



University of Groningen

Standardization and clinical implementation of liquid biopsy assays - IMI's CANCER-ID

Pantel, Klaus; Terstappen, Leon W. M. M.; manaresi, nicolo; Groen, Harry J. M.; Tamminga, Menno; Schuuring, Eduardus; Heitzer, Ellen; Speicher, Michael R.; Naume, Bjorn; kyte, jon amund

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Publication date: 2019

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

Pantel, K., Terstappen, L. W. M. M., manaresi, N., Groen, H. J. M., Tamminga, M., Schuuring, E., Heitzer, E., Speicher, M. R., Naume, B., kyte, J. A., & Schlange, T. (2019). *Standardization and clinical implementation of liquid biopsy assays - IMI's CANCER-ID*. Poster session presented at AACR annual meeting 2019, Atlanta, United States.

Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: https://www.rug.nl/library/open-access/self-archiving-pure/taverneamendment.

Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): http://www.rug.nl/research/portal. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

3190

Standardization and clinical implementation of liquid biopsy assays - IMI's CANCER-ID



¹University Medical Center Hamburg/Eppendorf, Center for Experimental Medicine, Institute of Tumor Biology, Hamburg, Germany; ²Faculty of Science and Technology, Medical Cell BioPhysics, University of Twente, Enschede, Netherlands; ³Menarini/Silicon Biosystems, Bologna, Italy; ⁴Department of Pulmonary Diseases, University Medical Center Groningen, The Netherlands; ⁵Department of Pathology, University Medical Center Groningen, and University Medical Center Groningen, The Netherlands; ⁵Department of Pathology, University Medical Center Groningen, The Netherlands; ⁵Department of Pathology, University Medical Center Groningen, and University Medical Center Groningen, The Netherlands; ⁵Department of Pathology, University Medical Center Groningen, The Netherlands; ⁵Department of Pathology, University Medical Center Groningen, The Netherlands; ⁵Department of Pathology, University Medical Center Groningen, The Netherlands; ⁵Department of Pathology, University Medical Center Groningen, The Netherlands; ⁵Department of Pathology, University Medical Center Groningen, The Netherlands; ⁵Department of Pathology, University Medical Center Groningen, The Netherlands; ⁵Department of Pathology, University Medical Center Groningen, The Netherlands; ⁵Department of Pathology, University Medical Center Groningen, The Netherlands; ⁵Department of Pathology, University Medical Center Groningen, The Netherlands; ⁵Department of Pathology, University Medical Center Groningen, The Netherlands; ⁵Department of Pathology, University Medical Center Groningen, The Netherlands; ⁵Department of Pathology, University Medical Center Groningen, The Netherlands; ⁵Department of Pathology, University Medical Center Groningen, The Netherlands; ⁵Department of Pathology, University Medical Center Groningen, The Netherlands; ⁵Department of Pathology, University Medical Center Groningen, The Netherlands; ⁵Department of Pathology, University Medical Center Groningen, The Netherlands; ⁵Department of Pathology, University Medical Center Groningen, The Netherlands; ⁵Department of Pathology, University Medical Center Groningen, The Netherlands; ⁵Department of Pathology, University Medical Center Groningen, The Netherlands; ⁵Department of Path Groningen, The Netherlands; ⁶Institute of Human Genetics, Medical University of Graz, Austria; ⁷University of Graz, Austria; ⁸Bayer AG, Pharmaceuticals, Wuppertal, Germany

INTRODUCTION

- CANCER-ID (www.cancer-id.eu) is a five-year (2015-2019) international public-private partnership project supported by Europe's Innovative Medicines Initiative (IMI). The consortium of currently 40 partners from 14 countries (Fig. 1) aims at the establishment of harmonized best practice protocols for patient sample collection, pre-analytical sample handling, sample and bioinformatic analyses, and actionable information guiding patient selection for personalized treatment.
- CANCER-ID tests and supports the development of standards for liquid biopsy as well as clinical implementation of liquid biopsy-based protocols in the clinical setting. This includes interaction with regulatory bodies, such as EMA's (European Medicines Agency) Innovation Task Force (ITF) and CDER/FDA's (U.S. Center for Drug Evaluation and Research/Food and Drug Administration) Critical Path Innovation Meeting (CPIM), to support future approval of liquid biopsies in multi-centered worldwide clinical studies.
- At the core of CANCER-ID's activities in the liquid biopsy field is the evaluation of technologies for circulating tumor cell (CTC), circulating tumor DNA (ctDNA), microRNA (miRNA) and exosome enrichment, isolation and analysis.
- Liquid biopsy protocols are being implemented in an observational study evaluating the utility of analyzing PD-L1 (programmed death-ligand 1) expression on CTCs in non-small cell lung cancer (NSCLC) and metastatic breast cancer. To this end, the potential predictive value of monitoring treatment response towards immune checkpoint inhibition (ICI) is assessed in advanced NSCLC patients at the University Medical Center Groningen (UMCG) as well as in two ICI-chemotherapy combination studies in triple-negative breast cancer and luminal B breast cancer, respectively, run by the University of Oslo (ALICE, ClinicalTrials.gov ID: NCT03164993 and ICON, ClinicalTrials.gov ID: NCT03409198).
- The aim is to assess whether the allelic frequency of mutations as a potential measure for tumor mutational burden (TMB) or the number of PD-L1-positive/overall CTCs at different time points is indicative of treatment success.
- As a follow-up activity of the CANCER-ID program, the European Liquid Biopsy Society (ELBS) is currently being established. The ELBS will be open to all interested liquid biopsy stakeholders worldwide as a platform for scientific exchange.

The CANCER-ID consortium

- The CANCER-ID consortium is funded by IMI (**Fig. 1**). This public-private partnership between the EU commission and the European Federation of Pharmaceutical Industries and Associations (EFPIA) provides a legal framework for addressing unmet challenges in the healthcare sector.
- In 2015, academic and clinical research groups, public research organizations, small and medium-sized enterprises (SME), and pharmaceutical and diagnostic corporations joined forces to evaluate technologies and establish analytical standards in the liquid biopsy field.
- The academic leaders of CANCER-ID, Professor Klaus Pantel (UKE, Germany), who has published >300 reports and high-impact review articles on disseminating tumor cells, and Professor Leon Terstappen (Universiteit Twente, The Netherlands), developer of the FDA-approved benchmark CELLSEARCH[®] CTC detection system, are pioneers in the field of blood-based cancer biomarkers.



Figure 1. The CANCER-ID consortium, funded by IMI.

Klaus Pantel¹, Leon Terstappen², Nicolò Manaresi³, Harry Groen⁴, Ed Schuuring⁵, Ellen Heitzer⁶, Michael Speicher⁶, Bjørn Naume⁷, Jon Amund Kyte⁷ and Thomas Schlange⁸ for the IMI CANCER-ID consortium

Standardization of liquid biopsy technologies

- The use of diverse input sample types (e.g. different blood fixatives, extraction protocols or analysis and substantially technologies) different user-developed protocols for blood-based analytes like CTCs, ctDNA or miRNA hampers the comparability of results (**Fig. 2**).
- Hence, there is a need to standardize liquid biopsy technologies. The multicenter ring trials for the evaluation of CTC, ctDNA and miRNA technologies include the analysis of standard materials (e.g. well-defined NSCLC spike-in controls, ctDNA reference material) by multiple CANCER-ID partners following consensus protocol or workflow to comparability of ensure the results [1, 2].

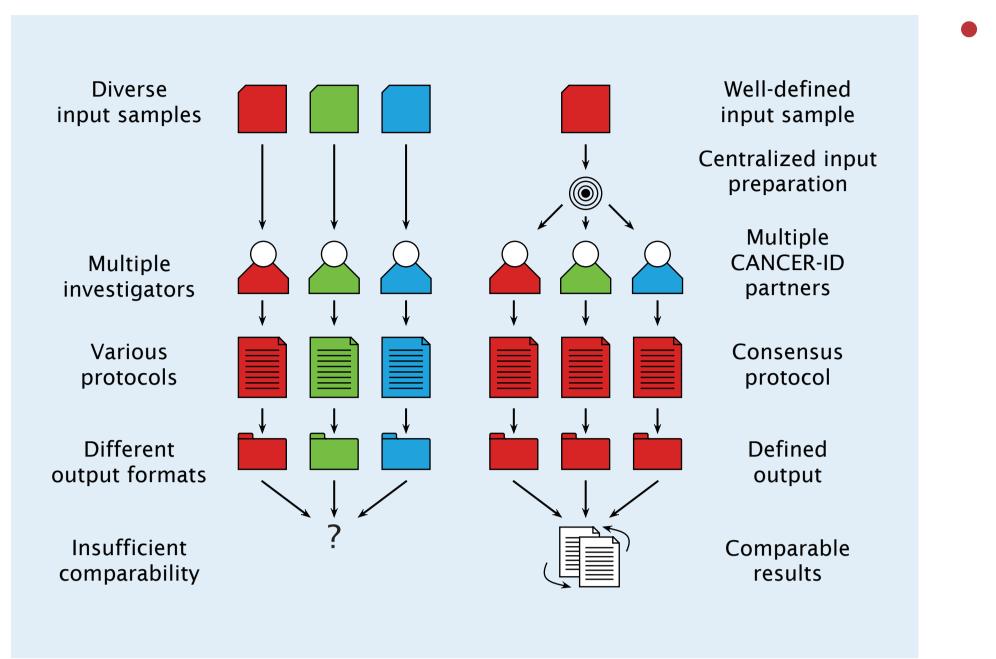
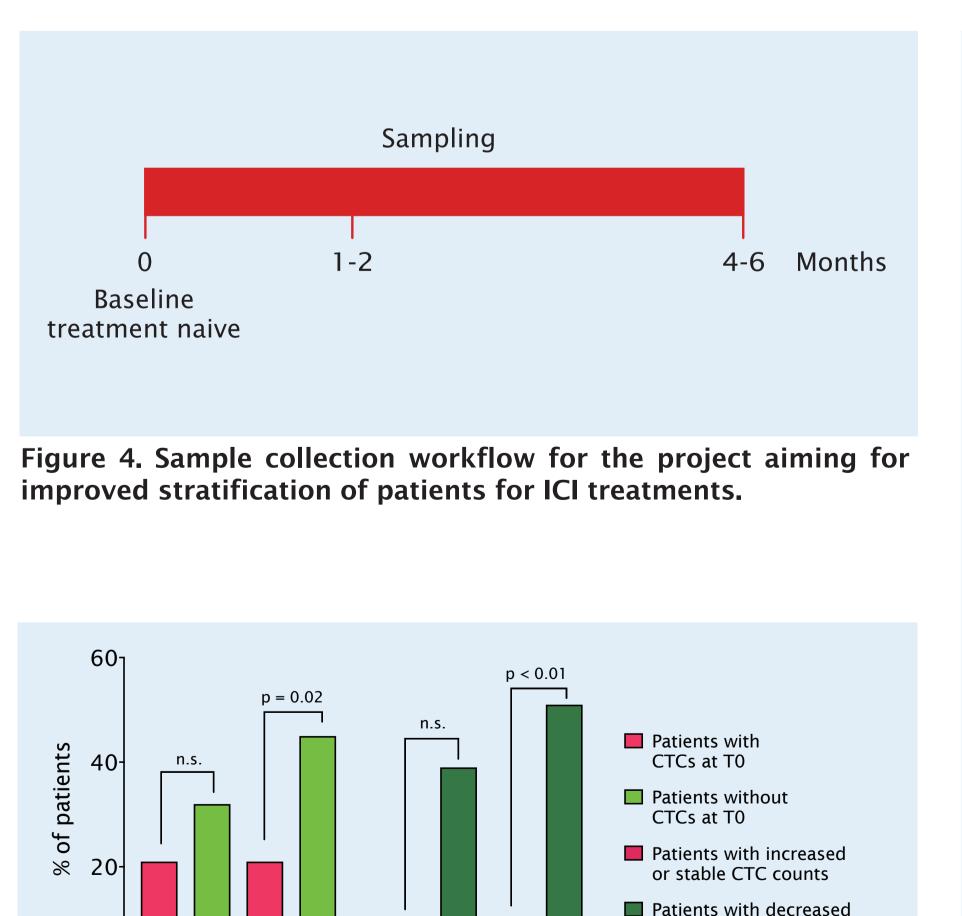


Figure 2. Comparison of workflows using either diverse input samples and protocols or well-defined input samples and

Improved patient selection for immune checkpoint inhibition (ICI) treatments

- The CANCER-ID partners are currently jointly analyzing samples from advanced NSCLC and • In this study, CTC presence at baseline and CTC change after therapy was used as a metastatic breast cancer patients who are participating in clinical studies at clinical partner stratification tool, and the percentage of early responders (partial and complete response sites, or are under ICI treatment at clinics (Table 1 and Table 2). The samples will be according to RECIST 1.1) and durable responders (stable disease, partial response and collected at baseline and 1-2 and 4-6 months after treatment initiation (**Fig. 4**). complete response according to RECIST 1.1 without progression in 6 months) to ICIs was determined.
- The aim of this study is to investigate whether CTC counts or mutational analysis of ctDNA using next-generation sequencing (NGS) panels combined with droplet digital PCR (ddPCR) Early response rates were not significantly different (T0: odds ratio, OR=0.67, p=0.56; Δ CTC OR=0.13, p=0.08), whereas, the durable response rate was significantly decreased in patients can be used for the selection of patients who may benefit from PD-1/PD-L1 inhibition and identify early signs of efficacy or relapse. with CTCs (T0: OR=0.28, p=0.02; Δ CTC OR=0.04, p<0.01) (Fig. 5).
- Preliminary data show that a decline in the ctDNA mutation variant allele frequency (VAF) This approach is supported by the results obtained from NSCLC patients treated with checkpoint inhibitors, a study performed at UMCG (**Table 1**). predicts progression-free survival (PFS) and overall survival (OS) (Fig. 6).



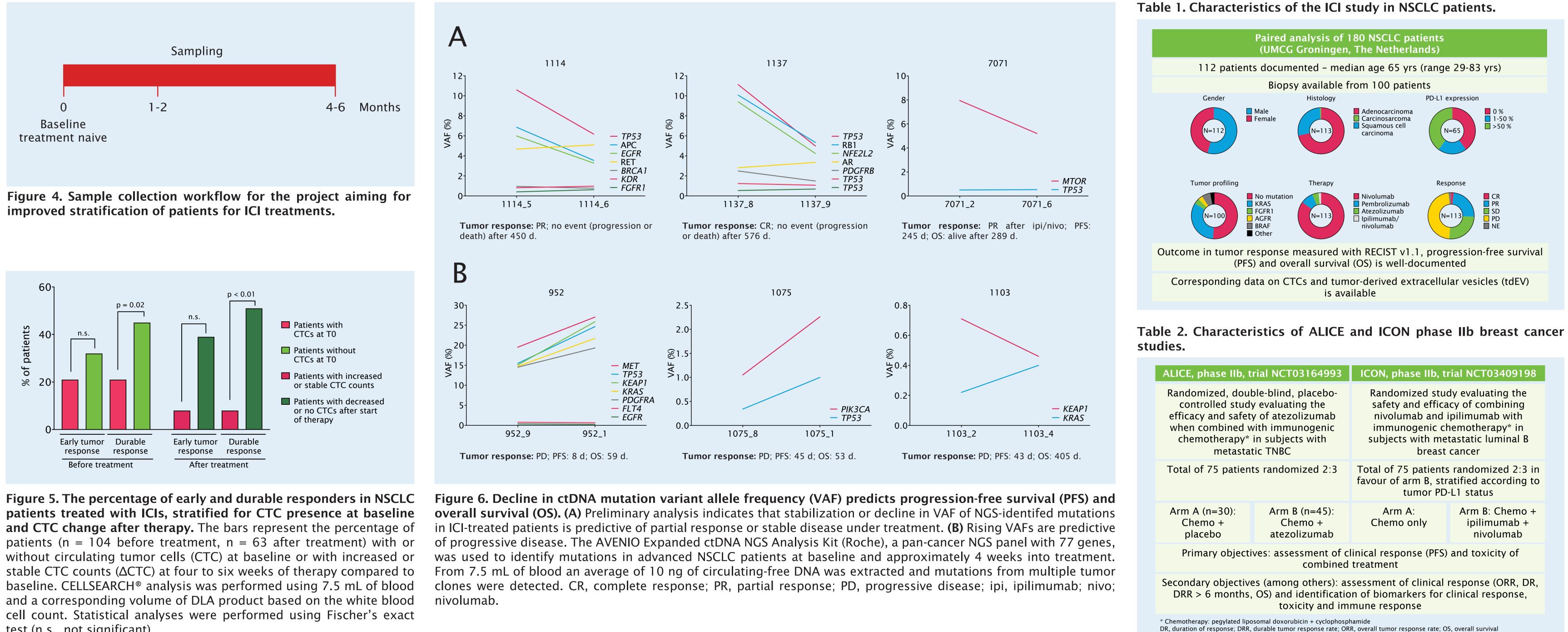
and a corresponding volume of DLA product based on the white blood nivolumab. cell count. Statistical analyses were performed using Fischer's exact test (n.s., not significant).

After treatment

Before treatment

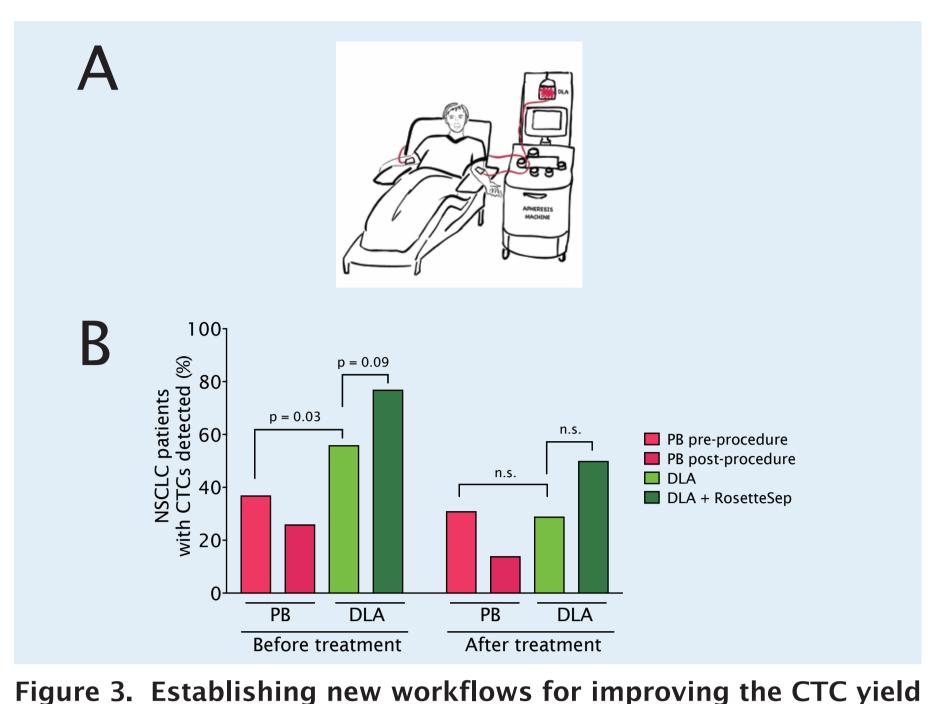
or no CTCs after start

of therapy



Efforts to improve the CTC yield in NSCLC patients

 To improve the CTC yield in NSCLC new workflows were patients, Diagnostic established using Leukapheresis (DLA) [3, 4] (Fig. 3). CTC detection frequency was significantly increased (p=0.03, Friedman's 2-way ANOVA by rank) when measured in a DLA product (~ 2 x 10⁸ cells, 1-3 mL of DLA product) compared to peripheral blood (15 mL) prior to ICI treatment. There was a trend towards further increase in the CTC detection frequency in DLA product (18 mL) when an immunodensity-based cell enrichment (RosetteSep[™], Stemcell Technologies) procedure (p=0.09) was used. After treatment, no differences were observed in the percentage of patients with CTCs detected in peripheral blood or DLA product. The cell enrichment by RosetteSep[™] again increased the yield o[™] CTCs in DLA product (p=0.007, Friedman's 2-way ANOVA by rank).



in NSCLC patients using Diagnostic Leukapheresis (DLA). (A) Schematic drawing of DLA. (B) The percentage of patients with CTCs detected in peripheral blood (PB) prior to and after DLA procedure and in DLA product with and without RosetteSep[™] enrichment. The percentage of NSCLC patients with CTCs was assessed in peripheral and DLA products before and after treatment with blood standard-of-care chemotherapy, tyrosine kinase inhibitors or ICI using CELLSEARCH[®]. Statistical analyses were performed using Fischer's exact test (n.s., not significant).



The European Liquid Biopsy Society

- IMI's CANCER-ID project ends in December 2019. The requirement for continued data and sample storage, further updating of best practice documents and standard operating procedures (SOPs) and scientific support of liquid biopsy proficiency testing have led to plans for sustained activity in the field by academic and industrial partners.
- The University Medical Center Hamburg-Eppendorf (UKE, Hamburg) is currently establishing the "European Liquid Biopsy Society" (ELBS) (Fig. 7) with the following goals
 - Foster the introduction of liquid biopsy into clinical practice.
 - Encourage interactions between academia and industry as well as other related initiatives (e.g. The US-based Blood Profiling Atlas in Cancer, BloodPAC; FNIH).
 - Provide a partner for regulatory agencies, healthcare providers and patient advocacy groups.
 - Support the implementation of liquid biopsy tests into clinical trials.
 - Develop guidelines and provide training in liquid biopsy for medical scientists.
 - Disseminate knowledge about liquid biopsies to the medical community through regular symposia, publications and press releases.
- International stakeholders worldwide are cordially invited to join the ELBS and support the advancement of liquid biopsy in cancer research and therapy. A kick-off meeting is being planned for May 3rd 2019 at UKE in Hamburg, Germany. For additional information please contact Prof. Klaus Pantel (pantel@uke.de).

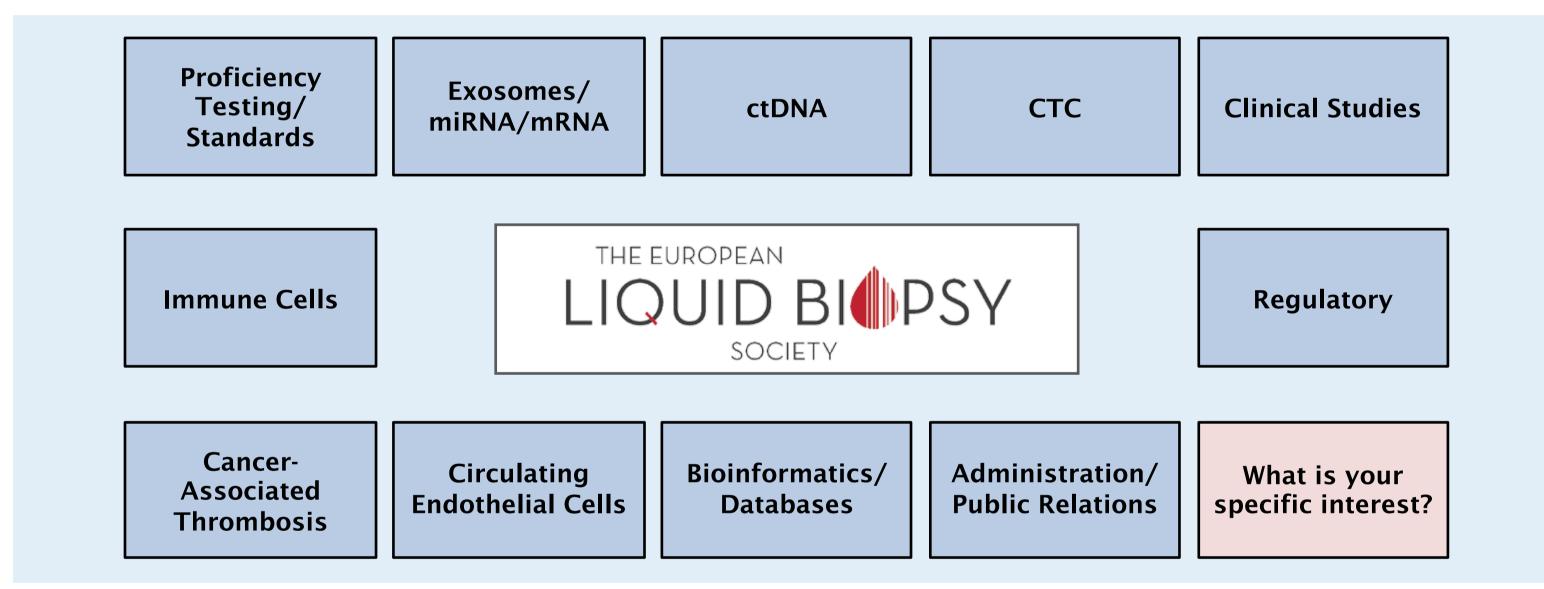


Figure 7. Scope of the European Liquid Biopsy Society (ELBS).

ACKNOWLEDGMENTS

CANCER-ID is supported by the Innovative Medicines Initiative (IMI) Joint Undertaking under Grant Agreement n° 115749, resources of which are composed of financial contribution from the European Union's Seventh Framework Programme (FP7/2007-2013) and EFPIA companies' in-kind contributions

Samples from patients were collected under signed informed consent.

Aurexel Life Sciences Ltd. (www.aurexel.com) is thanked for editorial assistance in the preparation of this poster, funded by Bayer AG.

REFERENCES

1. Lampignano et al. Dynamic changes of tumor-derived extracellular vesicles and related RNAs in blood samples of NSCLC patients. Session Category: Clinical Research; Session Title: Novel Strategies for Biomarker Identification and Use in Cancer 2; Session Date and Time: Tuesday Apr 2, 2019 1:00 PM - 5:00 PM; Location: Exhibit Hall B; Abstract Number: 3985.

2. Brudzewsky et al. Multicenter evaluation of circulating tumor DNA assays. Session Category: Clinical Research; Session Title: Current Developments in Non-invasive Biomarkers for Assessment of Cancer 1; Session Date and Time: Sunday Mar 31, 2019 1:00 PM - 5:00 PM; Location: Exhibit Hall B, Poster Section 18; Permanent Abstract Number: 438.

3. Fischer JC et al. Diagnostic leukapheresis enables reliable detection of circulating tumor cells of nonmetastatic cancer patients. *Proc Natl Acad Sci U S A.* 2013;110(41).

4. Fehm TN et al. Diagnostic leukapheresis for CTC analysis in breast cancer patients: CTC frequency, clinical experiences and recommendations for standardized reporting. *Cytometry A.* 2018;93(12).



Poster presented at the AACR Annual Meeting, March 29 - April 3, 2019, Atlanta, GA, USA