Publishable Summary for 22HLT06 GenomeMET
Metrology for genomic profiling to support early cancer detection and precision medicine

Overview

Cancer is a major burden on European society. Advances in genomics, driven by technologies such as Next Generation Sequencing (NGS) are transforming cancer care, enabling earlier and more accurate diagnosis, guiding therapy selection and driving development of targeted therapies (precision medicine), which improves patient outcomes and health system effectiveness. However, the quality and comparability of genomic profiling currently varies significantly and development of standards and metrological means to support the field are in their infancy. This project aims to address these needs by applying metrological principles to develop reference measurement systems (RMS) to support cancer genomic diagnostics in compliance with the In-vitro Diagnostic Device Regulation (IVDR EU 2017/746).

Need

Cancer is one of the most significant challenges for European societies and healthcare systems, being the second largest cause of death with more than 1.9 million deaths per year. Horizon Europe's Mission on Cancer has identified earlier diagnosis and implementation of precision medicine as key priorities for reducing deaths, improving health and the cost-effectiveness of health systems.

Precision medicine relies on molecular characterisation of a patient's disease, with genomic profiling central to new treatment models, enabling earlier and more accurate diagnosis/stratification and guiding targeted therapies. The EU Beating Cancer plan recommends genomic profiling for all cancer patients, with the "Cancer Diagnostic and Treatment for All" initiative improving access to new genomic diagnostics.

High quality genomic testing using technologies such as NGS and liquid biopsies is vital for successful implementation of precision medicine. However, NGS relies on complex multi-step workflows to simultaneously analyse large numbers of genomic variants. These are susceptible to major and poorly understood sources of uncertainty, resulting in significant variability and a current lack of comparability thereby impacting patient care and hindering wider implementation.

The standards and RMS to support assay validation and Quality Assurance (QA), including reference measurement procedures (RMP) and higher order methods, i.e., high-accuracy methods with low uncertainty that can be used as reference methods or for value assignment of reference materials, SI-traceable reference materials (RM) and measurement uncertainty (MU) guidance have yet to be established and are urgently needed to support new test development and approval under IVDR EU 2017/746 and implementation by clinical laboratories accredited to quality standards such as ISO 15189 or ISO 17025.

Developing and establishing novel metrological concepts, capabilities and RMS for genomic profiling will require a large-scale, multi-disciplinary and coordinated approach in collaboration with key end-user stakeholders to achieve the collective goals.

Objectives

The overall objective of this project is to develop metrological capability and to establish metrology frameworks to improve quality and reproducibility of critical processes within genomic profiling workflows as well as RMS for high accuracy SI-traceable cancer gene measurement to improve comparability and support assay validation as required by the IVDR (EU) 2017/746.

Report Status:
PU – Public, fully open

Publishable Summary

Issued: July 2024

Funded by the European Union. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or EURAMET. Neither the European Union nor the granting authority can be held responsible for them.

European Partnership Co-funded by the European Union

The project has received funding from the European Partnership on Metrology, co-financed from the European Union's Horizon Europe Research and Innovation Programme and by the Participating States.

METROLOGY PARTNERSHIP EURAMET

The specific objectives are:

1. To demonstrate the application of Reference Measurement Systems (RMS) to support development, validation, and quality assurance (QA) and external quality assessment (EQA) of genomic IVDs in accordance with EU IVDR 2017/746, including i) the establishment of an initial baseline framework (using outcomes from Objectives 2, 3 and 4), and ii) demonstration of proof of concept using key cancer genomic profiling models (NGS).
2. To establish Reference Measurement Procedures (RMPs) for high accuracy (VCs < 20 %) SI-traceable (to N=1) measurement of key cancer biomarkers and higher order methods to measure critical Quality Control (QC) parameters within genomic profiling workflows to support genomic RMS development.
3. To develop and characterise Reference Materials (RM) and external quality assessment (EQA) materials for genomic profiling in line with ISO 15194, ISO 15711 and JCTLM, with SI-traceable reference values and sequencing datasets, and to use these to establish a framework for SI traceable value assignment and commutability assessment of reference and EQA materials to support genomic IVDs.
4. To develop a framework for determining the measurement uncertainty (MU) in quantitative genomic data and nominal output data in multiparametric genomic profiles.
5. To facilitate take up of the measurement infrastructure, methods and materials developed in the project by the measurement supply chain (via EMN TLM), standards developing organisations (e.g., CEN TC 140 and ISO TC 212), and end users (e.g., healthcare, and medical laboratories, IVD developers, genomics/cancer/pathology institutes, EQA providers, RM producers, instrument/reagent developers, regulators).

Progress beyond the state of the art and results

GenomeMET will progress the state of the art by initiating development of novel metrological concepts, RMS and standards needed to support analytical validation and QA of genomic profiling IVDs for cancer patients. This will help enable implementation of accurate, comparable, and traceable genomic profiling for improved diagnosis, targeted treatment, and management of cancer. Some progress has been made since the beginning of the project. This includes the following advances described in the “Progress beyond the state of the art” sections:

Objective 1: To develop and demonstrate the application of Reference Measurement Systems (RMS) to support development, validation, and quality assurance of genomic IVD.

Current state of the art

Global and European efforts are underway to develop guidelines and standards to support the validation and QA/EQA of genomic profiling. However, current guidance for genomic test validation and QA lacks routes for independent comparability and assessment of analytical performance criteria.

Progress beyond the state of the art

This project will develop traceable methods for assessing critical quality attributes of key NGS genomic profiling workflow steps such as NA isolation (yield and quality) from clinical samples (tissue and liquid biopsy from cancer patients and NGS library preparation (yield and uniformity of coverage) feeding into frameworks to support assay QA. To date, the project Consortium has prioritised clinical model applications, including molecular stratification (disease sub-type), minimal residual disease (MRD) testing, and advanced cancer treatment selection, has chosen cancer types of lung and colorectal cancer, and has defined and prioritized the Critical Quality Metrics (CQM) for Laboratory Workflow QA/QC and Assay Validation and related examinands and measurands in both pre-analytical and analytical phases, including fragment size distribution, sample purity, instrument performance, procedural consistency, and adherence to standardized protocols. Information of genomic methodologies, sample requirements, sequencing parameters and data analysis information for tissue and liquid biopsy sample workflows have been collected by an extended literature review was made also on the state of the art on the latest ISO international standards, guidance, scientific publications, and initiatives in the field of early cancer detection. A clinical sample-planning document has been finalized to summarise and guide sample collection sharing and secure storage within the project.

Objective 2: To establish reference Measurement Procedures (RMP) for cancer biomarkers, and higher order methods.

Current state of the art

To date, there is only one primary RMP for quantification of a single cancer genetic variant in JCTLM DB, which is limited in scope to synthetic DNA controls.

Progress beyond the state of the art

The project will develop RMPs for high accuracy and SI-traceable measurements of key cancer biomarkers, assessment of the performance of RMPs using contrived RMs as well as demonstrating the applicability of RMPs to support validation of genomic profiling workflows. It will also establish novel sequencing (NGS/Sanger) strategies/capabilities for orthogonal validation of genomic variant calls, and identity and purity certification of genomic RM/EQA materials.

To date, the project has identified biomarkers representing key cancer variants including KRAS, BRAF, PIK3CA by an extended literature review and collected, in newly created tables, several information on those relevant biomarkers for early cancer detection and on the relative NA amplification-based methods for their quantification, including digital PCR. In addition, the project has selected the digital PCR (dPCR) as the best methodology for RMPs development for cancer gene variant quantification and total DNA quantity/fragment size measurements and has identified human specific (reference) genes that could be used for determination of total DNA concentration, by a literature survey and stakeholder consultations. Furthermore, the project has reviewed sequencing approaches for development of RMPs for nominal properties and characterization of reference materials and has selected the high accuracy NGS methods as sequencing technology to evaluate its potential as RMPs.

Objective 3: To establish SI-traceable frameworks for the development and characterisation of Reference Materials (RMs) and EQA materials for genomic profiling.

Current state of the art

WHO International Standards and commercial contrived RM/QCMs are only available for selected individual cancer biomarkers. Higher order RMs currently only exist for germline materials and only sequence identity is certified. In addition, these materials lack traceability to higher order standards and may not be commutable because frameworks for assessing the commutability of genomic RMs have not yet been established.

Progress beyond the state of the art

This project will support the roll-out of improved/new EQA schemes for cancer genomic profiling through provision of reference values to support traceability and comparability across schemes. It will establish routes for assessing commutability of complex multi-analyte genomic RMs taking into consideration nominal (variant identity) and quantitative Variant Allele Frequency (vAF) properties and will develop novel cell/tissue-based RM formats. To date, the Consortium investigated the availability of test/control/reference materials relevant to the cancer models/applications and cancer biomarkers and several exhaustive and informative tables are now available. In addition, materials for the development and assessment of RMPs for biomarkers/variant types and total NA quantity/quality have been selected. The project also identified EQA schemes relevant to the selected cancer models/applications, genetic variants, and/or selected technologies. Areas where new EQA schemes are needed to support cancer genomic testing have been recognized and reported. In addition, a very useful survey on standardisation requirements for cancer genomic profiling has been prepared and sent to relevant Stakeholders (mainly EQA schemes participants): the feedback was received from 95 laboratories and has given to the GenomeMET Consortium important information on their priority for standardisation in genomic workflow, reference genes, guidelines, etc, within the EQA schemes to support cancer genomic profiling.

The Consortium has initiated to investigate the feasibility of developing new RM/QCMs for cancer models/applications, where RM/QCMs are not available. The development of a potential "novel cell-based RM" for enrichment and genomic analysis of CTCs in liquid biopsy samples is started together with the optimization of cell culture systems for the development of novel cell-based RMs. Even the development of a strategy for new EQA scheme(s) aimed at accommodating emerging cancer testing modalities such as liquid biopsies has been initiated and the production/development of EQA materials for the schemes, as well as prototype samples for new schemes has begun. Fruitful new discussions on commutability for NGS were carried out in the

consortium at the last M9 meeting, giving rise to the beginning of a report on commutability for genomic profiling.

Objective 4: To develop a framework for determining measurement uncertainty (MU) of genomic profiling

Current state of the art

Traditional MU approaches for clinical chemistry and genetic testing focuses on single analytes which are incongruent with multiparametric genomic testing.

Progress beyond the state of the art

This project will establish a robust framework using statistical approaches for assessing MU for multi-parametric genomic profiling assays considering both quantitative (read count and vAF) and qualitative (sequence/variant identity) parameters. To date, the Consortium has made progress by reviewing statistical approaches for measurement uncertainty evaluation and sources of variation when working with multiparametric genomic datasets within genomic profiling workflows. Suitable ready-to-work-on datasets have been identified, reported and collected on the project database. The project has also initiated the compilation of information (meta-data) on the datasets to indicate the varying factors and the available output data, as well as a list of variants and additional variant information. Furthermore, the variation in the qualitative and quantitative output data related to the process of library preparation is under investigation.

The project has reviewed literature and software repositories to identify relevant bioinformatics pipelines and best practice guidance related to the cancer genomic profiling methods to be utilized in GenomeMET. In addition, the review of the availability of raw sequencing data files in the datasets identified within the project, has been initiated. Furthermore, the availability of matched test and reference datasets to be utilized for evaluating the qualitative accuracy of bioinformatic pipelines, as well as the availability of software capable of computing qualitative accuracy of genomic datasets, is being determined. Finally, the uncertainty in the output data associated with individual modules in a bioinformatic workflow, such as sequence alignment or variant calling programs, is currently under evaluation.

Outcomes and impact

Several key dissemination and communication activities were undertaken to promote the project. Engagement was established with 20 standard development organizations, including 17 international and 3 nationals, such as ISO and CEN, 3 industry working groups, and 1 regulatory body. Particularly, relevant standards committee meetings have been attended, among them ISO TC 212 on Clinical laboratory testing and in vitro diagnostic test systems and specifically meetings of WG4 on microbiology and molecular diagnostics and CEN TC 140 that specifies requirements and gives recommendations for next generation sequencing (NGS) workflows for in vitro diagnostics and biomedical research. The project was presented in 2 national and 3 international events through poster and oral presentations such as at the "Human and Medical Genetics Conference 2024" and at the "EMBL conference: Cancer Genomics". Additionally, 5 training course were conducted, such as one focused on statistical analysis, one interactive online course about applying digital PCR and measurement science to support early cancer detection and MRD and one invited seminar on Digital PCR and Measurement Procedure to Support Advances in Precision Medicine at the ELITech group, an international IVD developer company. To further enhance outreach, a project website (https://www.genomemet.org/) and a LinkedIn page (https://www.linkedin.com/company/genomemet/) were created and regularly updated. Dissemination efforts also included participation in discussions during meetings, the release of newsletters, utilization of social media platforms, and the publication of press releases.

Outcomes for industrial and other user communities

This project's outcomes support the implementation of precision medicine for cancer patients. It is envisaged that these will have impacts across multiple key stakeholder communities including, but not limited to:

- IVD developers - RMS will support the generation of enhanced performance validation data incorporating MU and metrological traceability, enabling genomic IVD developers to better demonstrate performance in line with the IVDR. This will lead to improved quality and comparability of IVDs and faster translation to market through more streamlined and consistent regulatory submissions.

- Clinical laboratories – Higher order methods and QC materials for monitoring key workflow quality metrics and performance will enable clinical laboratories to establish improved and standardised QA

frameworks, resulting in better quality and more comparable genomic profiling across laboratories and supporting accreditation (ISO 15189 or 17025).

- Healthcare providers – Frameworks for assessing analytical performance will enable healthcare providers to undertake improved Health Technology Assessments (HTA) of novel genomic IVDs, incorporating more robust data with defined uncertainties to support future test performance specifications and uptake of genomic profiling into health practice.

- RM producers – Frameworks for improved characterisation and SI-traceable value assignment of genomic RMs will enable RM producers to demonstrate metrological traceability and commutability in line with the IVDR and ISO 17511, leading to more streamlined RM development and a wider range of high quality RMs. A collaboration agreement with a major RM producer who is now actively working with the project and is involved in the Consortium meetings.

- EQA providers – Provision of SI-traceable reference values will enable EQA providers to demonstrate long term comparability and traceability of EQA materials and schemes, reducing reliance on arbitrary consensus values. This will improve robustness and quality of genomic EQAs, and support development of new schemes and harmonisation of EQAs in molecular pathology. In the first nine months of the project, the Consortium worked hard to plan two new EQA schemes which will be performed in the coming months.

- Drug developers will be able to undertake more streamlined development of targeted therapies through improved quality of genomic data from clinical trials, enabling more accurate selection of responders/non responders, leading to reduced development times, fewer failures, lower costs, and more effective cancer therapies.

- Clinical researchers will be able to generate more robust, reliable and reproducible genomic datasets, helping to address the current reproducibility crisis in clinical translational research, supporting faster translation of novel biomarkers to the clinic.

- Regulators – RMS and guidance for assay validation, incorporating metrological traceability, will inform IVD competent authorities /regulators / reference laboratories on performance metrics for genomic profiling assays, enabling more streamlined assessment of new IVDs and development of recommendations for implementation of genomic approaches in clinical practice.

Outcomes for the metrology and scientific communities

This project will provide a vehicle for joint activity, inter-laboratory comparisons, and knowledge sharing to support development of novel metrological concepts and capability for clinical genomics. Outcomes will support improved EU metrology infrastructure enabling provision of new RMS and measurement/calibration services allowing NMI/DIs to provide more reliable SI-traceable reference values and improving agreement between different laboratories worldwide. Outcomes include:

- Improved NMI/DI capabilities for quantification of cancer genomic biomarkers, quantification of total Nucleic acids and detection of panels of genomic variants, demonstrated through inter-laboratory comparisons. GenomeMET partners will be participating in a new CCQM study: K189 “Measurement of Single Nucleotide Variation (SNV) and Small Deletion in Cancer Biomarker of PIK3CA and EGFR.” This will allow to demonstrate cancer gene variants quantification capability, under development in GenomeMET, through recognized CCQM studies.

- Dissemination of case studies to advance development of metrological frameworks for multi-analyte clinical genomic profiling.

- Submission of new RMP for quantification of cancer genomic biomarkers and SI-traceable RMs to JCTLM database.

The metrological capabilities developed in this project will also support the wider clinical genomics sector (e.g., rare diseases and non-invasive prenatal testing (NIPT)) where Next Generation Sequencing (NGS) profiling is being applied and complement metrology development for other ‘omics sectors where multi parametric testing is needed, e.g., transcriptomics, proteomics, and metabolomics.

Outcomes for relevant standards

The RMS to support assay validation will enable IVD developers, clinical laboratories, and other end-users, e.g., EQA providers and RMs producers, to better comply with regulations and standards in the IVD field e.g., IVDR and ISO 15189, ISO 17025 and ISO 17511 through generation of more robust and comparable datasets incorporating metrological traceability and MU.

Higher order methods, i.e., high-accuracy methods with low uncertainty that can be used as reference methods or for value assignment of reference materials, e.g., dPCR and materials (RMs/EQA materials) will support stakeholder-driven standardisation initiatives, linked to GenomeMET, e.g., INSTAND-NGS4P project by providing the underpinning methods/materials required to assess performance.

Outputs from this project will be incorporated into relevant CEN TC 140 and ISO TC 212 standards in development for NGS and liquid biopsies through participant representation on drafting committees, and into periodic revisions of standards such as ISO 15193, ISO 15194 and ISO 20914.

Finally, proposals for new standards under CEN TC 140 or ISO TC 212 are expected during the lifetime of the project in order to support improved validation and QA of genomic profiling and accurate quantification of cancer gene biomarkers. To date, a new standard has been proposed under ISO TC 212 WG4 to support "NGS-based oncology applications" with GenomeMET partners as part of the Expert Drafting Team.

Longer-term economic, social and environmental impacts

Outputs from the project will support earlier cancer detection and implementation of precision medicine, through confident and valid uptake of genomic profiling. Cancer is the second largest cause of death in Europe, with more than 3.7 million new cases and 1.9 million deaths each year and carries an economic burden of €141.8 billion/pa (1.07 % of GDP). Earlier detection and genomics-guided targeted therapies with greater efficacy and less toxicity compared to traditional systemic therapies will significantly reduce healthcare costs and improve patient outcomes. High quality genomic testing will result in fewer diagnostic errors e.g., missed/incorrect diagnosis and support provision of the “right drug to the right patient at the right time” reducing the economic burden of cancer and allowing citizens to live longer and healthier lives.

The project’s outputs will also support growth of the European IVD and oncology therapeutics markets, valued at 33 billion Euros/pa and 75 billion Euros/pa respectively, through more streamlined routes for approval of new companion and precision genomic diagnostics, and improved genomic data from clinical trials resulting in more accurate selection of responders and more streamlined development of novel targeted therapies.

Environmental impacts include a reduction in the use of medical tools/devices and diagnostic kits/ components through more accurate “right first time” testing. These components are often single-use plastic products, the disposal of which presents an environmental risk.

List of publications

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This list is also available here: https://www.euramet.org/repository/research-publications-repository-link/

Project start date and duration:	36 months	
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Internal Beneficiaries: 1. INRIM, Italy 2. LNE, France 3. NIB, Slovenia 4. PTB, Germany 5. TUBITAK, Türkiye	External Beneficiaries: 6. CEA, France 7. FPO, Italy 8. INSTAND, Germany 9. MUG, Austria 10. UNITO, Italy	
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