

ICGEB WORKSHOP ON NEXT GENERATION DIAGNOSTICS, 22/03/2018-24/03/2018, MACEDONIAN ACADEMY OF SCIENCES AND ARTS, SKOPJE, REPUBLIC OF MACEDONIA

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ABSTRACT

More than 200 participants from Europe, Asia, Africa and South America attended the two days ICGEB Workshop on Next Generation Diagnostics, 22/03/2018-24/03/2018, at the Macedonian Academy of Sciences and Arts (MASA) in Skopje, Republic of Macedonia. The meeting provided an overview of the current and future use of next generation sequencing (NGS), proteomics and other high-throughput technologies in the diagnostic setup of malignant, inherited and communicable diseases. In addition, considerable emphasis was placed on the potential use of these techniques for disease prognostication, patient stratification and monitoring responses to therapy. Specific topics included NGS-based diagnostics of solid tumors, hematological malignancies, inherited and infectious diseases, proteomic-based approaches for biomarker discovery, predictive biomarkers for personalized treatment strategies, and bioinformatic analysis of NGS data. The meeting also provided a unique platform for fruitful discussions between internationally recognized experts and young researchers from developing countries, providing new perspectives and ideas on broader implementation of these techniques for personalized management and care.

Keywords: next generation sequencing; precision medicine; targeted therapy; predictive biomarkers

INTRODUCTION

Over the past several decades there have been tremendous discoveries in medical genetics and molecular biology, which have fundamentally changed our medical practice. Disease classifications are continuously being revised according to the new genetic data, treatment is individually tailored to molecular information and genetic counseling helps patients to deal with health challenges. It is not an exaggeration to say that every practicing physician – irrespective of his or her specialty will need a

strong foundation in the science that is at the heart of molecular medicine.

DNA sequencing is the process of determining the precise order of nucleotides within a DNA molecule. It includes any method or technology that is used to determine the order of the four bases – adenine, guanine, cytosine and thymine – in the DNA strand. The advent of rapid DNA sequencing methods has greatly accelerated the biological and medical research and discovery. Knowledge of

DNA sequences has become indispensable for basic biological research, and in numerous applied fields, such as medical diagnosis, biotechnology, forensic biology, virology and biological systematics. These topics were covered by 20 international experts during the three day workshop of the International Center for Genetic Engineering and Biotechnology (ICGEB) on Next Generation Diagnostics that was held at the Macedonian Academy of Sciences and Arts in Skopje on March 22-24, 2018. Below, a brief summary of the presentations is provided.

NGS DIAGNOSTICS: STATE OF THE ART AND BEYOND

The meeting was opened with introductory remarks by Momir Polenakovic (Macedonian Academy of Sciences and Arts, Skopje), who highlighted the potential of this rapidly expanding field in the future diagnostic workup of patients with various medical conditions.

The first lecture was given by Clare Turnbull (Institute of Cancer Research, London), who described the progress made during the Genomics England 100,000 Genomes Project. This project is a large-scale endeavor focused on delivering whole-genome sequencing within the UK National Health Service (NHS), covering both cancer and rare diseases. Her talk focused on NHS pathology pipelines for cancer sample collection and preparation, presentation of whole genome analyses back to the clinician, and future evolutions in provision of molecular testing.

The session was continued with a lecture by Richard Rosenquist (Karolinska Institute, Stockholm), who described the Swedish experience with the establishment of a national platform for next-generation sequencing (NGS) for clinical diagnostics. The platform was established in 2014 within the Science for Life Laboratory (SciLifeLab), a joint effort between the Karolinska Institute, the Royal Institute of Technology, Stockholm University and Uppsala University, and ever since has processed more than 10,000 patient samples for diagnostics of inherited diseases and cancer. To take the next step and facilitate implementation of precision medicine throughout the Swedish healthcare system, the same group recently launched the Genomics Medicine Sweden (GMS) initiative, which has the ambition to analyze up to 45,000 patient samples annually

using state-of-the-art sequencing technologies. The primary focus of this multidisciplinary project will be patients with inherited diseases, cancer and infectious diseases. The overall aim is to integrate findings from NGS into individual patient electronic healthcare records and registries enabling personalized management and care, and to provide a unique resource for population-based research projects within the field of precision medicine.

The last lecture of this session was given by Ulf Landegren (Uppsala University, Uppsala), who discussed novel and improved molecular technologies for diagnosis, patient stratification and monitoring response to treatment, which included a number of novel techniques developed in his lab. Among these, the proximity ligation or extension techniques (PLA and PEA, respectively) require only 1 μ l plasma sample to analyze sets of more than 90 proteins. The in situ PLA technique allows sensitive and specific imaging of proteins and their interactions and modifications in situ and in cells, to reflect states of signaling in cells and tissues. He also described a variant of these procedures that allows evaluation of target engagement by clinical low molecular weight drugs, such as kinase inhibitors, directly in patients' tissues. Finally, he described the recently developed super rolling circle amplification (sRCA) technique, which offers a convenient means to search and digitally enumerate tumor-specific mutations in plasma, as a means to monitor the progress or regression of tumors in patients under therapy.

NGS DIAGNOSTICS IN INHERITED AND INFECTIOUS DISEASES

The first lecture in this session was given by Marcel Nelen (Genome Diagnostics, Nijmegen), who described his experience with exome sequencing in the routine clinical setting. The clinical exome sequencing program in Nijmegen was launched in 2011 and since then has included >25000 clinical exomes. The program focuses primarily on inherited diseases, including blindness, deafness, movement disorders, craniofacial abnormalities, epilepsy, immunodeficiencies, multiple congenital anomalies, renal diseases, muscle diseases, neuropathies, metabolic diseases, and hypogonadotrophic hypogonadism. The use of small panel strategies to

reduce turnaround times, sequencing costs and make NGS-based diagnostic testing more affordable and adapted to the clinical setting were also discussed during his presentation.

The session was continued with a lecture by Anna Wedell (Karolinska Institute, Stockholm), who described the pipeline within the Science for Life Laboratory for whole genome sequencing (WGS) of inherited diseases. More than 3000 rare disease samples have been analyzed until now, with an average turnaround times are 10-12 days, allowing rapid diagnosis even in acute situations. Within the area of metabolic medicine, around 700 patients have been analyzed, with a diagnostic rate of 35% representing 170 different disease genes, illustrating a striking heterogeneity. A dozen novel diseases were discovered during these efforts and parallel mechanistic studies have opened novel possibilities for treatment.

Lars Engstrand (Karolinska Institute, Stockholm), described the pipeline of the Science for Life Laboratory for NGS analysis of infectious diseases. Whole genome sequencing of bacterial pathogens has been particularly useful for microbial subtyping and outbreak investigations, and can also provide information on virulence and resistance that may not be available through other currently available molecular diagnostics techniques. He also discussed approaches to improve the workflow for NGS, in particular shorten the turnaround time and reduce costs, and highlighted the potential use of NGS in driving targeted antimicrobial treatment decisions.

This session was closed with a lecture by Kristian Vlahovicek (University of Zagreb, Zagreb) on metagenomics analysis of human gut microbiome samples and implications for human disease.

NGS DIAGNOSTICS IN SOLID TUMORS

The first two lectures in this session focused on the current status and future perspectives of using liquid biopsies in the management of cancer. Simon Joosse from the University Medical Center Hamburg-Eppendorf discussed the use of monitoring circulating tumor cells as a surrogate marker for response to therapy. Johan Lindberg

(Karolinska Institute, Stockholm) described the use of NGS to profile circulating tumor DNA (ctDNA) in patients with metastatic prostate cancer, with the goal to enable biomarker-driven clinical trials. He presented data demonstrating that circulating tumor DNA is detectable in the majority of patients with metastatic prostate cancer and reflects the mutational landscape of the metastatic tissue. A prospective biomarker driven adaptive clinical trial is in progress in patients with metastatic prostate cancer, where the circulating tumor DNA profile established by his team guides the randomization process.

The session continued with a presentation by Dijana Plaseska-Karanfilska (RCGEB, Macedonian Academy of Sciences and Arts, Skopje), who gave an overview of the current status with the use of NGS for genetic testing of hereditary breast cancer syndromes and presented the results from her study using multi-gene panel testing among breast cancer patients from the Republic of Macedonia. The session was closed with a presentation by Katerina Davalieva from the same institution, who discussed the use of proteomics for the identification of novel biomarkers in patients with prostate cancer.

NGS DIAGNOSTICS IN HEMATOLOGICAL MALIGNANCIES

The session on hematological malignancies was opened with a lecture by Torsten Haferlach (Munich Leukemia Laboratory, Munich), who gave an overview on the use of NGS techniques in the diagnosis, classification, prognostication and treatment guidance of patients with myeloid malignancies. He also described the pipeline of the Munich Leukemia Laboratory for acute myeloid leukemia and discussed the next steps required to expand the use of NGS in the routine hematology diagnostic work-up, including improved automation, bioinformatic tools, accreditation and reimbursement procedures.

The use of NGS in the diagnosis of lymphoid neoplasms was covered by Davide Rossi from the Institute of Oncology Research, Bellinzona. He provided an overview of the pathways that are commonly affected by mutations in lymphoid tumors, and described how genetic alteration of these pathways may contribute to

disease pathogenesis, diagnosis, prognostication and treatment.

The last lecture of this session was given by Kostas Stamatopoulos (Institute of Applied Biosciences, CERTH, Thessaloniki), who discussed the use of NGS for the analysis of immunoglobulin and T cell receptor repertoires in patients with lymphoid malignancies. He described the advantages of using NGS for all main applications of immunogenetic analysis, including clonality assessment, MRD monitoring and analysis of stereotypy, and discussed challenges that still need to be overcome before NGS immunogenetics can replace PCR-based assays in the routine diagnostic setting.

PREDICTIVE BIOMARKERS AND PERSONALIZED TREATMENT STRATEGIES

Hematological malignancies were also the focus of the first lecture in the session on Predictive biomarkers and personalized treatment strategies. This lecture was given by Dimitar Efremov (ICGEB, Trieste), who discussed the use of kinase inhibitors for targeted therapy of patients with lymphoid malignancies. These drugs can selectively target oncogenic pathways that are deregulated because of genetic lesions or chronic stimulation by signals from the micro-environment and provide an opportunity for the development of individually-tailored treatment approaches. He gave an overview of the mechanism of action of these drugs and described ongoing efforts to incorporate these drugs into more effective rational combinations and identify factors that can predict response to treatment.

The second lecture was given by Päivi Östling (Karolinska Institute, Stockholm), who described the use of high throughput cell-based functional drug testing for identification of novel, clinically actionable therapeutic opportunities

in solid tumors. This approach combines genomic profiling by NGS of primary tumor tissue and drug sensitivity profiling with a large panel of approved and investigational oncology drugs. She described the results of their comprehensive drug testing program of castration-resistant prostate cancer and renal cell cancer, which resulted in the identification of distinct drug response profiles, confirming that genomic heterogeneity leads to variability in drug responses.

BIOINFORMATICS ANALYSIS OF NGS DATA

The workshop ended with a 3-hour session on bioinformatics analysis of NGS data. The goal of this session was to provide some practical examples of bioinformatics solutions for NGS data analysis through a diagnostic utility perspective. Building on the lectures provided during the preceding days of the workshop, this session showcased the basic computational pipelines and workflows for NGS data analysis and highlighted the different approaches that can be employed depending on the diagnostic study at hand. Lectures in this session were given by Valteri Wirta and Henrik Stranneheim from the Karolinska Institute in Stockholm and Fotis Psomopoulos and Andreas Agathaggelidis from the Institute of Applied Biosciences, CERTH, in Thessaloniki. The session ended with a lengthy discussion of the speakers and participants that highlighted the key concepts needed to be taken into consideration for the computational aspects of NGS data.

The workshop was closed with concluding remarks by Dimitar Efremov, who on behalf of all organizers thanked the speakers for their excellent presentations and expressed his gratitude to the International Centre for Genetic Engineering and Biotechnology and the Macedonian Academy of Sciences and Arts for their most generous sponsorship of this event.



Figure 1. Opening remarks by Momir Polenakovic and Dimitar Efremov



Figure 2. Clare Turnbull delivering lecture on "Genomics England and 100,000 genomes project: Developing a national genomics service"

Резиме

ICGEB РАБОТИЛНИЦА ЗА ДИЈАГНОСТИКА ОД СЛЕДНАТА ГЕНЕРАЦИЈА, 22/03/ 2018-24/03/2018, МАКЕДОНСКА АКАДЕМИЈА НА НАУКИТЕ И УМЕТНОСТИТЕ, СКОПЈЕ, РЕПУБЛИКА МАКЕДОНИЈА

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Повеќе од 200 учесници од Европа, Азија, Африка и Јужна Америка учествуваа на дводневната работилница на ICGEB за дијагностика од следната генерација, 22/03/2018-24/03/2018, во Македонската академија на науките и уметностите (МАНУ) во Скопје, Република Македонија. На состанокот беше даден преглед на сегашната и на идната употреба на следната генерација на секвенционирање (NGS), протеомика и други технологии во дијагностиката на малигни, наследни и заразни болести. Покрај тоа, значителен акцент беше ставен на потенцијалната употреба на овие техники за прогноза на болеста, стратификација на пациентот и следење на одговорите на терапијата. Специфични теми вклучуваа дијагностика на цврсти тумори базирани на NGS, хематолошки малигноми, наследни и заразни болести, пристапи базирани на протеоми за откривање на биомаркери, предвидувачки биомаркери за персонализирани стратегии за третман и биоинформатичка анализа на податоците од NGS. Состанокот, исто така, обезбеди единствена платформа за плодни разговори меѓу меѓународно признати експерти и млади истражувачи од земјите во развој, обезбедувајќи нови перспективи и идеи за поширока имплементација на овие техники за персонализирано управување и нега.

Клучни зборови: секвенционирање од следната генерација, прецизна медицина, насочена терапија, предвидувачки биомаркери