OTKA K-129065 FINAL REPORT

1st year

As described in the Workplan of the ESLA-7 research project, we have collected and verified the formalin fixed paraffin embedded tissue blocks of surgically resected stage I lung adenocarcinomas suitable for further molecular genetic analysis.

2nd year

During the second year of the research project, we have performed the planned IHC stainings on the verified formalin fixed paraffin embedded tissue blocks of surgically resected stage I lung adenocarcinomas. Within the framework of a scientific collaboration with the internationally renowned Francis Crick Institute in London more than 1/4 of the tumor samples had already undergone genome testing planned for year 3, and the results of the WES *(whole exome sequencing)* have been used in part in our publication *(Biswas et al., Nature Medicine, 2019, „A clonal expression biomarker associates with lung cancer mortality")*. The remaining samples were prepared for WES. In cases where EGFR and/or KRAS mutation status analysis was not performed during routine patient care, EGFR and KRAS molecular analysis were performed during NGS (next generation sequencing). Moreover, these gene sequencing analyses also included the examination of the ESLA-7 gene pattern, so they could be used during the final evaluation in the final phase of the OTKA-supported project. Given that, in addition to the prognostic factor of ESLA-7, we also wanted to study the predictive factor for cytotoxic chemotherapy, resected surgical tumor samples for stage II lung adenocarcinoma were also included in the tumor samples to be examined.

3rd year

We, in collaboration with Prof. Charles Swanton from the Francis Crick Institute in London, previously published a method to prognosticate the clinical outcome of early-stage lung cancer *(Biswas et al., Nature Medicine, 2019, „A clonal expression biomarker associates with lung cancer mortality.")*. During the third year of the research project, we have performed the validation process that was necessary for widespread clinical introduction. Altogether, we provided surgically resected FFPE tumor material of 629 early-stage LUAD (lung adenocarcinoma) patients. We completed the NanoString measurements and analyzed data on 216 cases, for another 144 cases data generation was ongoing and the rest of the cases were in preparation for NanoString measurements. Analysis was completed on the first 216 cases and our measurements indicated that our proposed ORACLE method *(ORACLE = Outcome Risk Associated Clonal Lung Expression)* is a robust predictor of clinical outcome. The hazard ratio for overall survival was 1.8 by the ORACLE gene expression classifier.

4th year

During the fourth grant year, we continue our validation project with a special emphasis on predicting which early lung cancer patients would benefit from adjuvant chemotherapy.

The ultimate aim of this project was to produce a clinically accepted, CLIA *(Clinical Laboratory Improvement Amendments)* certified diagnostic test that is officially accepted by the relevant authorities such as EMA *(European Medicines Agency)*. In order to do this, we needed to migrate our RNAseq based invention to a methodology, NanoString, that is more amenable for everyday clinical practice. We performed this methodology transfer by the side-by-side comparison of RNAseq and NanoString based measurements on the same RNA samples extracted from 90 LUAD cases. This allowed us to convert he RNAseq based classifiers to NanoString based classifier that will in turn be applied to our clinical validation cohorts.

We have completed RNA extraction and NanoString based gene expression measurements for the entire validation cohort, encompassing more than 1000 FFPE lung adenocarcinoma tumor samples (including tumor materials from the National Korányi Institute of Pulmonology, Budapest, Hungary). The final analysis of the validation cohort was performed after the risk score normalization strategy (e.g. household gene selection, etc.) was finalized.

The final step of the analysis included combining the NanoString data with the survival data by sending ORACLE, RAS84 and our ESLA-7 estimates over to UCL Clinical Trials Centre for a blinded OS (overall survival) analysis. This included subgroup analyses such as chemotherapy versus non-chemotherapy treatment, stage I versus stage IIA etc..

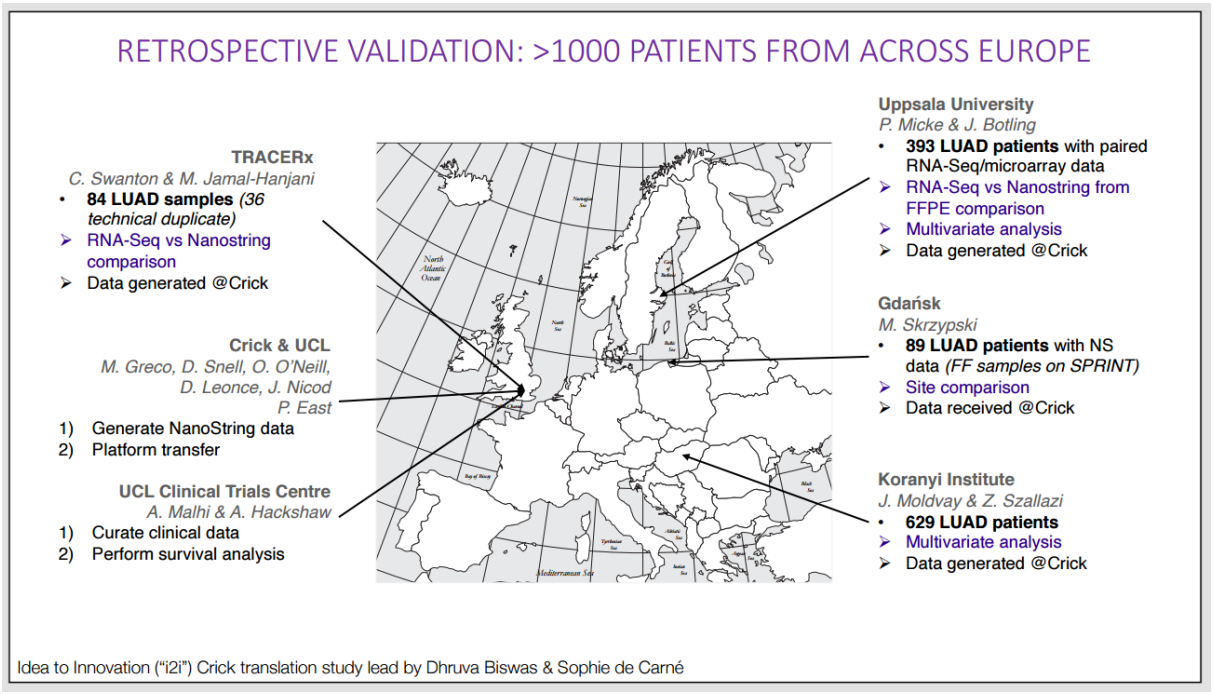
5th year

The main goal of our work is the most effective clinical management of early-stage lung cancer. In addition to the validation of the method described in our previous publication in Nature Medicine *(Biswas et al, 2019)* that will identify high risk versus low-risk early-stage lung cancer, we also started to explore diagnostic methods for 1) early detection of lung cancer using liquid biopsies and 2) biological characterization of lung cancer using plasma derived cfDNA epigenomics. We have completed the preliminary studies of this work and based on the promising results obtained we are currently applying for funding to perform a comprehensive validation of this diagnostic methods.

Summary of the OTKA K-129065 research project

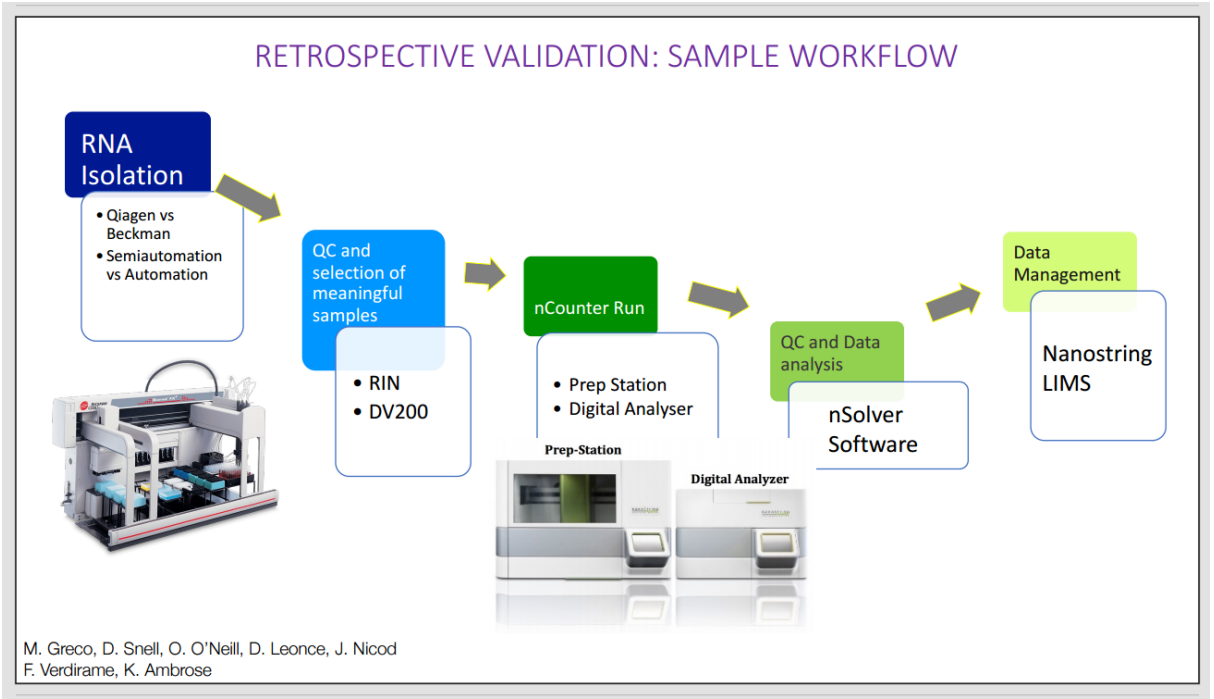
The determination of the prognostic and predictive value of the ESLA-7 (**E**arly **S**tage **L**ung **A**denocarcinoma-7) gene pattern in early-stage lung adenocarcinoma, which is the subject of the K-129065 research project, was part of an international collaboration led by Prof. Charles Swanton at the Crick Institute in London. He is also the leader of TRACERx *(TRAcking Cancer Evolution through therapy/Rx)* clinical study (https://www.crick.ac.uk/research/labs/charles-swanton/areas-of-interest/tracking-cancer-evolution -through-therapy/rx-tracerx-clinical-study).

During the five-year duration of the project molecular genetic analyses of the tumor tissue of more than 1,000 patients were performed including 629 LUAD (lung adenocarcinoma) patient samples from the National Korányi Institute of Pulmonology, Budapest, Hungary.

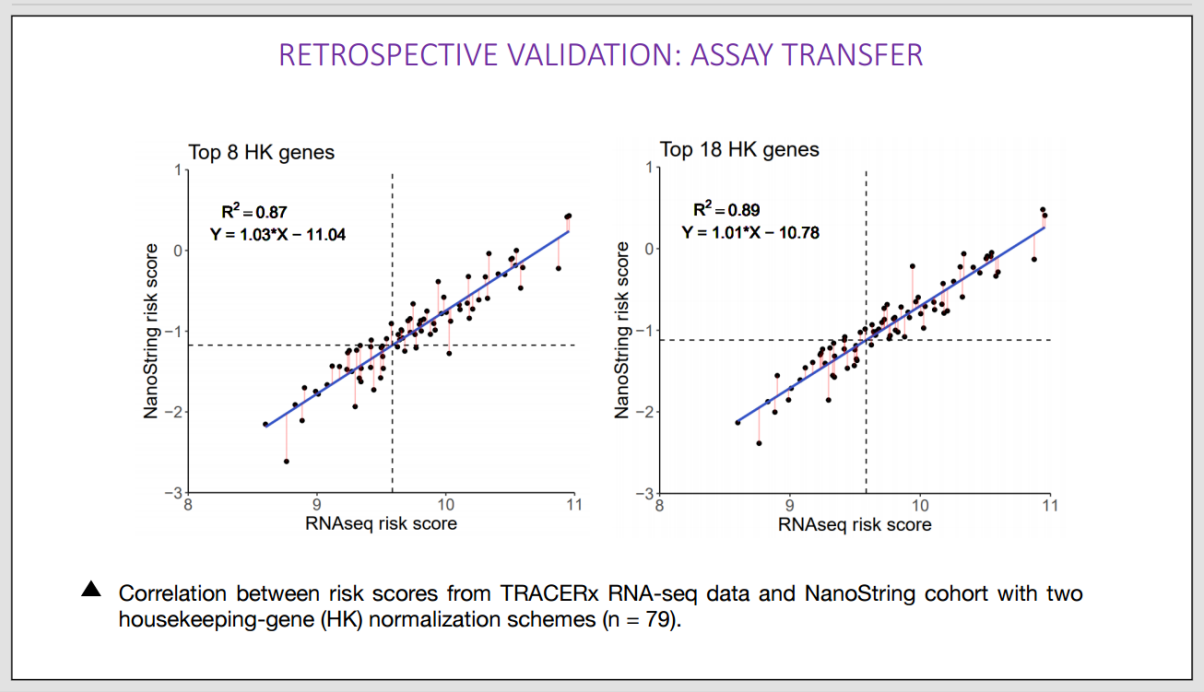


In the final study, cohorts from several centers from across Europe were combined to increase the statistical power of analysis. As shown in the figure above, the National Korányi Institute of Pulmonology provided the majority of the clinical validation samples.

The ultimate goal of this work is to produce a clinically applicable NanoString technology-based gene expression-based classifier to identify early-stage lung cancer cases with high and low risk of recurrence. Introduction to the everyday practice required several steps of quality controls as shown on the figure below. These technical details are essential for the development of an assay that conforms with the requirements of a CLIA certification level diagnostic test.



The original ESLA-7 and the ORACLE (**O**utcome **R**isk **A**ssociated **C**lonal **L**ung **E**xpression) gene expression signatures were derived from microarray and RNAseq data sets. Therefore, we needed to validate whether gene expression levels correlated between the high throughput technologies, such as RNAseq and the medium throughput technology (NanoString). As the figure below indicates, the gene expression signatures can be reliable transferred from the high throughput technologies to the NanoString platform.



▲ Correlation between risk scores from TRACERx RNA-seq data and NanoString cohort with two housekeeping-gene (HK) normalization schemes (n = 79).

Our data have been evaluated, and the results are summarized in our manuscript entitled “A clonal expression biomarker in patients with lung adenocarcinoma: a prospective observational cohort study" that has been accepted for publication in Nature Cancer on 15.11.2024. In this article Judit Moldvay and János Fillinger appear as co-authors [NATCANCER-A12684B]. In this work we investigated clonally expressed genes as a solution to the sampling bias problem by analyzing multi-region whole-exome and RNA sequencing data for 450 tumor regions from 184 patients with non-small cell lung cancer. We prospectively validated the survival association of a clonal expression biomarker, in combination with clinicopathological and molecular risk factors, and in stage I disease. We expanded mechanistic understanding of clonal transcriptional signals, discovering these are “hard-wired” prior to tissue invasion, can serve as a molecular fingerprint for the lethal metastatic clone, and significantly correlate with chromosomal instability. We evaluated the therapeutic relevance of clonally expressed genes, identifying a relationship with sensitivity to cytotoxic chemotherapy, and nominating novel markers for the targeting of cellular immunotherapies. Lastly, we developed clonally expressed genes as a general strategy to refine biomarker design across cancer types.

Besides, we have published the results of a total of 32 investigations on lung cancer – supported partly by the K-129065 grant – with a cumulative impact factor of 220.853 and independent citations of 433.

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IF: 5,5

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IF: 2,874

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IF: 2,874

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Összes idéző: 1, Független idézők: 0, Önidézet: 1, Nem vizsgált idézők: 0
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IF: 2,874

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IF: 6,603

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