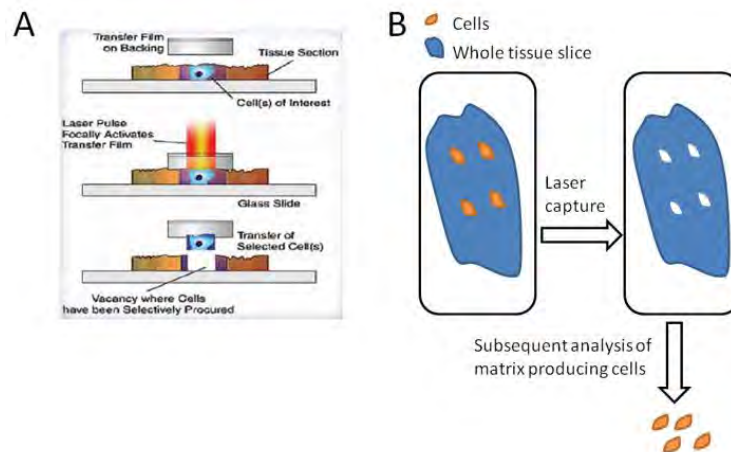


Figure 3: Laser capture microdissection enables a fast, simple and accurate procurement of cells from a heterogeneous tissue under microscopic visualization. (A) via microscopic visualization single cells of interest can be dissected using IR laser capturing, resulting in an enriched cell sample. (B) inspection of the tissue slide reveals successful capture of cells of interest. LCM can be combined with different DNA, RNA, or protein analysis with high sensitivity to enable molecular profiling. Image from Arcturus 2002 website.

In the present project, liver samples from the Gubra NASH model will constitute the principal source of tissue material for LCM of specific hepatic cell populations. Focus will be on hepatocytes during NASH progression as well as hepatic stellate cells that are believed to play a key role in fibrosis development. Isolation of distinct cell types will allow detection of gene regulatory events in sub-stoichiometric cell types, that would be impossible to detect in biopsies due to the strong signal from highly prevalent cell types (Figure 4). This concept is at the core of project and will build on the vast LCM experience at Gubra - an experience that will be important for the success of the project. Additionally, this phase of the project will be performed in close collaboration with the University partner and benefit from the histopathological experience of the University partner, for identification of rare cells. Such tissue samples are unique in the sense that they have been obtained in a manner that allows LCM, RNA extraction and downstream analyses without comprising the RNA quality.

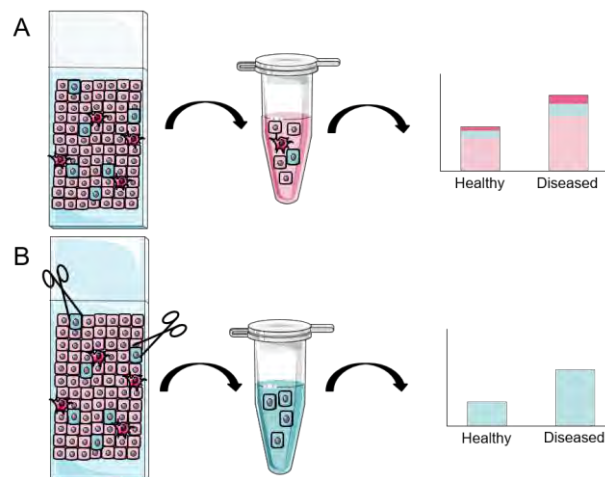


Figure 4. Expression profiling of whole tissue (A) and single cells isolated by use of LCM (B). Gene expression variations of rare cells will be difficult to detect using gene expression analysis of a heterogeneous tissue section (A), however, isolation of specific single cells allow for specific quantification of an enriched cell population, enabling gene expression analysis of rare cells in a heterogeneous tissue section (B).

NGS sequencing and bioinformatics

NGS-based transcriptome analysis is a highly attractive platform for biomarker due to its high sensitivity, that allow detection and quantification of genes expressed at very low levels. Furthermore, the technique is virtually unbiased and will therefore identify the expression of all expressed genes without the need for any *a priori* selection of genes of interest. In combination, this technique serves as read out for the gene expression in the different cell types. Genome-wide association studies have previously been used to identify single nucleotide polymorphism in the PNPLA3 gene which was strongly associated with hepatic fat content and the histologic features of NAFLD (Romeo et al., 2008). However, to date no research group have combined the isolation of specific cell groups using LCM with gene expression profiling to identify novel biomarkers.

The PhD student will assist in setting up RNA sequencing at Gubra, which will be followed by comprehensive bioinformatics analysis performed by the PhD applicant in close collaboration with already established bioinformatic expertise at Gubra. In short, the data will be subjected to quality control analyses and genes differentially expressed between samples will be extracted. Subsequently differentially expressed genes will be organized into pathways to investigate the biological processes affected by the progression of the NASH. Finally, extensive efforts will be made to integrate the information obtained from bioinformatic analysis with the existing understanding of NASH to arrive at a shortlist of the most interesting targets for functional validation and follow-up.

Biomarker validation

The results from RNA sequencing will aid in the discovery of putative biomarkers. Differentially expressed genes will be validated in rodent model and patient material. Location of potential genes of interest in liver samples from mouse and human NASH will be identified using chromogen based in-situ hybridization techniques (e.g. RNAscope) that allows for detection of even rare mRNAs histologically. Genes expressed at levels that correlate with disease severity and in the right cell types (e.g. ballooning hepatocytes or in stellate cells/inflammatory cells) will be further investigated by assay techniques. Hence, putative translated protein products will be used to raise antibodies (Gubra has already used this method to in other projects) and subsequently those antibodies will be used to detect gene-products in plasma samples. It is anticipated that the PhD student will be involved only to a smaller degree in these studies as they are not anticipated to be started until the end of the PhD project.

E. Publication plan

Proposed title and date of publication	Proposals for one or more acknowledged research journals as desired place of publication
Characterization of a rodent model for Nonalcoholic Steatohepatitis	Submitted to <i>"Hepatology"</i>
Global profiling of the transcriptome changes in Nonalcoholic Steatohepatitis	Submitted to <i>"Bioinformatics"</i>
Identification of a novel marker of Nonalcoholic Steatohepatitis	Submitted to <i>"Journal of Hepatology"</i>

F. Courses, conferences and stays abroad (max. ½ page)

- PhD courses

Course	Institution	Time	ECTS points
Next Generation Sequencing Analysis	Technical University of Denmark	Summer 2016	5
Summer School on Diabetes and Metabolism	University of Copenhagen	Summer 2016	3,5
Basic Statistics for Experimental Medical Researchers	University of Copenhagen	Fall 2016	3,5
Bioinformatics methods for Analysis of High-Throughput Sequencing Data	University of Copenhagen	Fall 2016	7,5
Project Management	University of Copenhagen	Fall 2016	2,5
Scientific Writing - a Framework for Writing a Scientific Paper	University of Copenhagen	Spring 2017	0,7
Introduction to Bioinformatics	Technical University of Denmark	Spring 2017	5
Basal Metabolism and Molecular Metabolism	University of Copenhagen	Summer 2017	3,5
Total			31,2

- Conferences, seminars
 - AASSLD, USA, November 2016
 - The 16th international conference on Systems Biology, Barcelona, Spain, Winter 2016
 - EASL Meeting, Stockholm, Sweden, April 2017
 - 52nd EASD Annual Meeting, Munich, Germany, September 2017
- Stays abroad

The PhD student has expressed a strong wish to include one or more stays at foreign Universities during the PhD period, which is fully supported by the supervisors. Both the company and the university partner have a large network within the scientific community and are in contact with internationally recognized researchers within diabetes and inflammatory bowel diseases.

G. Dissemination plan (max. ½ page)

- Dissemination plan
 - Regular internal seminar presentations at weekly group meetings and weekly laboratory meetings: 4 presentations a year; 6 hours each = 24 hours per year = 71 hours in total.
 - Weekly journal club: 4 presentations a year; 6 hours each = 24 hours per year = 71 hours in total.
 - Communication of research results at national and international meetings, approx. 100 hours in total.
 - Presentation and publication of results in international peer reviewed journals and/or patent applications, approx. 3 papers: 1 month each = 450 hours in total.
 - Preparation of PhD thesis and the Industrial PhD report, approx. 3 months = 450 hours.
 - Attending PhD courses including preparation, approx. 100 hours.

Total approximately 1250 hours.

H. Structure and time schedule (max. 1 side)

- The structure of the education and project, divided into work packages with corresponding milestones and success criteria, and illustrated in a time schedule chart

Phase 1: Collection of sample material

The overall aim of first phase is to collect the tissue samples needed for subsequent histology and LCM. This phase will include two parallel activities: The first activity is to setup and validate the rodent models at the company partner and the second part is to, in collaboration with the University partner, collect the samples from patient donors at the University. As well as retrieve the NAFLD fresh-frozen biopsies from the collaborator.

Phase 2: Histological analysis and preparation

The aim of this phase is to characterize the NASH phenotype of collected material and to isolate hepatocytes and hepatic stellate cells for further analysis. Characterization of the NASH phenotype of the rodent models and the NAFLD progression of the patient material will be completed using a panel of histological techniques. Specifically, histological tissue preparations will be used to assess the presence of both steatosis, inflammation, fibrosis and ballooning degenerations. In addition, histological preparations will be validated and further developed for incorporation of LCM of hepatocytes and hepatic stellate cells from rodent liver tissue and NAFLD fresh-frozen biopsies, for subsequent investigation of gene expression profiles during disease stages.

Phase 3: Characterization of global gene regulation

The third phase will focus on setting up RNAsequencing at Gubra, the subsequent global analysis of the transcriptome of the samples from RNAsequencing and bioinformatics analysis of the data. The primary goals of the bioinformatics analyses will be to obtain a detailed and comprehensive understanding of the differential impact on signal pathways in the different samples. Furthermore, the bioinformatics will provide a catalogue of putative biomarkers for validation and follow-up.

Phase 4: Biomarker validation and analysis

Target genes will be validated using tissue samples, tissue homogenates, and if commercial antibodies are available targets will be validated histologically, e.g. using in situ hybridization and in circulation using plasma samples and subsequently test this in biochemical setups such as ELISA to verify and further

characterize the findings from the global gene expression analysis. A critical activity in the biomarker analysis phase will be to ensure that the findings from the rodent samples can be observed also in human samples.

Phase 5: Academic phase

The course activity is planned for the beginning of the PhD period to allow acquirement of academic tools beneficial for the PhD project and to allow for scientific reflection. The last quarter of the PhD will be devoted to completing the thesis and preparing the last manuscript for publication.

Activity	2016		2017				2018				2019	
	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2
Phase 1												
Setup NASH animal model												
Characterize NASH animal model												
Collection of human patient samples												
Phase 2												
Histological analysis of rodent liver tissue												
LCM of hepatic cell types												
Evaluating and writing paper												
Phase 3												
Global gene expression analysis												
Bioinformatic analysis												
Nomination of primary candidate genes												
Evaluating and writing paper												
Phase 4												
Target validation in rodent liver samples												
Target validation in sections from NASH/NAFLD patients												
Target validation by biochemical analysis in samples from rodent and human material												
Evaluating and writing paper												
Phase 5												
Courses												
Finalizing PhD thesis												

I. Time allocation

Allocation of the Industrial PhD candidate's time	in months	in % of project time
In Danish division of host company	20	55 %
In non-Danish divisions of host company	0	%
At other companies or organizations	0	%
At the host university	10	28 %
At other universities and research institutions	6	17 %

J. Company (max. 2 pages)

- The company and its activities

Gubra ApS was founded in October 2008. Gubra is a privately held company that delivers services (consulting, pharmacology, histology) for the pharmaceutical industry within the main focus areas of Type 2 Diabetes, Obesity and NASH. Gubra has grown organically since 2008 and today the company consists of 50 full time employees. Since its inception Gubra has vested its revenues from its CRO activities into own research (target discovery and early peptide drug discovery projects). On average discovery activities take up approximately 25% of the resources at Gubra. Gubra currently employs 3 industrial PhD students (two is two years into the program and one is one year into the program).

Currently, Gubra has established long term (FTE (Full Time Employee) based) contracts with a total of 4 different mid to big-size pharmaceutical companies. One of the long term collaborations is a 3-4 year research collaboration with Sanofi that was initiated in 2013 around the discovery of new peptide drug targets in the gut, a project of which part of was co-funded by a grant from the HTF in 2012. Currently Gubra is negotiating discovery deals with other companies around a target discovery project in the brain and NASH target discovery project.

- The candidate's placement in the company

The candidate is currently employed in the company as Research Scientist since September 2015. In the period until the beginning of the project period will work with both histology, bioinformatics and in vivo pharmacology. In this period the candidate will mainly be involved in contract research, to gain valuable knowledge on how to direct and manage projects.

- Any exit strategy

The company is in a very stable period and therefore the need for an exit strategy seems not to be relevant.

K. University (max. 1 page)

- Description of the university and center / institute

University of Copenhagen, Faculty of Health and Medical Sciences, Department of Biomedical studies.

The department employs close to 500 researchers, including 28 full professors, 51 assistant professors and more than 90 PhD students, and thus this department conducts active and widespread research. The scientific focus of the department is to conduct basic research with clinical perspectives in order to improve diagnostics and patient therapies. Specifically, the focus areas of the department are endocrinology, Heart and Circulatory Research, Molecular Pathology, Renal and Vascular Research, Cell and metabolism and Systems Biology Research. Based on this, it is clear that the Department of Biomedical studies is the optimal University partners since it harbors extensive knowledge and expertise in essentially all scientific areas relating directly, or indirectly, to NAFLD/NASH. In addition to the professorship at the Department of Biomedical studies the university partner is also employed at the Pathology division at the Diagnostics center at Rigshospitalet. The pathology division performs diagnostics on patient biopsies using a wide array of histological procedures, but is also a site of active research within several fields relating to histopathology, such as NASH/NAFLD, cancer, diabetes and neuropathology. Due to the collaboration with the pathology division the candidate will have access to patient material which will be pivotal for the project. It will enable both a global characterization of steatosis development in human patients and validation of the observations made in rodents in humans, emphasizing the translational perspective of the project.

During her stay at the University partner the candidate will obtain hands-on experience with cutting-edge approaches in biomedical research and clinical diagnostics. This will be a crucial factor in the project since a large part of NAFLD/NASH research is driven by histopathological techniques. In addition, the candidate will gain access to the large collection of facilities and instrumentation available at the biomedical research via collaborations within the department and from the core facilities. In summary, both the technical opportunities as well as the academic and scientific expertise present at the institutions of the university partner will be of crucial importance to both the impact of the proposed project and the scientific training of the candidate

References

- Alkhour, N., & McCullough, A. J. (2012). Noninvasive diagnosis of NASH and liver fibrosis within the spectrum of NAFLD. *Gastroenterology and Hepatology*, 8(10), 661–668.
- Browning, J. D., Szczepaniak, L. S., Dobbins, R., Nuremberg, P., Horton, J. D., Cohen, J. C., ... Hobbs, H. H. (2004). Prevalence of hepatic steatosis in an urban population in the United States: Impact of ethnicity. *Hepatology*, 40(6), 1387–1395. <http://doi.org/10.1002/hep.20466>
- Carter-Kent, C., Yerian, L. M., Brunt, E. M., Angulo, P., Kohli, R., Ling, S. C., ... Feldstein, A. E. (2009). Nonalcoholic steatohepatitis in children: A multicenter clinicopathological study. *Hepatology*, 50(4), 1113–1120. <http://doi.org/10.1002/hep.23133>
- Clapper, J. R., Hendricks, M. D., Gu, G., Wittmer, C., Dolman, C. S., Herich, J., ... Roth, J. D. (2013). Diet-induced mouse model of fatty liver disease and nonalcoholic steatohepatitis reflecting clinical disease progression and methods of assessment. *American Journal of Physiology. Gastrointestinal and Liver Physiology*, 305(7), G483–95. <http://doi.org/10.1152/ajpgi.00079.2013>
- Day, C. P., & James, O. F. W. (1998). Steatohepatitis: A tale of two “Hits”? *Gastroenterology*, 114(4 I), 842–845. [http://doi.org/10.1016/S0016-5085\(98\)70599-2](http://doi.org/10.1016/S0016-5085(98)70599-2)
- Dyson, J. K., Anstee, Q. M., & McPherson, S. (2014). Non-alcoholic fatty liver disease: a practical approach to diagnosis and staging. *Frontline Gastroenterology*, 5(3), 211–218. <http://doi.org/10.1136/flgastro-2013-100403>
- Guha, I. N., Parkes, J., Roderick, P., Chattopadhyay, D., Cross, R., Harris, S., ... Rosenberg, W. M. (2008). Noninvasive markers of fibrosis in nonalcoholic fatty liver disease: Validating the European liver fibrosis panel and exploring simple markers. *Hepatology*, 47(2), 455–460. <http://doi.org/10.1002/hep.21984>
- Harrison, S. a, Oliver, D., Arnold, H. L., Gogia, S., & Neuschwander-Tetri, B. a. (2008). Development and validation of a simple NAFLD clinical scoring system for identifying patients without advanced disease. *Gut*, 57(10), 1441–1447. <http://doi.org/10.1136/gut.2007.146019>
- Kalhan, S. C., Guo, L., Edmison, J., Dasarathy, S., McCullough, A. J., Hanson, R. W., & Milburn, M. (2011). Plasma metabolomic profile in nonalcoholic fatty liver disease. *Metabolism: Clinical and Experimental*, 60(3), 404–413. <http://doi.org/10.1016/j.metabol.2010.03.006>
- Larter, C. Z., & Farrell, G. C. (2006). Insulin resistance, adiponectin, cytokines in NASH: Which is the best target to treat? *Journal of Hepatology*, 44(2), 253–261. <http://doi.org/10.1016/j.jhep.2005.11.030>
- Lazo, M., Hernaez, R., Eberhardt, M. S., Bonekamp, S., Kamel, I., Guallar, E., ... Clark, J. M. (2013). Prevalence of nonalcoholic fatty liver disease in the United States: The third national health and nutrition examination survey, 1988-1994. *American Journal of Epidemiology*, 178(1), 38–45. <http://doi.org/10.1093/aje/kws448>

- Lee, S. S., & Park, S. H. (2014). Radiologic evaluation of nonalcoholic fatty liver disease. *World Journal of Gastroenterology : WJG*, 20(23), 7392–7402. <http://doi.org/10.3748/wjg.v20.i23.7392>
- Milic, S., Mikolasevic, I., Krznaric-Zrnic, I., Stanic, M., Poropat, G., Stimac, D., ... Orlic, L. (2015). Nonalcoholic steatohepatitis: emerging targeted therapies to optimize treatment options. *Drug Design, Development and Therapy*, 9, 4835–45. <http://doi.org/10.2147/DDDT.S64877>
- Moylan, C. a., Pang, H., Dellinger, A., Suzuki, A., Garrett, M. E., Guy, C. D., ... Diehl, A. M. (2014). Hepatic gene expression profiles differentiate presymptomatic patients with mild versus severe nonalcoholic fatty liver disease. *Hepatology*, 59(2), 471–482. <http://doi.org/10.1002/hep.26661>
- Neuschwander-Tetri, B. a. (2010). Hepatic lipotoxicity and the pathogenesis of nonalcoholic steatohepatitis: The central role of nontriglyceride fatty acid metabolites. *Hepatology*, 52(2), 774–788. <http://doi.org/10.1002/hep.23719>
- Peverill, W., Powell, L. W., & Skoien, R. (2014). Evolving concepts in the pathogenesis of NASH: Beyond steatosis and inflammation. *International Journal of Molecular Sciences*, 15(5), 8591–8638. <http://doi.org/10.3390/ijms15058591>
- Ratziu V, Bellentani S, Cortez-Pinto H, Day C, M. G. (2010). A position statement on NAFLD/NASH based on the EASL 2009 special conference. *J Hepatol*, 53(2), 372–384.
- Robinson, S. W., Fernandes, M., & Husi, H. (2014). Current advances in systems and integrative biology. *Computational and Structural Biotechnology Journal*, 11(18), 35–46. <http://doi.org/10.1016/j.csbj.2014.08.007>
- Romeo, S., Kozlitina, J., Xing, C., Pertsemlidis, A., Cox, D., Pennacchio, L. a, ... Hobbs, H. H. (2008). Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease. *Nature Genetics*, 40(12), 1461–1465. <http://doi.org/10.1038/ng.257>
- Schwimmer, J. B., Deutsch, R., Kahen, T., Lavine, J. E., Stanley, C., & Behling, C. (2006). Prevalence of fatty liver in children and adolescents. *Pediatrics*, 118(4), 1388–1393. <http://doi.org/10.1542/peds.2006-1212>
- Su, Z., Łabaj, P. P., Li, S., Thierry-Mieg, J., Thierry-Mieg, D., Shi, W., ... Shi, L. (2014). A comprehensive assessment of RNA-seq accuracy, reproducibility and information content by the Sequencing Quality Control Consortium. *Nature Biotechnology*, 32(August). <http://doi.org/10.1038/nbt.2957>
- Tilg, H., & Moschen, A. R. (2010). Evolution of inflammation in nonalcoholic fatty liver disease: The multiple parallel hits hypothesis. *Hepatology*, 52(5), 1836–1846. <http://doi.org/10.1002/hep.24001>
- Trevaskis, J. L., Griffin, P. S., Wittmer, C., Neuschwander-Tetri, B. a., Brunt, E. M., Dolman, C. S., ... Roth, J. D. (2012). Glucagon-like peptide-1 receptor agonism improves metabolic, biochemical, and histopathological indices of nonalcoholic steatohepatitis in mice. *AJP: Gastrointestinal and Liver Physiology*, 302(8), G762–G772. <http://doi.org/10.1152/ajpgi.00476.2011>
- Wang, Z., Gerstein, M., & Snyder, M. (2009). RNA-Seq: a revolutionary tool for transcriptomics. *Nature Reviews. Genetics*, 10(1), 57–63. <http://doi.org/10.1038/nrg2484>

- Wieckowska, A., McCullough, A. J., & Feldstein, A. E. (2007). Noninvasive diagnosis and monitoring of nonalcoholic steatohepatitis: Present and future. *Hepatology*, 46(2), 582–589. <http://doi.org/10.1002/hep.21768>
- Williams, C. D., Stengel, J., Asike, M. I., Torres, D. M., Shaw, J., Contreras, M., ... Harrison, S. a. (2011). Prevalence of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis among a largely middle-aged population utilizing ultrasound and liver biopsy: A prospective study. *Gastroenterology*, 140(1), 124–131. <http://doi.org/10.1053/j.gastro.2010.09.038>

7. 03. Cv for kandidaten

Maria Nicoline Baandrup Kristiansen

Cand. Scient in Biology-Biotechnology



Personal Information

Sallingvej 24, 1.th 2720 Vanløse
26855131
mbaandrup@gmail.com
Viborg 01.07-1987

Profile

I am an adaptable and curious molecular biologist with a great interest in human disease biology. I have gained intimate knowledge within working with human cell cultures, development of antibodies as well as experimental animal models, histology and several molecular biology techniques.

During my education I combined studying with work, student politics and sports, showing myself to be organised and self-motivated. I enjoy working on my own initiative as well as working in a team; I am social, which helps me when entering a new team.

Work Experience

Sep. 2015-: Gubra Aps., Bioinformatics and Biology Group

Agern Allé 1, 2970 Hørsholm

- Research Scientist
 - Laser Capture Microdissection
 - Cryosectioning and isolation of cell areas of interest of pancreas and brain
 - Histological examination of animal models of non-alcoholic steatohepatitis
 - Presentation of experimental results and relevant scientific research

Sep. 2014-2015: Finsen Laboratory, Engelholm Group

Finsen Laboratory, Ole Maaløes vej 5, 3rd floor 2200 København N

- Research Assistant
 - Staining techniques for circulating tumour cells in sarcomas in particular osteosarcomas
 - Collaboration with CytoTrack
 - Presentation of experimental results and relevant scientific research

2012-2013: Master Thesis student, Fibrosis Department, Supervisor Sanne Skovgård Veidal

Nordic Bioscience, Herlev Hovedgade 207, 2730 Herlev

Master thesis entitled: "Investigation of Extracellular Matrix Remodeling Associated with Liver Fibrosis and Hepatocellular Carcinoma".

- Achievements
 - Early development of an *in vivo* rodent model for hepatocellular carcinoma
 - Improvement of a competitive ELISA for diagnosis of liver fibrosis, hereunder development of monoclonal antibodies
 - Investigation and early development of a cell system in order to investigate early events leading to fibrotic cells
 - Presentation of experimental results and relevant scientific research

2009-2010: Fulltime employment Copenhagen Fur
Langagervej 60, 2600 Glostrup

2008-2009: Student helper at Radiometer Medical
Åkandevej 21, 2700 Brønshøj

Education

2010-2013: MSc Biology-Biotechnology

University of Copenhagen, Faculty of Science

My interest in chemistry, human health and treatment possibilities led me to choose a base in my elective courses within the mammalian organism; expression patterns as well as applied chemistry.

2006-2009: BSc Biologi-Bioteknologi

University of Copenhagen, Faculty of Life Science

Conference

June 2015: Cancer Markers & Liquid Biopsies, GTCBio, San Diego, USA

Oral presenter and poster presentation

Course

January 2015: CFIM Light Microscopy Course

University of Health, Core Facility for Integrated Microscopy, Department of Biomedical Sciences

A two-day course on the basics of light microscopy

Publications

Vassiliadis E, Veidal SS, **Kristiansen MNB**, Hansen C, Jorgensen M, Leeming DJ, Karsdal M. Peptidyl arginine deiminase inhibitor effect on hepatic fibrogenesis in a CCl4 pre-clinical model of liver fibrosis. Am J Transl Res 2013;5(4):465-469.

Kristiansen MNB, Veidal SS, Rigbolt KTG, Tølbøl KS, Roth J, Jelsing J, Vrang N and Feigh M. Obese mouse models of diet-induced nonalcoholic steatohepatitis – Tracking disease progression by liver biopsy. World Journal of Gastroenterology -in revision.

References

Sanne Skovgård Veidal, Gubra Aps, available upon request.

Lars Henning Engelholm, Group Leader, The Finsen Laboratory, available upon request.

Political Representative

2011-2012: Chairman of The Student Council

University of Copenhagen, Faculty of Life Sciences

- Collaboration with:
 - Student politicians
 - Management at the Faculty
 - Employees at The Student Council

2009-2011: Chairman of the Union of Biology-Biotechnology students

University of Copenhagen, Faculty of Life Sciences

- Collaboration with:
 - Student politicians
 - Management at the Faculty
 - Professors at the Biology-Biotechnology programme
 - The industry about which focus of the education is needed
 - The trade-union "JA"

Volunteering

2014-: "Børn, Unge og Sorg"

Kejsergade 3, 1155 København K

- Volunteering at "Linjen"

Personal

I am 28 years old and live in Vanløse. I live an active lifestyle; I am a former tumbler at elite level and in my spare time I work out as part of my rehabilitation of my knee that hopefully can get me back on track as a tumbler. Furthermore I use the outdoor possibilities and cultural events of Copenhagen as often as I can, preferably with my friends.

8. 04. Fulde eksamensdiplomer for kandidatuddannelse

Maria Nicoline Baandrup Kristiansen

Cpr.: 010787-2388

har gennemført kandidatuddannelsen i
Biologi-bioteknologi
10. september 2013



Oversigt over prøver og bedømmelser side 1 af 2

Følgende resultater er opnået	Resultat 7-trinsskala	Resultat ECTS-skala	ECTS point
Speciale			
Investigation of the Extracellular Matrix Remodelling associated with Liver Fibrosis and Hepatocellular Carcinoma.....	10	B	60,0
Obligatoriske kurser			
Enzymology and Experimental Biochemistry	7	C	7,5
Heterologous Expression	7	C	15,0
Immunology - Theoretical.....	4	D	7,5
Advanced Biotechnology and Intellectual Property Rights	7	C	15,0
Lab Animal Science cat C.....	Bestået		7,5
Valgfrie aktiviteter			
RNA Biology	4	D	7,5

17. oktober 2013

Charlotte Louise Friis Rundsten
SCIENCE Uddannelse

Maria Nicoline Baandrup Kristiansen

Cpr.: 010787-2388

har gennemført kandidatuddannelsen i
Biologi-bioteknologi
10. september 2013

Oversigt over prøver og bedømmelser side 2 af 2

Kandidatuddannelsen i Biologi-bioteknologi er normeret til 120 ECTS-point iht. Bekendtgørelse om bachelor- og kandidatuddannelser ved universiteterne (uddannelsesbekendtgørelsen), som ændret ved bekendtgørelse nr. 814 af 29. juni 2010 med senere ændringer, nr. 338 af 06. maj 2004.

Aktiviteter herover, der er angivet på engelsk, er gennemført på engelsk. Aktiviteter, der er angivet på øvrige sprog, er tilsvarende gennemført på disse sprog.

Adgangsgrundlaget til kandidatuddannelsen

BSc inden for naturvidenskab eller tilsvarende

17. oktober 2013



Charlotte Louise Friis Rundsten
SCIENCE Uddannelse



MARIA NICOLINE
BAANDRUP KRISTIANSEN

cpr. 010787-2388

har den 10. september 2013
opnået
kandidatgraden i

*has on 10 September 2013
been awarded the degree of
Master of Science in*

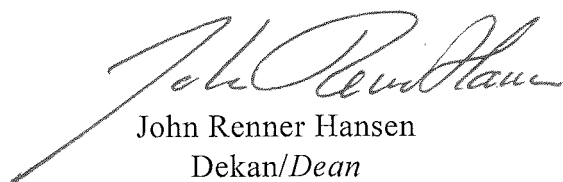
Biologi-bioteknologi


Biology - Biotechnology

og titlen

and the title

cand.scient.
candidata scientiarum


John Renner Hansen
Dekan/Dean


Karen Rønnow
Studiechef/Director of Studies

DET NATUR- OG BIOVIDENSKABELIGE FAKULTET
FACULTY OF SCIENCE

9. 05. Fulde eksamensdiplomer for bacheloruddannelse

Maria Nicoline Baandrup Kristiansen


Cpr.: 010787-2388

har gennemført bacheloruddannelsen i
Biologi - Bioteknologi
13. juli 2010



Oversigt over prøver og bedømmelser side 1 af 2

Følgende resultater er opnået	Resultat 7-trinsskala	Resultat 13-skala	Resultat ECTS-skala	ECTS point
Obligatoriske kurser				
Kemi	4	7	D	15,0
Biofysik	7	9	C	7,5
Cellebiologi	02	6	E	7,5
Matematik og databehandling	7		C	7,5
Bioteknologi	Bestået			7,5
Genetics 1	7	9	C	7,5
Genetics 2	4	7	D	7,5
Biokemi 1	4		D	7,5
Organismebiologi	10		B	15,0
Mikrobiologi	7		C	7,5
Tema: Eksperimentel molekylærbiologi	10		B	30,0
Valgfrie aktiviteter				
Organisk kemi og spektroskopi	10		B	7,5
Bioinformatik 1	4		D	7,5
Mikrobielle interaktioner	7		C	7,5


Sofie Christensen
Uddannelse og Studerende

Maria Nicoline Baandrup Kristiansen

Cpr.: 010787-2388

har gennemført bacheloruddannelsen i
Biologi - Bioteknologi
13. juli 2010




Oversigt over prøver og bedømmelser side 2 af 2

Følgende resultater er opnået	Resultat 7-trinsskala	Resultat 13-skala	Resultat ECTS-skala	ECTS point
Mammalian Genomics	7		C	7,5
Makromolekyler, cofaktorer og metalioner og deres kemi i biologiske systemer	Bestået			7,5
Bioorganisk kemi og lægemiddelkemi	7		C	7,5
Bachelorprojekt				
Bachelorprojekt	7		C	15,0
<i>The Minipig as a Model for Obesity</i>				

Bacheloruddannelsen i Biologi - Bioteknologi er normeret til 180 ECTS-point iht. Bekendtgørelse om bachelor- og kandidatuddannelser ved universiteterne.

Aktiviteter herover, der er angivet på engelsk, er gennemført på engelsk. Aktiviteter, der er angivet på øvrige sprog, er tilsvarende gennemført på disse sprog.


Sofie Christensen
Uddannelse og Studerende

10. 06. Karakterberegninger for kandidaten

ErhvervsPhD-kandidat, beregning af vægtet karaktergennemsnit		
ECTS	Karakter	ECTS x karakter
15	4	60
7,5	7	52,5
7,5	2	15
7,5	7	52,5
7,5	7	52,5
7,5	4	30
7,5	4	30
15	10	150
7,5	7	52,5
30	10	300
7,5	10	75
7,5	4	30
7,5	7	52,5
7,5	7	52,5
7,5	7	52,5
15	7	105
165		1162,5
Gennemsnit for BA		7,0

60	10	600
7,5	7	52,5
15	7	105
7,5	4	30
15	7	105
7,5	4	30
112,5		922,5
Gennemsnit for KA		8,2

	Samlet (1):	7,5
	Speciale (2):	10

11. 07. Generelt karakterniveau og -spredning

Maria Nicoline Baandrup Kristiansen


Cpr.: 010787-2388

har gennemført bacheloruddannelsen i
Biologi - Bioteknologi
13. juli 2010



Oversigt over prøver og bedømmelser side 1 af 2

Følgende resultater er opnået	Resultat 7-trinsskala	Resultat 13-skala	Resultat ECTS-skala	ECTS point
Obligatoriske kurser				
Kemi	4	7	D	15,0
Biofysik	7	9	C	7,5
Cellebiologi	02	6	E	7,5
Matematik og databehandling	7		C	7,5
Bioteknologi	Bestået			7,5
Genetics 1	7	9	C	7,5
Genetics 2	4	7	D	7,5
Biokemi 1	4		D	7,5
Organismebiologi	10		B	15,0
Mikrobiologi	7		C	7,5
Tema: Eksperimentel molekylærbiologi	10		B	30,0
Valgfrie aktiviteter				
Organisk kemi og spektroskopi	10		B	7,5
Bioinformatik 1	4		D	7,5
Mikrobielle interaktioner	7		C	7,5


Sofie Christensen
Uddannelse og Studerende

Maria Nicoline Baandrup Kristiansen

Cpr.: 010787-2388

har gennemført bacheloruddannelsen i
Biologi - Bioteknologi
13. juli 2010




Oversigt over prøver og bedømmelser side 2 af 2

Følgende resultater er opnået	Resultat 7-trinsskala	Resultat 13-skala	Resultat ECTS-skala	ECTS point
Mammalian Genomics	7		C	7,5
Makromolekyler, cofaktorer og metalioner og deres kemi i biologiske systemer	Bestået			7,5
Bioorganisk kemi og lægemiddelkemi	7		C	7,5
Bachelorprojekt				
Bachelorprojekt	7		C	15,0
<i>The Minipig as a Model for Obesity</i>				

Bacheloruddannelsen i Biologi - Bioteknologi er normeret til 180 ECTS-point iht. Bekendtgørelse om bachelor- og kandidatuddannelser ved universiteterne.

Aktiviteter herover, der er angivet på engelsk, er gennemført på engelsk. Aktiviteter, der er angivet på øvrige sprog, er tilsvarende gennemført på disse sprog.


Sofie Christensen
Uddannelse og Studerende

12. 07. Generelt karakterniveau og -spredning

Maria Nicoline Baandrup Kristiansen

Cpr.: 010787-2388

har gennemført kandidatuddannelsen i
Biologi-bioteknologi
10. september 2013



Oversigt over prøver og bedømmelser side 1 af 2

Følgende resultater er opnået	Resultat 7-trinsskala	Resultat ECTS-skala	ECTS point
Speciale			
Investigation of the Extracellular Matrix Remodelling associated with Liver Fibrosis and Hepatocellular Carcinoma.....	10	B	60,0
Obligatoriske kurser			
Enzymology and Experimental Biochemistry	7	C	7,5
Heterologous Expression	7	C	15,0
Immunology - Theoretical.....	4	D	7,5
Advanced Biotechnology and Intellectual Property Rights	7	C	15,0
Lab Animal Science cat C.....	Bestået		7,5
Valgfrie aktiviteter			
RNA Biology	4	D	7,5

17. oktober 2013

Charlotte Louise Friis Rundsten
SCIENCE Uddannelse

Maria Nicoline Baandrup Kristiansen

Cpr.: 010787-2388

har gennemført kandidatuddannelsen i
Biologi-bioteknologi
10. september 2013

Oversigt over prøver og bedømmelser side 2 af 2

Kandidatuddannelsen i Biologi-bioteknologi er normeret til 120 ECTS-point iht. Bekendtgørelse om bachelor- og kandidatuddannelser ved universiteterne (uddannelsesbekendtgørelsen), som ændret ved bekendtgørelse nr. 814 af 29. juni 2010 med senere ændringer, nr. 338 af 06. maj 2004.

Aktiviteter herover, der er angivet på engelsk, er gennemført på engelsk. Aktiviteter, der er angivet på øvrige sprog, er tilsvarende gennemført på disse sprog.

Adgangsgrundlaget til kandidatuddannelsen

BSc inden for naturvidenskab eller tilsvarende

17. oktober 2013



Charlotte Louise Friis Rundsten
SCIENCE Uddannelse



MARIA NICOLINE
BAANDRUP KRISTIANSEN

cpr. 010787-2388

har den 10. september 2013
opnået
kandidatgraden i

*has on 10 September 2013
been awarded the degree of
Master of Science in*

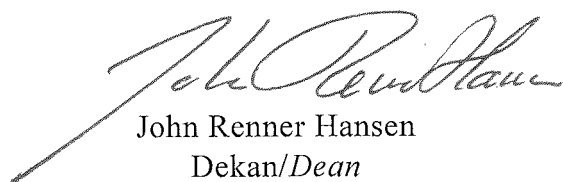
Biologi-bioteknologi


Biology - Biotechnology

og titlen

and the title

cand.scient.
candidata scientiarum


John Renner Hansen
Dekan/Dean


Karen Rønnow
Studiechef/Director of Studies

DET NATUR- OG BIOVIDENSKABELIGE FAKULTET
FACULTY OF SCIENCE

13. 08. Cv for virksomhedsvejleder

Curriculum vitae

Kristoffer Rigbolt

Experience	01/2014 - Present	Gubra	Hørsholm, Denmark
	<i>Bioinformatic Research Scientist</i>		
	02/2012 – 01/2014	Albert-Ludwigs-Universität	Freiburg, Germany
	<i>Postdoc</i>		
Education	07/2011 – 02/2012	University of Southern Denmark	Odense, Denmark
	<i>Postdoc</i>		
	04/2008 – 07/2011	University of Southern Denmark	Odense, Denmark
	<i>PhD, Department of Biochemistry and Molecular Biology</i>		
	09/2002-06/2008	University of Southern Denmark	Odense, Denmark
	<i>Master, Department of Biochemistry and Molecular Biology</i>		

Honors and awards	Sapere Aude – Young Elite Researcher, Danish Research Counsel
	Post doc scholarship – , Danish Research Counsel
	Novo Scholarship Grant, Novo Nordisk A/S

- | | |
|---------------------|--|
| Publications | <ul style="list-style-type: none">▪ Dalbøge LS, Pedersen SL, van Witteloostuijn SB, Rasmussen JE, Rigbolt KT, Jensen KJ, Holst B, Vrang N, Jelsing J. Synthesis and evaluation of novel lipidated neuromedin U analogs with increased stability and effects on food intake. <i>J Pept Sci.</i> 2015 Feb;21(2)▪ Chylek LA, Akimov V, Dengjel J, Rigbolt KT, Hu B, Hlavacek WS, Blagoev B. Phosphorylation site dynamics of early T-cell receptor signaling. <i>PLoS One.</i> 2014 Aug 22;9(8)▪ Akimov V, Henningsen J, Hallenborg P, Rigbolt KT, Jensen SS, Nielsen MM, Kratchmarova I, Blagoev B. StUbEx the global investigation of cellular ubiquitination. <i>J Proteome Res.</i> 2014 Sep 5;13(9)▪ Rigbolt KT, Zarei M, Sprenger A, Becker AC, Diedrich B, Huang X, Eiselein S, Kristensen AR, Gretzmeier C, Andersen JS, Zi Z, Dengjel J. Characterization of early autophagy signaling by quantitative phosphoproteomics. <i>Autophagy.</i> 2014 Feb;10(2)▪ Abeliovich H, Zarei M, Rigbolt KT, Youle RJ, Dengjel J. Involvement of mitochondrial dynamics in the segregation of mitochondrial matrix proteins during stationary phase mitophagy. <i>Nat Commun.</i> 2013;4▪ Francavilla C, Rigbolt KT, Emdal KB, Carraro G, Vernet E, Bekker-Jensen DB, Streicher W, Wikstrøm M, Sundstrøm M, Bellusci S, Cavallaro U, Blagoev B, Olsen JV. Functional proteomics defines the molecular switch underlying FGF receptor trafficking and cellular outputs. <i>Mol Cell.</i> 2013 Sep 26;51(6) |
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- Halbach S, Rigbolt KT, Wöhrle FU, Diedrich B, Gretzmeier C, Brummer T, Dengjel J. Alterations of Gab2 signalling complexes in imatinib and dasatinib treated chronic myeloid leukaemia cells. *Cell Commun Signal*. 2013 Apr 22;11(1)
 - Küttner V, Mack C, Rigbolt KT, Kern JS, Schilling O, Busch H, Bruckner-Tuderman L, Dengjel J. Global remodelling of cellular microenvironment due to loss of collagen VII. *Mol Syst Biol*. 2013 Apr 16;9
 - Müller PJ, Rigbolt KT, Paterok D, Piehler J, Vanselow J, Lasonder E, Andersen JS, Schaper F, Sobota RM. Protein tyrosine phosphatase SHP2/PTPN11 mistargeting as a consequence of SH2-domain point mutations associated with Noonan Syndrome and leukemia. *J Proteomics*. 2013 Jun 12;84
 - Rigbolt KT, Blagoev B. Quantitative phosphoproteomics to characterize signaling networks. *Semin Cell Dev Biol*. 2012 Oct;23(8)
 - Akimov V, Rigbolt KT, Nielsen MM, Blagoev B. Characterization of ubiquitination dependent dynamics in growth factor receptor signaling by quantitative proteomics. *Mol Biosyst*. 2011 Dec;7(12)
 - Rigbolt KT, Vanselow JT, Blagoev B. GProX, a user-friendly platform for bioinformatics analysis and visualization of quantitative proteomics data. *Mol Cell Proteomics*. 2011 Aug;10(8)
 - Rigbolt KT, Prokhorova TA, Akimov V, Henningsen J, Johansen PT, Kratchmarova I, Kassem M, Mann M, Olsen JV, Blagoev B. System-wide temporal characterization of the proteome and phosphoproteome of human embryonic stem cell differentiation. *Sci Signal*. 2011 Mar 15;4(164)
 - Broemer M, Tenev T, Rigbolt KT, Hempel S, Blagoev B, Silke J, Ditzel M, Meier P. Systematic in vivo RNAi analysis identifies IAPs as NEDD8-E3 ligases. *Mol Cell*. 2010 Dec 10;40(5)
 - Rigbolt KT, Blagoev B. Proteome-wide quantitation by SILAC. *Methods Mol Biol*. 2010;658
 - Henningsen J, Rigbolt KT, Blagoev B, Pedersen BK, Kratchmarova I. Dynamics of the skeletal muscle secretome during myoblast differentiation. *Mol Cell Proteomics*. 2010 Nov;9(11)
 - Bartoi T, Rigbolt KT, Du D, Køhr G, Blagoev B, Kornau HC. GABAB receptor constituents revealed by tandem affinity purification from transgenic mice. *J Biol Chem*. 2010 Jul 2;285(27)
 - Mortensen P, Gouw JW, Olsen JV, Ong SE, Rigbolt KT, Bunkenborg J, Cox J, Foster LJ, Heck AJ, Blagoev B, Andersen JS, Mann M. MSQuant, an open source platform for mass spectrometry-based quantitative proteomics. *J Proteome Res*. 2010 Jan;9(1)
 - Prokhorova TA, Rigbolt KT, Johansen PT, Henningsen J, Kratchmarova I, Kassem M, Blagoev B. Stable isotope labeling by amino acids in cell culture (SILAC) and quantitative comparison of the membrane proteomes of self-renewing and differentiating human embryonic stem cells. *Mol Cell Proteomics*. 2009 May;8(5)
 - Ditzel M, Broemer M, Tenev T, Bolduc C, Lee TV, Rigbolt KT, Elliott R, Zvelebil M, Blagoev B, Bergmann A, Meier P. Inactivation of effector caspases through nondegradative polyubiquitylation. *Mol Cell*. 2008 Nov 21;32(4)

14. 09. Cv for medvejleder ved virksomheden

Curriculum Vitae

Personal details

Name	Sanne Møller Knudsen
Address	Højeloft Vænge 162; 3500 Værløse
Civil status	married, two children
Telephone	44484988 (private); 31652276 (job)
E-mail	smk@gubra.dk
Date of birth	26 th September 1963

Current position

Director of Chemistry and Biology Research (January 2015 -)
Gubra ApS
Denmark

Previous employments

Director of Incretin & Islet Biology (April 2013 - January 2015)
Diabetes Biology
Novo Nordisk A/S, Måløv
Denmark

Head of Biology Departments (Aug 2007 – April 2013)
Diabetes Biology & Hagedorn Research Institute
Novo Nordisk A/S, Gentofte and Måløv

Research Scientist (March 2000 – Aug. 2007)
In vitro Biology Departments
Novo Nordisk A/S, Måløv

PostDoc (NeuroScience PharmaBiotec Centre) (Nov. 1997 – March 2000)
Clinical Biochemistry, Bispebjerg Hospital

Molecular biologist and PhD student in the Department of Clinical Biochemistry,
Bispebjerg Hospital (Feb. 1994 – June 1997)

- PhD thesis: Structural and functional analysis of the human VIP receptor by site-directed mutagenesis

Scientific assistant at the University of Aarhus (Sep. 1993 – Jan. 1994) – lectures and examination in biochemistry.

Patent applications

- WO2006003096-A1; EP1765814-A1; JP2008504345-W; ...
Title: [New condensed thiophene derivatives are non-peptide glucagon like peptide-1 agonists useful in the treatment of e.g. type 1 diabetes, type 2 diabetes, obesity, hypertriglyceridemia, syndrome X, and insulin resistance](#)
Assignee: NOVO NORDISK AS
Inventor(s): KNUDSEN S M, PETTERSSON I, LAU J, et. al
- **Title:** [Use of glucagon like peptide-1 agonist for the treatment of drug-induced obesity which is induced by administration of an antipsychotic or steroid](#)
Assignee: NOVO NORDISK AS
Inventor(s): BOCK C, KNUDSEN S M, RIMVALL K, et. al
- **Title:** [Identifying compounds capable of affecting G-protein coupled receptor activity, by contacting test compound with cell membrane from the receptor expressing arrestin and determining the receptor bound arrestin](#)
Assignee: NOVO NORDISK AS, KNUDSEN S M, POULSEN F, et. al
Inventor(s): KNUDSEN S M, POULSEN F, HEDING A

- **Title: Long-Acting Y2 and/or Y4 Receptor Agonists**
Assignee: Novo Nordisk AS
Inventor(s): ØSTERGAARD S, KNUDSEN S M, SPETZLER J, JØRGENSEN R

Publications after 2000:

- Sanne Møller Knudsen, Jeppe W. Tams and Jan Fahrenkrug. "Mutagenesis of the Second Extracellular Loop of the human Vasoactive Intestinal Polypeptide/Pituitary Adenylate Cyclase Activating Polypeptide 1 (VPAC₁) Receptor Identifies Residues Involved in Receptor Activation". J. Mol. Neuroscience 14, 137-146 (2000)
- Jeppe W. Tams, Sanne Møller Knudsen and Jan Fahrenkrug. "Characterization of a G protein coupling "YL" motif of the human VPAC1 receptor. Equivalent to the first two amino acids in the "DRY" motif of the Rhodopsin family". J. Mol. Neuroscience 17, 325-330 (2001)
- H. S. Nielsen, J. Hannibal, S. M. Knudsen and J. Fahrenkrug. "Pituitary adenylate cyclase activating polypeptide induces period1 and period2 gene expression in the rat suprachiasmatic nucleus during late night" Neuroscience 103, 433-441 (2001)
- Sanne Møller Knudsen, Jeppe W. Tams and Jan Fahrenkrug. "Functional roles of conserved transmembrane prolines in the human VPAC1 Receptor". FEBS Letters 503, 126-130 (2001)
- Jens Hannibal, Peter Hindersson, Sanne M. Knudsen, Birgitte Georg and Jan Fahrenkrug. "The photopigment melanopsin is exclusively present in pituitary adenylate cyclase-activating polypeptide-containing retinal ganglion cells of the retinohypothalamic tract". J. Neuroscience 22, 1-7 (2002)
- Florencio Zaragoza, Henrik Stephensen, Sanne M. Knudsen, Lone Pridahl, Birgitte S. Wulff, and Karin Rimwall. "1-Alkyl-4-acylpiperazines as a New Class of Imidazole-Free Histamine H3 Receptor Antagonists". J. Med. Chem. 47, 2833-2838 (2004)
- Marie Skovgaard, Janos T. Kodra, Dorte Xenia Gram, Sanne Møller Knudsen, Dennis Madsen and David A. Liberles. "Using Evolutionary Information and Ancestral Sequences to Understand the Sequence-Function Relationship in GLP-1 Agonists". J. Mol. Biol. 363, 977-988 (2006)
- Steffen Runge, Susan Schimmer, Jan Oschmann, Christine Bruun Schiødt, Sanne Møller Knudsen, Claus Bekker Jeppesen, Kjeld Madsen, Jesper Lau, Henning Thøgersen and Rainer Rudolph. "Differential structural properties of GLP-1 and Exendin-4 determine their relative affinity for the GLP-1 receptor N-terminal extracellular domain" Biochemistry (2007)
- Bosse-Doenecke E, Weininger U, Gopalswamy M, Balbach J, Knudsen SM, Rudolph R. High yield production of recombinant native and modified peptides exemplified by ligands for G-protein coupled receptors. Protein Expr Purif. Mar;58(1):114-21 (2008)
- Rye Underwood C, Møller Knudsen S, Schjellerup Wulff B, Bräuner-Osborne H, Lau J, Bjerre Knudsen L, H. Petersen G, Reedtz-Runge S. Transmembrane α -Helix 2 and 7 Are Important for Small Molecule-Mediated Activation of the GLP-1 Receptor. Pharmacology 88:340-348 (2011)
- Sarah Norklit Roed, Pernille Wismann, Christina Rye Underwood, Barbara Maurer, Nikolaj Kulahin, Helle Iversen, Karen Averad Cappelen, Trine Devantier, Jesper Mosolff Mathiesen, Hans Bräuner Osborne, Lauge Schäffer, Jennifer L. Whistler, Sanne Møller Knudsen, and Maria Waldhoer. Real-time trafficking and signaling of the Glucagon-like Peptide-1 Receptor. Molecular and cellular endocrinology 382, 938-949 (2014)
- Sarah Noerklit Roed, Anne Cathrine Nøhr, Pernille Wismann, Sanne Moeller Knudsen, and Maria Waldhoer "Functional Consequences of Glucagon-Like Peptide-1 Receptor Crosstalk and Trafficking". Journal of Biol Chem 290, 1233-1243 2015
- Jesper Lau, Paw Bloch, Lauge Schäffer, Ingrid Pettersson, Jane Spetzler, Jacob Kofoed, Kjeld Madsen, Lotte Bjerre Knudsen, James McGuire, Dorte Bjerre Steensgaard, Holger Martin Strauss, Dorte X. Gram, Sanne Møller Knudsen, Flemming Seier Nielsen, Peter Thygesen, Steffen Reedtz-Runge and Thomas Kruse. "The discovery of the once weekly glucagon like peptide-1 (GLP-1) analog semaglutide". Journal of Medicinal Chemistry Manuscript ID: jm-2015-00726n.R2

15. 10. Cv for universitetsvejleder

EDUCATION:

1995: MD from University of Copenhagen

2005: Board certification in surgical pathology, histopathology and cytopathology

CURRENT EMPLOYMENTS:

2008 - : Senior consultant (liver and urological pathology), Dept. of Pathology, Rigshospitalet.

2008 - : Director of Education, Dept. of Pathology, Rigshospitalet.

2013 - : Professor of pathology, Dept. of Pathology, Rigshospitalet, and Department of Biomedical Science, University of Copenhagen. Focus areas: digitalization of the diagnostic process and implementation of digital methods in pathology research and education.

As consultant at Dept. of Pathology at Rigshospitalet, which is the largest pathology department in Denmark, I have developed liver pathology, including transplantation pathology, into international standards, and have start up cancer research in the area of uropathology with close cooperation with leading cancer researchers in Copenhagen and internationally.

As Professor and Associate Professor at University of Copenhagen (since 2008), I have implemented digital methods in the teaching curriculum in the courses of pathology for medical students and students in odontology and human biomedicine, including virtual microscopy, lecture streaming, blogs, and remote education. Please visit www.virmik.sund.dk.

TEACHING APPOINTMENTS:

1997-2001 : Clinical teacher at Dept. of Internal Medicine, Glostrup Hospital

1997-2004 : Teaching assistant, medical science theory and ethics, Univ. of Copenhagen

2005- : Organiser of national postgraduate courses in liver and gastroenterological pathology in the pathology speciality training programme

2008-2013 : Associate Professor, Dept. of Biomedical Sciences, University of Copenhagen

2013- : Professor of pathology, Dept. of Biomedical Sciences, Univ. of Copenhagen, and Dept. of Pathology, Rigshospitalet

SCIENTIFIC APPOINTMENTS:

External reviewer at several scientific journals, including Scand J Gastroenterol, Clin Exp Immunol, Virchows Archiv, Inflamm Res, Histol Histopathol and J Urol.

- Chief editor at APMIS (the Scandinavian journal of pathology, microbiology and immunology)
- Member of the Advisory Board at Inflammation Research
- Member of the Advisory Board at World Journal of Hepatology
- Member of the Advisory Board at Visiopharm (image analysis company)

SCIENTIFIC SUPERVISION AND EVALUATION:

Presently, supervisor of 9 PhD students (4 as primary supervisor) and 2 pre-graduate students (topics: morphological and expressional characteristics of colorectal liver metastases; TMPRSS2/ERG fusion in prostate cancer; digital imaging and analysis of biomarker assessment, and implementation of digital methods in routine surgical pathology). Previous supervisor of 5 PhD and 17 pre-graduate students.

PUBLICATIONS ETC.:

- Gold medal, Univ. of Copenhagen, 1996, on a thesis on adhesion molecules in inflammatory bowel disease
- PhD, Univ. of Copenhagen, 2001, on a thesis on chemotactic effects of ICAM-1 on neutrophils in ulcerative colitis
- DMSc, Univ. of Copenhagen, 2010, on a doctoral thesis on morphological studies of ICAM-1 in ulcerative colitis
- Additional 104 peer-reviewed publications, 22 as first author, nine as senior author. Recent studies have focused on cancer in the gastrointestinal tract and in male genitals and application of digital image analysis.
- H-index: 22; citations: 1557.

- Berg KD, Brasso K, Thomsen FB, Røder MA, Rossing HH, Toft BG, Vainer B. ERG protein expression over time – from diagnostic biopsies to radical prostatectomy specimens in clinically localized prostate cancer. *J Clin Pathol* 2015; June 9. doi: 10.1136/jclinpath-2015-202894. (Epub ahead of print)
- Berg KD, Røder MA, Thomsen FB, Vainer B, Gerds TA, Brasso K, Iversen P. The predictive value of ERG protein expression for development of castration-resistant prostate cancer in hormone-naïve advanced prostate cancer treated with primary androgen deprivation therapy. *The Prostate* 2015; 75: 1499-1509
- Holten-Rossing H, Talman MLM, Kristensson M, Vainer B. Optimizing HER2 assessment in breast cancer – application of automated image analysis. *Breast Cancer Res Treat* 2015; 152: 367-75.
- Raft MB, Jørgensen EN, Vainer B. Gene mutations in hepatocellular adenomas. *Histopathology* 2015; 66: 910-21.
- Eefsen RL, Vermeulen PB, Christensen IJ, Laerum OD, Mogensen MB, Rolff HC, Van den Eynden GG, Høyer-Hansen G, Østerlind K, Vainer B, Illemann M. Growth pattern of colorectal liver metastasis as a marker of recurrence risk. *Clin Exp Metastasis* 2015; 32: 369-81.
- Nielsen MJ, Veidal SS, Karsdal M, Leeming DJ, Vainer B, Gardner SD, Hamatake R, Goodman ZD, Schuppan D, Patel K. Plasma Pro-C3 (N-terminal type III collagen propeptide) as marker of fibrosis progression in chronic hepatitis C. *Liver Int* 2015; 35: 429-37.
- Seidelin J, Larsen S, Linnemann D, Vainer B, Coskun M, Troelsen JT, Nielsen OH. Cellular inhibitor of apoptosis protein 2 controls human colonic epithelial restitution, migration, and Rac1 activation. *Am J Physiol* 2015; 308: G92-9.
- Perell K, Vincent M, Vainer B, Petersen BL, Federspiel B, Møller AK, Madsen M, Hansen NR, Friis-Hansen L, Nielsen FC, Daugaard G. Development and validation of a microRNA based diagnostic assay for primary tumor site classification of liver core biopsies. *Mol Oncol* 2015; 9: 68-77.
- Nielsen K, Rolff HC, Eefsen R, Vainer B. The morphological growth pattern of colorectal liver metastases are prognostic for overall survival. *Modern Pathology* 2014; 27: 1641-8.
- Nielsen K, Clemmesen O, Vassiliadis E, Vainer B. Liver collagen in cirrhosis correlates with portal hypertension and liver dysfunction. *APMIS* 2014; 122: 1213-22.
- Nygård SB, Christensen IJ, Smith DH, Nielsen SL, Jensen NF, Nielsen HJ, Vainer B, Brünner N. Underpinning the repurposing of anthracyclines towards colorectal cancer: assessment of Topoisomerase II alpha gene copy number alterations in colorectal cancer. *Scand J Gastroenterol* 2013; 48: 1436-43.
- Rømer MU, Nygård SB, Christensen IJ, Nielsen SL, Nielsen KV, Müller S, Smith DH, Vainer B, Nielsen HJ, Brünner N. Topoisomerase I (TOP1) gene copy number in stage III colorectal cancer patients and its relation to prognosis. *Molecular Oncology* 2013; 7: 101-11.
- Vassiliadis E, Olilveira CP, Alvares-da-Silva MR, Zhang C, Carrilho FJ, Stefano JT, Rabelo F, Nguyen QHT, Henriksen K, Veidal SS, Vainer B, Duffin KL, Leeming DJ, Christiansen C, Qvist P, Karsdal M. Evaluation of citrullinated and MMP-degraded vimentin as a liver fibrosis biomarker. *Am J Translat Med* 2012; 4: 403-14.
- Eefsen RL, van den Eynden GG, Høyer-Hansen G, Brodt P, Laerum OD, Vermeulen PB, Christensen IJ, Wettergren A, Federspiel B, Willemoe GL, Vainer B, Østerlind K, Illemann M. Histopathological growth pattern, proteolysis and angiogenesis in chemo-naïve patients resected for multiple colorectal liver metastases. *J Oncol* 2012; Article ID 907971.
- Leeming DJ, Nielsen MJ, Dai Y, Veidal SA, Vassiliadis E, Zhang C, He Y, Vainer B, Zheng Q, Karsdal MA. Enzyme-linked immunosorbent serum assay specific for the 7S domain of Collagen Type IV (P4NP 7S): A marker related to the extracellular matrix remodeling during liver fibrogenesis. *Hepatology* 2012; 42: 482-93.
- Madsen DH, Jørgensen HJ, Ingvarsen S, Melander M, Vainer B, Egerod KL, Hald A, Rønø b, Madsen CA, Bugge TH, Engelholm LH, Behrendt N. Endocytic collagen degradation: a novel mechanism involved in the protection against liver fibrosis. *J Pathol* 2012; 227: 94-105.
- Veidal SS, Karsdal MA, Nawrocki A, Larsen MR, Dai Y, Zheng Q, Hägglund P, Vainer B, Skjøt-Arkil H, Leeming DJ. Assessment of proteolytic degradation of the basement membrane: a fragment of type IV collagen as a biochemical marker for liver fibrosis. *Fibrogenesis & Tissue Repair* 2011; 4: 22.
- Veidal SS, Karsdal MA, Vassiliadis E, Nawrocki A, Larsen MR, Nguyen QHT, Hägglund P, Luo Y, Zheng Q, Vainer B, Leeming DJ. MMP mediated degradation of type VI collagen is highly associated with liver fibrosis – identification and validation of a novel biochemical marker assay. *PloS ONE* 2011; 6: e24753.
- Vassiliadis E, Veidal SS, Simonsen H, Larsen DV, Vainer B, Chen X, Zheng Q, Karsdal MA, Leeming DJ. Immunological detection of the type V collagen propeptide fragment, PVCP-1230, in connective tissue remodelling associated with liver fibrosis. *Biomarker* 2011; 16: 426-33.
- Thorsteinsdottir S, Gudjonsson T, Nielsen OH, Vainer B, Seidelin JB. Carcinogenesis in inflammatory bowel disease – pathogenesis and biomarkers. *Nature Rev Gas Hep* 2011; 8: 395-404.
- Veidal SS, Vassiliadis E, Barascuk N, Zhang C, Segovia-Silvestre T, Klickstein L, Larsen M, Qvist P, Christiansen C, Vainer B, Karsdal M. Matrix metalloproteinase-9-mediated type III collagen degradation as a novel serological biochemical marker for liver fibrogenesis. *Liver Int* 2010; 30: 1293-1304.
- Veidal SS, Vassiliadis E, Bay-Jensen A-C, Tougas G, Vainer B, Karsdal M. Procollagen type I N-terminal propeptide (PINP) is a marker for fibrogenesis in bile duct ligation-induced fibrosis in rats. *Fibrogen & Tissue Repair* 2010; 3: 5
- Veidal SS, Bay-Jensen A-C, Tougas G, Karsdal MA, Vainer B. Serum markers of liver fibrosis: combining the BIPED classification and the neo-epitope approach in the development of new biomarkers. *Disease Markers* 2010; 28: 15-28.

16. 13. Evt. supplerende bilag



Rigshospitalet

THE FINSEN LABORATORY

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Copenhagen Biocenter
Ole Maaløes Vej 5
DK-2200 Copenhagen N
Denmark

Lars H. Engelholm:
Telephone: +45 3545 6031
Cell phone: +45 3143 2077
E-mail: lhe@finsenlab.dk

Anbefaling cand.scient Maria N Baandrup Kristiansen

27. august 2015

Jeg har haft den store fornøjelse at have Maria tilknyttet i min forskningsgruppe siden september 2014. Hun har med stor succes har været primus motor på et samarbejde mellem min forskningsgruppe og det lille biotek firma CytoTrack. I min gruppe arbejder vi med en collagen receptor som er vigtig bl.a. i leverfibrose og cancer invasion. Maria blev ansat til at udvikle en ny biomarkør til detektion af cirkulerende tumorceller i blodprøver fra patienter med kræftsygdomme af mesenkymal oprindelse (sarkom). Maria opnåede meget hurtigt en serie vigtige fremskridt i projektet, og hun har vist sig som en særdeles kompetent og engageret molekylærbiolog med afgjort potentiale for eksperimentelt forskningsarbejde. Maria arbejder med stor selvstændighed, grundigt og effektivt, og hendes indsats bærer præg af både et stærkt teoretisk fundament og et naturligt talent for laboratoriearbejde af høj kvalitet.

Maria har ud over at drive projektet med at udvikle en biomarkør været et vigtigt aktiv i flere af min forskningsgruppes andre projekter. Vi har derfor draget stor nytte af hendes store viden om biomarkører og leversygdomme herunder leverfibrose og tekniske kunnen inden for dyremodeller af leverskader, som netop er et fokusområde i vores videnskabelige arbejde.

Marias kompetencer er derfor helt optimale til et PhD projekt, som omhandler udvikling af biomarkører i relation til NAFLD/NASH. Foruden at have det teoretiske grundlag på plads og at mestre hovedparten af de nødvendige teknikker, så besidder Maria et exceptionelt forskningstalant, Maria har således en sjælden evne til at tænke ud af boksen når det er påkrævet, en stor stædighed til at løse videnskabelige problemstillinger og hun er hurtig til at tilegne sig ny viden. Udover sin store faglighed, så har Maria et stort overskud til at hjælpe, og hun står altid klar med en hjælpende hånd både på arbejdspladsen og i sit frivillige arbejde hos "Børn, Unge og Sorg".

Jeg vil med glæde uddybe min anbefaling, hvis det skulle være nødvendigt.

Venlig hilsen

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17. 13. Evt. supplerende bilag

Appendix 5: Other Industrial PhD Projects



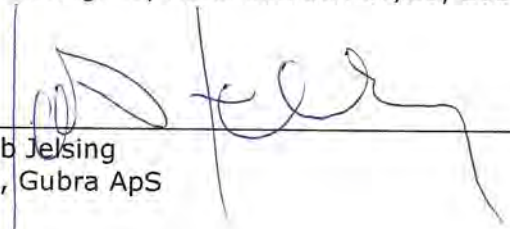
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Industrial-PhD projects at Gubra ApS

Currently ongoing PhD projects at Gubra ApS and their relation to the current project:

Sagsnummer	Begin	End	Title	Relation to current project
13-135470	2013-04-01	2016-04-01	Effects of obesity surgery on brain appetite centres: Searching for novel pharmacological strategies to combat obesity.	This project does not bear any direct connection with the current application.
1355-00066	2013-08-01	2016-10-07	Hydrophilic lipid conjugates for the modification of peptide therapeutics.	This project does not bear any direct connection with the current application.
4135-00073B	2015-02-01	2018-01-31	The intestinotrophic and anti-inflammatory effects of GLP-1 and GLP-2 in rodent models of inflammatory bowel disease.	This project does not bear any direct connection with the current application.
5016-00132B	2015-11-23	2018-11-23	Extending the systemic half-life of peptide drugs	This project does not bear any direct connection with the current application.
5016-00168B	2016-02-01	2019-02-01	Development of novel models reflecting human fatty liver disease and nonalcoholic steatohepatitis.	This project has indirect connection with the current application but is focused on development of novel animal models.

For vitterlighed, Hørsholm den 07/03, 2016



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18. 13. Evt. supplerende bilag



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07. Marts 2016

Gubra ApS blev stiftet i oktober 2008. Gubra er privatejet og leverer serviceydelser (konsulentbistand, farmakologi, histologi) til den farmaceutiske industri i ind- og udland indenfor fokusområderne fedme og type 2 diabetes og deraf ledte følgesygdomme. Gubra har haft vækst i antal medarbejdere og omsætning siden firmaets etablering i 2008.

Gubras omsætning (>50%) udgøres i stigende grad af langsigtede forskningsaftaler med big pharma således at driften i 2015, 2016 på nuværende tidspunkt næsten er sikret. Driftsoverskuddet fra Gubra anvendes dels til at udbygge Gubras infrastruktur, dels til at udvide Gubras serviceportefølje og dels til egen forskningsaktivitet.

Per 1. marts 2016 har Gubra ialt 65 ansatte. Gubra har pt 5 igangværende erhvervs-PhD projekter (to kandidater afsluttes 2016, én afsluttes i 2018 og 2 kandidat afslutter i 2019). Derudover har Gubra tidligere uddannet kandidater i henholdsvis 2014 og 2015.

Gubras øvrige personale (teknisk og videnskabeligt) personale anvender ca. 25% af deres tid på forskning.



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19. 13. Evt. supplerende bilag

Anbefaling

Maria Nicoline Baandrup Kristiansen har arbejdet med sin Master Thesis hos Nordic Bioscience 2012-2013, hvor jeg har haft den fornøjelse at være hendes vejleder.

Maria har, som en del af sin Master Thesis, arbejdet på at finde biomarkører til diagnosis og/eller prognosis af leverfibrose og hepatocellulær carcinoma. Dette arbejde har involveret udvikling af monoklonale antistoffer, screening af antistoffer og udviklingen af den færdige ELISA. Sideløbende har Maria implementeret en rotte model for hepatocellulær carcinoma for at teste den biologiske relevans af hendes ELISA. Endvidere har Maria arbejdet med et *in vitro* system, hvor hun benyttede LX-2 celler for at undersøge, hvilke pathways der er involveret i aktiveringen af hepatic stellate cells. Maria har i sin Master Thesis arbejdet meget selvstændig og giver ikke let op, men er god til at komme med konstruktive løsninger på svære problemstillinger.

I arbejdet med sin Master Thesis har Maria udvist stort potentiale som forsker ved nysgerrigt at kaste sig over leverfibrose feltet, deltaget aktivt i at formulere hypoteser og validere både velkendte og nye modeller. Hun har forholdt sig kritisk til data og har udvist talent for at formidle resultater på gruppemøder og til science club.

Udover arbejdet med sin Master Thesis, har Maria, på eget initiativ, været involveret i flere projekter i fibrosis-gruppen hos Nordic Bioscience. Som eksempel har hun stået for den histologiske evaluering af en rotte fibrose model, hvilket har resulteret i et medforfatterskab.

Jeg kan på baggrund af ovenstående give Maria min varmeste anbefaling.

Med venlig hilsen



Sanne Skovgård Veidal

PhD, Research Scientist

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