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TITLE: **Clinical Validation of a miRNA Blood Test To Identify High-Risk Individuals Eligible for Low-Dose Computed Tomography Screening for Lung Cancer Early Detection**

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14. ABSTRACT The overall objective of this project is to validate whether a lung cancer diagnostic blood test that is based on the detection of a signature of 34 circulating miRNAs (miR-Test) can be used to identify individuals who require further investigation by LDCT screening. The project requires the use of serum samples obtained from at-risk subjects (heavy smokers with defined characteristics) undergoing LDCT screening. Such samples can be classified into two risk classes (Bach-high, and Bach-low), as determined by a risk model developed at IEO. Under separate funding, samples from Bach-high subjects are being analyzed using the miR-Test. The DoD-funded project aims to improve the clinical applicability of the miR-Test blood test by analyzing ~1000 serum samples of Bach-low individuals. A parallel study of 1000 individuals undergoing LDCT screening at the Mount Sinai School of Medicine (MSSM) in New York will provide validation of the miR-Test in an independent, non-Italian cohort of at-risk individuals to assess its applicability internationally. At 12 months, work is in the early phases of profiling the miRNA signatures of the collected serum samples.					
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Table of Contents

	<u>Page</u>
1. Introduction.....	4
2. Keywords.....	4
3. Accomplishments.....	5
4. Impact.....	7
5. Changes/Problems.....	8
6. Products.....	9
7. Participants & Other Collaborating Organizations.....	10
8. Special Reporting Requirements.....	11
9. Appendices.....	11

1. INTRODUCTION

The overall objective of this project is to validate a blood test based on the detection of a signature of 34 circulating miRNAs (miR-Test) developed at IEO as a first-line diagnostic screening tool for early lung cancer detection. The miR-Test can potentially be used to identify individuals who require further investigation by LDCT screening.

The project requires the use of serum samples obtained from at-risk subjects (heavy smokers with defined characteristics) that were collected for the COSMOS II lung cancer screening program coordinated by the European Institute of Oncology (10,000 samples planned to be collected). COSMOS II has received ethical clearance from the European Institute of Oncology (IEO) Ethics Committee.

The COSMOS II samples can be classified into two risk classes (called Bach-high and Bach-low), as determined by a risk model developed at IEO. COSMOS II was granted ethical clearance for the collection of the 10,000 blood samples from at-risk subjects, and for the analysis of the 34-miRNA miR-Test within the higher-risk subset of these individuals (Bach-high). The miRNA screening was not originally planned in the lower-risk individuals (Bach-low) arm, although serum samples were planned for collection in these individuals. The purpose of the DoD-funded project is to improve the clinical applicability of the miRNA blood test by analyzing ~1000 serum samples, collected within COSMOS II, of Bach-low individuals. Furthermore, the project plans to recruit ~1000 individuals undergoing LDCT screening at the Mount Sinai School of Medicine (MSSM) in New York. This will provide validation of our miR-Test in an independent, non-Italian cohort of high-risk individuals to assess its applicability internationally.

Finally, upon confirmation of the diagnostic accuracy of the miR-Test, this project aims to investigate whether our miRNA biomarkers can be used to refine existing lung cancer risk models, in order to select an optimal target population for LDCT lung cancer screening.

2. KEYWORDS

Lung Cancer
Early detection
Computed tomography
microRNA
serum

3. ACCOMPLISHMENTS

3.1 Major goals of the project

List (as stated in the approved SOW)

Major Task 1. Identification of 1000 ‘Bach-low’ participants from the COSMOS II trial (IEO)

Start date: Month 1
Target end date: Month 24
Completion end date: Month 0
Percentage completion: 100%

Major Task 2. Recruitment and radiological screening of the 1000 MSSM cohort

Start date: Month 1
Target end date: Month 24
Percentage completion: 0%

Major Task 3. Serum miRNA screening of 2000 subjects

Start date: Month 6
Target end date: Month 24
Percentage completion: 10%

Major Task 4. Evaluation of miR-Test performance

Start date: Month 24
Target end date: Month 30
Percentage completion: 0%

Major Task 5. Integration of the miR-Test and LDCT for the development of lung cancer early detection programs.

Start date: Month 30
Target end date: Month 36
Percentage completion: 0%

3.2 Achievements

Major Task 1. Identification of 1000 ‘Bach-low’ participants from the COSMOS II trial (IEO)

This task has been completed. Major Task 1 is part of an ongoing observational trial (COSMOS II) being conducted at IEO. Due the successful recruitment strategy of COSMOS II, this task was completed prior to the beginning of the project (3072 serum samples were collected at IEO. A total of 1926 (62.7%) were classified as being Bach-high and 1146 (37.3%) were classified as being Bach-low).

HRPO approval was obtained on 25th June 2015 for the miRNA analysis of samples classified as Bach-low, therefore we started with the processing of samples and analysis using the miR-test (see Major Task 3).

Major Task 2. Recruitment and radiological screening of the 1000 MSSM cohort

This Task has not yet commenced. The MT Sinai IRB cleared the study on 13th July 2015. Clearance from HRPO was obtained on 18th August, 2015. Subaward contract details have not yet been finalized between IEO and MSSM, therefore work remains pending.

Major Task 3. Serum miRNA screening of 2000 subjects

This task is in progress. HRPO approval was obtained for the miRNA analysis of COSMOSII Bach-low samples. In June 2015, after the HRPO clearance of study, we began processing the 1000 COSMOSII sample. So far ~600 samples have been processed for miRNA extraction, of these 119 have been profiled by TaqMan low density custom array. Work is currently on stand-by, pending continuing review by the IEO IRB (see section 5.2 below).

Major Task 4. Evaluation of miR-Test performance

This task has not yet started. The project is currently at Month 13. The start date of Major Task 4 was forecast at Month 24.

Major Task 5. Integration of the miR-Test and LDCT for the development of lung cancer early detection programs.

This task has not yet started. The project is currently at Month 13. The start date of Major Task 4 was forecast at Month 30.

Other Activities

None.

3.3 Opportunity for training and professional development

Nothing to report.

3.4 Disseminated results to communities of interest

Nothing to report.

3.5 Planning for the next reporting period to accomplish the goals

Work on Major Task 3 is currently on stand-by at IEO, pending continuing review by the IEO IRB (see section 5.2 below). The next meeting of the IEO IRB is planned for 25th November 2015, at which time we expect to obtain renewal of clearance. Considering the unexpected time required to obtain final HRPO clearance, we assigned maximum priority to this project in terms of machine time. Because of this, the processing rate at IEO for miRNA extraction and qRT-PCR profiling of serum samples is estimated at approximately 200/samples a month. With this throughput we expect that completion of the screening of COSMOSII subjects will require a further 4-5 months after renewal of clearance. Considering that all '*Bach-low*' subjects have already been identified and all serum samples were collected prior to the beginning of the project (Major Task 1), we nonetheless expect to complete this part of the Task 3 well within the time schedule it was assigned.

As described above, subaward contract details have not yet been finalized between IEO and MSSM. However, negotiations are at an advanced phase. Once the subaward contract details have been approved by both IEO and MSSM, our collaborators will begin collecting serum samples from their patients. We expect that we will have completed processing of the IEO cohort by the time we receive samples from MSSM, so we will be in a position to profile the samples we receive from MSSM (1000 subjects) without delay. Considering the high throughput of our miRNA-analysis pipeline that has been achieved as a result of prioritizing this project (serum processing and miRNA profile is fully automatized with Hamilton Robotics; the execution of miR-Test analysis is completely automatized using a R (GNU S) script) we expect that the processing and analysis of MSSM samples will require some 5-6 months.

4. IMPACT

4.1 Impact on the development of the principal discipline(s) of the project

Nothing to report.

4.2 Impact on other disciplines

Nothing to report.

4.3 Impact on technology transfer

Nothing to report.

4.4 Impact on society beyond science and technology

Nothing to report.

5. CHANGES PROBLEMS

5.1 Changes in approach and reasons for change

Nothing to report.

5.2 Actual or anticipated problems or delays and actions or plans to resolve them

Work on Major Task 3 began late as HRPO clearance for the project was granted only on 25th June 2015. The continuing review date was set by HRPO for 19th September 2015. IT was not possible for us to obtain IEO IRB clearance by this date, so work is currently on stand-by at IEO, pending continuing internal review. The next meeting of the IEO IRB is planned for 25th November 2015, at which time we hope to obtain renewal of clearance. As a consequence of these unplanned delays, we have assigned maximum priority to this project in terms of machine time. Therefore, this unexpected delay is not expected to have an impact on the overall timeline of the project, as described in Section 3.5 above.

Clearance from the HRPO for MSSM was obtained on 18th August, 2015. However, subaward contract details have not yet been finalized between IEO and MSSM (they are at an advanced phase). So far, the delay caused to the sample collection timeline of MSSM is estimated to be 10 months. As described in Section 3.5 above, we expect that we will have completed processing of the IEO cohort by the time we receive samples from MSSM, so we will be in a position to will then profile the samples we will receive from MSSM (1000 subjects) without delay. Consequently, we do not expect any delay that would impact the overall workflow of the project.

5.3 Changes that had a significant impact on expenditures

Nothing to report.

5.4 Significant changes in use or care of human subjects

Nothing to report.

5.5 Significant changes in use or care of vertebrate animals.

Not applicable.

5.6 Significant changes in use of biohazards and/or select agents

Not applicable.

6. PRODUCTS

6.1 Publications, conference papers, and presentations

Nothing to report.

6.2 Website(s) or other Internet site(s)

Nothing to report.

6.3 Technologies or techniques

Nothing to report.

6.4 Inventions, patent applications, and/or licenses

Nothing to report.

6.5 Other Products

Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

The following people have been principally involved in the project during the current period:

Name: Dr. Pier Paolo Di Fiore Project Role: Principal Investigator, IEO Effort to the nearest person year: <1 Contribution to project: Coordination of entire project (IEO).
Name: Dr. Fabrizio Bianchi Project Role: Co-investigator, IEO Effort to the nearest person year: <1 Contribution to project: Coordination and development of technical protocols required for the project (IEO).
Name: Dr. Francesca Montani Project Role: Post doctoral researcher, IEO Effort to the nearest person year: 1 Contribution to project: Processing of IEO serum samples (miRNA extraction and qRT-PCR profiling).
Name: Dr. Pascale Romano Project Role: Project manager, IEO Effort to the nearest person year: <1 Contribution to project: Project coordination and preparation of documentation for submission to HRPO (IEO)
Name: Dr. Giulia Veronesi Project Role: Co-investigator, PI of COSMOS II study, IEO Effort to the nearest person year: <1 Contribution to project: Preparation and submission of documents to the Ethics Committee (IEO)
Name: Dr. Claudia Henschke Project Role: Collaborator, MSSM Effort to the nearest person year: <1 Contribution to project: Preparation and submission of documents to the Ethics Committee (MSSM)
Name: Dr. Vivian Recoppa Project Role: Project manager, MSSM Effort to the nearest person year: <1 Contribution to project: Project management (MSSM)

7.1 Change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period

Nothing to report.

7.2 What other organizations were involved as partners

Nothing to report.

8. SPECIAL REPORTING REQUIREMENTS

No special reporting requirements are applicable.

9. APPENDICES

None.