NOVARTIS

HOSPITAL GRANT AGREEMENT

This Grant Agreement ("**Agreement**") is entered into as of December, 2015 ("**Effective Date**") by and between Novartis Healthcare A/S, Reg. No. 20575786, a company incorporated under the laws of Denmark, located at Edvard Thomsens Vej 14, DK-2300 Copenhagen S, Denmark ("**Novartis**") and Dr. Peter Meldgaard and Professor Boe Sandal Soerensen, Oncology Department at University Hospital of Aarhus a company incorporated under the laws of Denmark, located at Norrebrogade 44 8000 Aarhus C Denmark, ("**Grant Recipient**"). Novartis and Grant Recipient may hereinafter be referred to individually as a "**Party**" and collectively as the "**Parties**".

WHEREAS, Grant Recipient has specifically requested Novartis' financial contribution in order to support the Grant Activity (as defined in Exhibit A), through a Grant Request Letter, which is attached hereto as Exhibit B;

WHEREAS, in accordance with the Grant Request Letter mentioned above, Novartis wishes to support the Grant Activity with the grant amount or grant in-kind ("Grant") (as defined in Exhibit A); and

WHEREAS, Grant Recipient accepts the Grant subject to the terms and conditions of this Agreement.

NOW THEREFORE, in consideration of the premises and the mutual covenants herein contained, it is mutually agreed as follows:

1. GRANT BY NOVARTIS

1.1 **Grant.** Novartis will provide the Grant as set forth in Exhibit A solely to support Grant Recipient in performing the Grant Activity as set forth in Exhibit A in accordance with agreed timelines if any..

1.2 **Statement of Purpose.** The Grant Activity is for scientific and/or educational purposes only and will not promote Novartis' products, directly or indirectly. The Grant is not being given in exchange for any explicit or implicit agreement to purchase, prescribe, recommend, influence or provide favorable formulary status for any of Novartis' products. The Grant is based upon a budget provided to Novartis by Grant Recipient reflecting a good faith estimate of the actual cost of the Grant Activity. The Grant has not been determined in a manner that takes into account the volume or value of referrals or business, if any, generated between Novartis and Grant Recipient or any of their respective officers, directors, employees, agents, affiliates, parents or subsidiaries.

1.3 **Novartis Responsibility.** Grant Recipient agrees that Novartis' responsibility is solely to provide the Grant. Novartis will not be liable to Grant Recipient or to any other person for the Grant Activity or the use of the Grant (including any claims or losses related thereto). Novartis may terminate this Agreement and require Grant Recipient to return the Grant and take other corrective action if Grant Recipient breaches this Agreement.

2. OBLIGATIONS OF GRANT RECIPIENT

2.1 Use of Grant.

(a) Grant Recipient verifies by signing this Agreement that the Grant Recipient is willing to receive the Grant.

(b) Grant Recipient has appointed Activity and Amount Manager(s) to manage the Grant and the Grant Activities as set forth in Exhibit A.

(c) Grant Recipient shall use the Grant solely for the Grant Activity and shall not use the Grant for any activity that is inconsistent with, or prohibited by any law, rule or regulation. The Grant Recipient undertakes to independently contact Novartis in the event any part of the Grant has not been used for the Grant Activity so that such amount can be refunded to Novartis without undue delay.

(d) Grant Recipient will comply with (and shall be solely responsible for any failure to comply with) all relevant laws, rules and regulations (including any code of practice or other guidelines generally followed by pharmaceutical companies in the relevant country) in connection with the Grant Activity. Grant Recipient warrants that the Grant Activity is compliant with all such requirements.

(e) Grant Recipient is solely responsible for the manner in which the Grant is disbursed, recorded and accounted and for all contractual and other relationships with third parties relating to the Grant Activity and the use of the Grant. Any claims for payment from third parties involved in the Grant Activity are the sole responsibility of Grant Recipient and Novartis will not fund any additional amounts for the Grant Activity.

2.2 Objectivity & Balance.

The Grant Activity will be independent, non-promotional and free from commercial influence or bias.

(a) If the Grant Activity involves the discussion of Novartis products, or the comparison of Novartis products with other products, that discussion and/or comparison must be objective, balanced, accurate, not misleading or deceptive and in compliance with all applicable laws, rules and regulations. Where appropriate, the Grant Activity will include a discussion of multiple treatment options, and will not focus on a single product.

(b) Grant Recipient will ensure that any titles or overview information relating to the Grant Activity will fairly and accurately represent the scope of the planned activity.

(c) If required, Grant Recipient is responsible for selection of presenters, moderators and collaborators for the Grant Activity. Novartis will not control the planning, content, speaker selection or execution of any Grant Activity. If Novartis suggests presenters, moderators or

collaborators, Grant Recipient will record the role of Novartis in making the suggestion, seek other sources and make a final selection based on balance and independence.

2.3 **Disclosure of Financial Relationships.**

(a) Grant Recipient will: (i) disclose, to all audiences and in all publications relating to the Grant Activity, that Novartis has provided a grant to support the Grant Activity; (ii) acknowledge support from Novartis in brochures, syllabi, and other materials related to the Grant Activity; and (iii) disclose any other relationships Novartis has with any individual speakers, moderators, collaborators or Grant Recipient which a reasonable and ethical person would expect to be disclosed.

(b) Novartis may disclose publicly the financial and non-financial support provided to Grant Recipient, including, without limitation, the Grant Recipient's identity, the Grant and purpose of the support.

2.4 **Ancillary Activities.**

(a) If the Grant Activity occurs as part of an overall activity that includes commercial activities, such activities will neither influence planning nor interfere with the Grant Activity. No commercial activities will be permitted in the same room as an educational activity, unless (i) this is allowed in the country in which the activity will take place and (ii) only to the extent that such commercial activity does not interfere with the purpose of the Grant Activity.

(b) The scheduling of meals and/or receptions, if any, in connection with any portion of the Grant Activity is at the sole discretion of Grant Recipient. Meals and/or receptions, if any, will be modest and conducive to the Grant Activity, and the amount of time at the meals or receptions will be clearly subordinate to the overall amount of time.

(c) Reconciliation of Expenses. At the conclusion of the Grant Activity, Grant Recipient will provide to Novartis a reconciliation of the actual expenses versus estimated expenses and will issue a refund to Novartis for any portion of the Grant not incurred in the implementation of the Grant Activity. In addition, Grant Recipient will retain appropriate records of the Grant Activity and the use of the Grant and will provide copies of the records to Novartis on request to confirm that the Grant has been used in accordance with this Agreement.

3. **GENERAL**

3.1 **Entire Agreement.** This Agreement, together with its Exhibits, sets forth the entire agreement and understanding of the Parties as to the subject matter hereof and supersedes all proposals, oral or written, and all other prior communications between the Parties with respect to such subject matter. In the event of any conflict between a substantive provision of this Agreement and any Exhibit hereto, the substantive provisions of this Agreement shall prevail.

3.2 **Governing Law and Jurisdiction.** This Agreement shall be governed by and construed under the laws of Denmark, without giving effect to the conflicts of laws provision thereof. Any dispute or claim arising out of or in connection with this Agreement which cannot be settled amicably between the Parties, is to

be brought before the Maritime and Commercial Court in Copenhagen or, if this court is not competent, before a competent court of law in the Kingdom of Denmark.

3.3 **Counterparts**. This Agreement may be executed in two or more counterparts, each of which shall be deemed an original, but all of which together shall constitute one and the same instrument.

IN WITNESS WHEREOF, the Parties intending to be bound have caused this Agreement to be executed by their duly authorized representatives.

NOVARTIS HEALTHCARE A/S

By: _____

Name: Erik Heegaard

Title: Medical Head Oncology

Date: 01-12-2015

By: _____

Name: Magnus Gisel

Title: Business Unit Head

Date: 1/12-2015

Aarhus University Hospital

By: _____

Name: Anni Ravnsbæk Jensen

Title: Head of department

Date: 1/12 2015

By: _____

Name: Peter Meldgaard

Title: MD, PhD

Date: Dec. 1st, 2015

By: _____

Name: _____

Title: _____

Date:

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EXHIBIT B

GRANT REQUEST LETTER INCLUDING BUDGET

Til Novartis,

From: Boe Sandahl Sørensen [mailto:boesoere@rm.dk]
Sent: 26. november 2015 13:55
To: Byberg, Lone
Cc: Peter Meldgaard
Subject: Ansøgning

Kære Lone Byberg,

Hermed fremsendes ansøgning om støtte til projekt. Det vil have stor betydning for projektet hvis det vil være muligt for jer at støtte os.

De bedste hilsner

Boe Sørensen og Peter Meldgaard

Professor Boe Sorensen, Ph.D.
Department of Clinical Biochemistry
University Hospital of Aarhus
Norrebrogade 44
8000 Aarhus C
Denmark

Detection of ALK translocations in blood samples from Non-Small Cell Lung Cancer patients

Associate professor Peter Meldgaard, Ph.D.
Department of Oncology
Aarhus University Hospital

Professor Boe Sorensen, Ph.D.
Department of Clinical Biochemistry
Aarhus University Hospital

NOVARTIS

Aarhus University Hospital
November 2015

Short introduction and aim

The methods used today for selection of patients with non-small cell lung cancer (NSCLC) for treatment with agents directed against ALK translocations are not optimal, and might exclude ALK translocated patients from treatment with these agents. To improve the selection of patients the present project aims at establishing and validating new methodology to detect ALK translocations. We will use next generation sequencing to identify all known and un-known translocation types involving the ALK gene. A major diagnostic problem in determining if lung cancer patients should be treated with ALK inhibitors is that the patients often have advanced disease and that different metastases are likely to differ with respect to their genetic rearrangements. Therefore, it can be problematic to select the tumor where the biopsy should be taken. A second problem is that the samples that can be isolated for ALK analysis often have degraded RNA and DNA due to fixation with formalin and storage in paraffin. A third problem is that the IHC and FISH based assays are highly dependent on the specificity and sensitivity of the antibodies and probes used. Recently a solution for these problems has been offered with the discovery of tumor RNA and DNA in blood samples and the use of deep sequencing to analyze it. This new technique allows us to identify the translocation specific for the individual patient.

The aim of this study is to investigate two methods for detection of ALK translocations. One uses circulating tumor RNA and the other tumor DNA as in-put for testing with next generation sequencing. Establishment and validation of these techniques will allow us, in future studies, to investigate if these new sequencing approaches of DNA and RNA in the blood samples identify additional ALK translocations, which have been mis-diagnosed using tissue. This will in turn enable us to demonstrate that the incidence of ALK translocations is higher than known today.

Background.

The EML4-ALK translocation was first identified in lung cancer by screening of a cDNA library from a NSCLC patient in 2007 (Soda et al., 2007). Shortly after it was generally recognized that additional EML4-ALK variants, than the ones observed by Soda et al., existed. Today approximately 27 different variants have been described and more will most likely be detected. These variants are both variations of the original EML4-ALK translocation and ALK translocations involving other fusion partners. TKI's that targets the ALK protein was tested in patients harboring an ALK translocation. The study was successful, and a six month progression free survival of 72 % was found (Kwak et al., 2010). Even though the area of ALK rearrangements has been greatly enlightened since its discovery in 2007, the diagnostic methods for detecting the translocation still depend on combinations of IHC and FISH testing. The IHC test is used to identify ALK overexpression and is followed by FISH investigations to confirm the translocations. Both IHC and FISH are labor intensive and require that adequate tumor

material is present in the biopsy taken. IHC and FISH are dependent on the sensitivity and specificity of the employed antibodies and probes. Furthermore the EML4-ALK translocation, which remains the most frequent, is caused by an intra-chromosomal inversion, which makes it hard to determine if a translocation has occurred when using FISH. There has been different attempts to develop a PCR based method for detection of the rearrangements, but the major problem here is the many different translocation variants known today, which requires that an individual assay is set up for each variant.
When taking tumor biopsies to identify mutations that could predict a specific cancer treatment two issues are to be discussed. First of all taking biopsies is an invasive procedure. Secondly, due to the fact that most NSCLC patients have metastatic disease, taking a biopsy from the primary tumor might not reflect the heterogeneity of the cancer. To fully enlighten the mutational status of the cancer a blood sample can be used instead. Blood plasma contains DNA and RNA from all tumors of the patient and represents an easily assessable and non-invasive source of tumor DNA and RNA for further analysis that reflects the heterogeneity of the cancer.

Project description

Establishment and validation of methods for detection of ALK translocations with NGS

We wish to investigate two different methods for detection of ALK translocations that use circulating tumor RNA and DNA, respectively.
To identify ALK translocated RNA, the Archer™ FusionPlex™ ALK, RET, ROS1 Panel in combination with NGS will be used. The general principle of the panel is to sequence from a known sequence (ALK, RET or ROS1) into an unknown sequence (fusion partner) by using Anchored Multiplex PCR technology. The panel detects the translocations and mutations shown in Table 1. RNA purified from plasma with the QIAamp Circulating Nucleic Acid Kit (Qiagen) will be converted to cDNA. Since the fusion partner is unknown, Molecular Barcode Adapters (Archerdx) are ligated to the ends of all cDNA fragments. These adapters contain primer sites for target amplification, Ion Torrent specific elements, and molecular barcodes for multiplex identification. The cDNA is purified and amplified by PCR. This is possible by using a primer specific for the added adapter and a primer specific for the portion of the ALK, RET or ROS1 transcript that is included in the fusion transcript. All PCR reactions are performed on LightCycler 480 instruments (Roche). This method will also amplify wild-type ALK, RET or ROS1 fragments and thus it is crucial to employ sequencing to detect whether the RNA in fact is translocated and in that case which variant is present. We will use deep NGS sequencing. The Archer™ FusionPlex™ ALK, RET, ROS1 Panel results in a prepared sample library and the Ion PGM™ Hi-Q™ Chef Kit will be employed to prepare templates and load them on Ion 316 Chips with an Ion Chef™ instrument. Sequencing will be performed on an Ion Torrent Personal Genome Machine™ instrument according to the manufacturer's protocol. All instruments are available in our lab. For each sample we will generate approximately 500,000 sequences of individual DNA or RNA molecules ensuring detection of even rare translocations.

Identification of ALK-translocated DNA will also be performed with a different method. Circulating DNA molecules will be purified from plasma as described above for RNA. Libraries for sequencing will be prepared with the Ion AmpliSeq™ Library Kit 2.0 (Thermo Fischer Scientific), which will ligate sequencing-specific adapters to both ends of the DNA fragments. Due to the fact that the breakpoint between ALK and its fusion partner can be present at multiple positions throughout the entire intron 19 of the ALK gene, it is impossible to use the same approach as with RNA. Thus, we will use an enrichment method to capture the DNA fragments originating from intron 19 in order to enhance the sensitivity of the sequencing. This will be done using approximately 20 oligos spanning intron 19, which have been designed and validated to hybridize solely to intron 19 of ALK DNA. The oligos will contain pentabases (PentaBase A/S), which will contribute to a higher sensitivity of the oligos. They will furthermore be biotinylated, which allow us to isolate oligo-bound DNA with Dynabeads® M-270 Streptavidin (Thermo Fischer Scientific). When the target DNA fragments have been isolated, we will be able to amplify them with primers complementary to the sequencing-specific adapters. Template preparation and sequencing will be performed as described for RNA above.

None of the methods have been properly validated in our laboratory yet. However, we have both patient plasma samples and an ALK-translocated NSCLC cell line (NCI-H2228 ATCC® CRL5935) to initiate the establishment and validation. We will firstly optimize the methods on 100 % DNA from the cell ALK translocated lung cancer cell line and afterwards test the sensitivity by performing spike-in measurements of the cell line DNA into DNA without ALK translocations. We estimate that the sensitivity will be <1 % enabling us to detect translocated ALK molecules in the blood sample although it only represents 1% of all the ALK DNA molecules in the blood.

We will validate our findings using droplet digital PCR (Biorad). This method allows precise quantification of known ALK translocations. We will use this technique to validate the results of both the RNA and DNA methods.

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Table 1: Archer™ FusionPlex™ ALK, RET, ROS1 Panel Targets

Gene	Accession	Targets	Direction	Assay Type
ALK	NM_004304	Exons 19, 20, 21, 22	5'	Fusion
ALK	NM_004304	C1156Y, G1202R, D1203N, S1206Y, F1174L, L1196M, G1269A	N/A	Mutation
RET	NM_020975	Exons 8, 9, 10, 11, 12, 13	5'	Fusion
RET	NM_020975	V804M/L	N/A	Mutation
ROS1	NM_002944	Exons 31, 32, 33, 34, 35, 36, 37	5'	Fusion

Time plan:

We expect the study to span no more than six months from start of funding.

Feasibility and economy

The costs of this project are related to blood analysis by NGS. We will use our own resources to validate the methods with droplet digital PCR and for salary for a molecular biologist to perform the experiments.

All the facilities and staff expertise needed for this project is available at the Department of Clinical Biochemistry at Aarhus University Hospital. The laboratory is experienced in using Next Generation Sequencing and droplet digital PCR. See budget for further details.

Budget

Price for DNA NGS per sample:

Sample preparation	406 kr.
Library preparation	961 kr.
Oligonucleotides	2300 kr.
Template preparation and NGS sequencing	4750 kr.
Total	**8417 kr.**

Price for RNA NGS per sample:

Sample preparation	706 kr.
Library preparation	1700 kr.
Template preparation and sequencing	4750 kr.
Total	**7156 kr.**

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Establishment of a method for detection of ALK translocations with NGS

Optimizing RNA method (4 samples tested with NGS x 7156 DKr.)	28.624 DKr.
Optimizing DNA method (4 samples tested with NGS x 8417 DKr.)	33.668 DKr.
Validating RNA method (6 samples tested with NGS x 7156 DKr.)	42.936 DKr.
Validating DNA method (6 samples tested with NGS x 8417 DKr.)	50.502 DKr.
Reruns (Estimated 30 %)	46.719 DKr.
Total	**202,449 DKr.**