World Cancer Research Journal WCRJ 2016; 3 (1): e632

PERSONALIZED MEDICINE IN THE CLINICAL PRACTICE: THE ROLE OF FUNCTIONAL GENOMICS

RESEARCH CENTRE CETAC" CASERTA (ITALY) THE 04/05 DECEMBER 2015

S. DI MARTINO[1], A. TOMMASELLI[1], A. GRILLO[2], A. PETRUZZIELLO[3], S. DI NOTO[4], I. DI BIASE[5], A. SANGERMANO[6], G. LUS[7], A. D'ORTA[8], M. BERRETTA[9], A. PEZONE[10], R.DI FRANCIA[11]

[1]CETAC Research Center, Caserta, Italy
[2]Labogen s.r.l., Catania, Italy
[3]Laboratory of Virology and Molecular Biology "V. Tridente", Transfusion Service, Department of Haematology, IRCCS Italia – Fondazione G. Pascale, Naples, Italy
[4]Centro Medico "Le Zagare", Catania, Italy
[5]MeriGen Molecular Biology Laboratory, Naples, Italy
[6]DIACHEM s.r.l., Naples, Italy
[7]Centro per la Sclerosi Multipla, II Clinica Neurologica, Second University of Naples, Naples, Italy
[8]DD Clinic s.r.l., Caserta, Italy
[9]Department of Medical Oncology, National Cancer Institute, Aviano (PN), Italy
[10]Department of Molecular Medicine & Biotechnology, University "Federico II", Naples, Italy
[11]National Cancer Institute "Fondazione G. Pascale", Naples, Italy

INTRODUCTION

Over the past 20 years, the clinical medicine has evolved into a multifaceted technology-driven activity whose major purposes are diagnosed and screening for disease, monitoring health and therapeutic response, and gauging deviations from ordinary physiology in humans.

Advances in diagnostic medicine, have been achieved during the application of science and technology as result of a synergic effort among universities, industries, governments and private institutions. We are now entering the era of *Personalized Medicines*, which is bringing forth the newest and most powerful science and technology available for the modern-day practice of diagnostic-based medicine. Among the several important areas to consider with diagnostics, there are the emerging issues concerning the development of genomic assays and their use for testing individual patient responses for tailored therapy. Here, we highlight the most relevant discussion presented by speakers in the conference "Personalized medicine in the clinical practice" held in Caserta at "Research Centre CETAC on December 04/05, 2015 (Figure 1). The most recent report from literature in the field of the newest genomic tests suitable in clinical practice have been presented at the above faculty.

KNOWLEDGE-BASE SESSION

Dr. Oriana Catapano introduced the knowledge-base of ***"The new "OMICS" sciences: from research laboratory to clinical practice"***

The "omics" sciences (genomics, transcriptomics, proteomics and metabolomics) explore biological components in a global view to improve understanding of physiological and pathological processes. Their development comes from the evolution of new "omics" technologies (Microarray, Next Generation Sequencing, Tandem Mass Spectrometry, Nuclear Magnetic Resonance Spectroscopy), and by the implementation of sophisticated bioinformatics and statistical software.

Corresponding Author: Salvo Di Martino, MD; e-mail: salvo-dimartino@libero.it

Fig. 1.

More and more applications of these technologies are possible in genetic laboratory for screening programs, diagnosis, prognosis evaluation, monitoring of therapies; moreover, their high sensibility allows performing analysis starting from few amount of biological samples[1].

In prenatal field, Non-Invasive Prenatal Chromosomal Aneuploidy Testing (NIPT) on free-placental Circulating DNA in maternal blood improves the detection rate of biochemical screening of first trimester. In Invasive Prenatal and Post-natal Diagnosis, traditional karyotype can be supplemented with the study of microdeletion/microduplication syndromes by array-CGH, and with NGS of the entire exome or costumed gene panels for inherited disease, carrier screening or specific clinical areas[2].

Dr. Antonio Pezone clarified the Knowledge-base of
"DNA methylation is a "mark" induced by damaged repaired DNA. The new diagnostic tool for personalized treatments"

DNA is modified by methylation, which is layered on the primary genetic information and modifies gene expression. Somatic DNA methylation is unstable or metastable and varies with age and diseases.

Using a defined genetic system, we find that faithful repair of a DNA lesion in a reference gene leaves methylation marks, as "scars" on one strand of the repaired segment, which are transmitted to half of the daughter cells[3]. These DNA methylated sites are stable and inherited. If the scars occur in genes, which inhibit growth, the silencing of these genes by methylation will "foster" the growth of the cells and favor cancer. In cancer, cells with the same scars accumulate and evolve with malignancy[3].

We are deciphering the code of these epigenetic signatures by deep sequencing methylated alleles (epialleles) in cancer[4]. For example, by analyzing the growth suppressor, methylation traits of p16 5' promoter region (UTR) in myeloid leukemia, we have monitored the progression of the disease from the beginning to the course of disease (remission and/or relapse). We are formerly able to detect patient-specific signatures (corresponding to DNA damage events) and partially disease–specific markers. More samples should be analyzed to validate epigenetic traits specific of the disease and its stage. In both cases, this represents a viable method to personalize treatment[4].

NEUROLOGY SESSION

Prof. Giacomo Lus proceeded to examine
"The molecular markers of neurodegeneration in multiple sclerosis as predictors of the clinical evolution"

The complex etiopathogenesis and the considerable heterogeneity of multiple sclerosis (MS) disease have led to identify it as a complex disorder that can be further subdivided into different subtypes, each possibly characterized by a common pathophysiological molecular mechanism and probably by a similar prognosis of the disease and treatments response. The study of biomarkers can be considered as the most promising indicator of numerous pathological disorders; in MS identification of these parameters, especially if used in clinical practice, is still in the early stages[5]. The identification of anti - aquaporin -4 as another

specific form of demyelinating disease of the central nervous system, such as the neuromyelitis optica (NMO), is by far the most successful example of a diagnostic biomarker. Ultimately, in the next few years the research should be focused on the identification and validation of biomolecular parameters that combined with those clinical and neuroimaging already present may seek to give greater predictability to the prognosis of MS[6].

DISCUSSION PANEL SESSION

Dr. Agata Grillo and **Dr. Imma Di Biase** moderated the panel issue regarding ***"Diagnostic appropriateness of genetic testing"***

In the last decade, the biomolecular and genetic request has been growing due to the introduction of methods that can also be used in a clinical diagnostic laboratory. Since 2008 these exams have become reimbursable by the Regional Health Service. The appropriateness of prescribing is governed by Decree 40 and its modifications, which describes the organizational arrangements, the methods and the criteria of the prescription. The request of benefits marked with the R letter is not linked to the specific investigation, but rather is defined by general character codes referred to the method used. The great flexibility of the system led to a significant lack of homogeneity in the request for reimbursement, resulting in early depletion of regional budget. Minister Lorenzin has highlighted the issue and has determined that the benefits are subjected to conditions of deliverability and indications regarding the appropriateness of prescribing. Specifically, the genetic investigations, as decided in agreement with representatives of SIGU, will be prescribed only in case of a specific diagnosis. Regional Campania's team recently is promoting new guidelines for the highly specialized requirements to allow more consistent reimbursement.

PHARMACOGENOMICS AND MOLECULAR ONCOLOGY SESSION

Dr. Raffaele Di Francia reported a study on ***"Pharmacogenomics of Cytochrome P450 Family enzymes: implications for drug-drug interaction in anticancer therapy"***

Current pharmacogenomics era, which integrates the uniqueness of an individual genetic profile with respect to the pharmacokinetics and pharmacodynamics of a drug, provides greater safety and efficacy in drug therapy[7]. Personalized medicine is particularly important in oncology whereby most clinically used anticancer drugs have a narrow therapeutic window and exhibit a large interindividual pharmacokinetic and pharmacodynamic variability. This variability can lead to therapeutic failure or severe toxicity[8]. Understanding of how genetic variations influence drug disposition and achievement could help in tailoring cancer therapy based on individual's genetic composition. Each drug, after it distribution in the body, interacts with several proteins, such as carrier proteins, transporters, metabolizing enzymes, and multiple types of receptors. These protein interactions determine drug pharmacokinetics (i.e. drug absorption, distribution, metabolism, and excretion) and pharmacodynamics (i.e. target site of action, pharmacological and toxicological effects)[2]. Moreover, drugs activate downstream secondary events which may impact additional gene or protein expression responses and can also vary among patients. As a result, the overall response to a drug is determined by the interplay of multiple genes that are involved in the pharmacokinetic and pharmacodynamic pathways of a drug. In general, important genetic variation in drug effect can be envisioned at the level of drug metabolizing enzymes, drug transporters, and drug targets. This issue provides an overview on the commonly occurring, functionally and/or clinically relevant genetic polymorphisms within the genes encoding Cytochrome P450 (CYP) super family, with a particular highlight of examples whereby genetic variations in these genes influence the pharmacokinetics and/or response of Tamoxifen-based chemotherapy[9].

The retrospective and prospective trials evaluating the pharmacoeconomic impact of genotyping testing in these phase I and phase II metabolizing enzymes will provide answers on the possibility to incorporate Pharmacogenomics testing into routine clinical practice.

Dr. Antonio Pasquale Tommaselli clarified the important role of ***"Functional genomics and thyroid cancer"***

Thyroid cancer incidence has dramatically increased worldwide over the last two decades. Papillary thyroid cancer (PTC) is the most common endocrine malignancy that accounts for 80% to 85% of thyroid malignancies. The rise is mostly due to an increased detection of small papillary thyroid carcinomas (PTCs) (≤20 mm), predominantly microPTC (≤10 mm).

Although small tumors have an excellent outcome, a considerable percentage may show a more aggressive pattern with a worse prognosis.

Targeted radioiodine therapy for thyroid cancer is based on selective stimulation of Na+/ I- Symporter (NIS)-mediated radioactive iodide uptake (RAIU) in thyroid cells by thyrotropin. Patients with advanced thyroid cancer do not benefit from radioiodine therapy due to reduced or absent NIS expression.

Inhibitors targeting Akt, MEK, PI3K, Hsp90 or BRAF increase RAIU in thyroid cells expressing BRAFV600E or RET/PTC3 oncogene.

The genetic pattern evaluation, by separating patients with thyroid neoplasia into different risk groups, particularly by defining the group with the most aggressive disease, has important prognostic and therapeutic implications[10].

New approaches to diagnosis of cancer in thyroid nodules are based on detection of aforementioned mutational markers, which can be reliably detected in cells aspirated during the FNA procedure. These markers offer significant improvement in the diagnostic accuracy of FNA cytology and are poised to make a profound effect on the management and therapy of patients with thyroid nodules[11].

Dr. Massimiliano Berretta
introduced the known
"Pharmacogenomic markers for toxicity prevention in chemotherapy."

Despite the advances in dosing and scheduling of chemotherapy in both adjuvant and advanced settings, and a greater emphasis on early detection, the outlook remains poor for most patients.

Molecular analyses have shown that not all cancer diseases have the own natural history[12].

Cancers belonging to a particular pathologic stage may display significant clinical heterogeneity, which may reflect underlying molecular heterogeneity.

Individual patients with same stage tumours may have different long term prognosis and response to therapy. In addition, some prognostic factors are likely to be more important than others. These findings led, over the last eight decades, to extensive research of other possible prognostic factors, in attempt to improve the identification of patients more likely to have a poorer clinical outcome and therefore more likely to benefit from more aggressive treatment strategies[13].

The selection of the most beneficial treatment regimes in cancers remains a challenge and is hindered by a lack of well established prognostic markers that correlate with survival or disease-free survival (DFS) and predictive markers for response to a particular therapy[14].

Therefore, information regarding which parameters influence the prognosis would be valuable in the interpretation and design of clinical trials, and could also have implications for the clinical management decisions in the palliative setting.

Dr. Sabatino Dinoto
clarified the
"Pharmacogenomics in obstetrics and gynecology"

Pharmacogenomics applied to gynecology and oncology can be considered a useful and interesting tool to improve therapy efficacy.

Pharmacogenomics in obstetrics has a very interesting role: pregnant women and non-pregnant patients undergo different physiologic changes due to pharmacokinetics. Moreover, the genetic profile of fetus influences the mother's drug response.

During this presentation, we focus on official documents approved by the scientific community about several drugs prescribed by gynecologist containing pharmacogenomics labeled by FDA.

Today obstetricians are able to apply a much more efficient therapy to patients thanks to the knowledge coming from different scientific branches. However what is still missing now is a selection and validation of standardized models which may bring along patient's pharmacogenetic make up and physiologic changes due to pregnancy: this kind of model could be the guide to the right selection of drugs and dosages during pregnancy to optimize the therapy and reduce risks for the mother and the fetus[15].

Dr. Armando D'Orta
clarify the issue
"Personalized Diet in the oncology field".

In recent years, in the world of cancer research, are slowly growing new models of integrated medicine and more effective intervention strategies, in a clear attempt to influence microenvironment in which cells live, proliferate and die. This is now possible by modulating diet, food and lifestyle. People with less healthy diets are more likely to develop cancer. Many studies have been conducted looking at the association between diet and cancer, experts agree the food we eat can affect our risk of cancer. Scientists have estimated that less healthy diets cause nearly one in ten (9%) cancer cases in the UK. Diet and nutraceutical modulation, in association with the usual chemo/radio regimens, is called Nutritional Manipulation[16]. The ultimate purpose of this new discipline is to make the extracellular matrices inhospitable and unsuited to

the neoplastic cells, intervening on the expression of these oncogenes that are involved directly in the pathogenesis or in the maintenance of the disease.

HEPATIC SESSION

Dr. Amalia Sangermano/
Dr. Massimiliano Ammirabile,
clarified the polymorphisms related to
"Non-Alcoholic Fatty Liver Disease (NAFLD)".

Non-Alcoholic Fatty Liver Disease (NAFLD) is a term used to cover a wide range of liver conditions that range from mild to extremely serious. Uncontrolled, it can progress to cirrhosis, cancer and liver failure[17]. Over 1/3 of the general U.S. population, and 60% of women with PCOS, have confirmed diagnosis of NAFLD. NAFLD is characterized by the accumulation of fat in the liver and it is correlated to Insulin Resistance, therefore women with PCOS will be at high risk as well. There are two stages: 1) steatosis (usually benign and often reversible showing an increased fat in the hepatocytes); 2) a more severe stage called nonalcoholic steatohepatitis (NASH). It may get worse with obesity and metabolic syndrome, particularly insulin resistance as well as type 2 diabetes mellitus. NASH may lead to cirrhosis (~30%), hepatic failure and sometimes hepatocellular carcinoma. Nowadays molecular biology can evaluate the genetic predisposition of these liver diseases. The analysis of four polymorphisms of four different genes (Kruppel-like factor 6 (KLF6), Dependent phosphatidic acid phosphohydrolase (LPIN1), Patatin-like Phosholipase containing 3 (PNPLA3) and superoxide dismutase manganese-dependent (SOD2) with their genotypes will allow the prediction of development of different pathological aspects associated with NALFD such as steatohepatitis NASH and liver fibrosis.

Dr. Arnolfo Petruzziello
introduced the known
"Distribution pattern of Hepatitis C Virus (HCV) genotypes and correlation with viral load and risk factors in chronic positive patients"

Hepatitis C virus (HCV) infection has epidemic proportions worldwide, with more than one million new cases of infection reported annually. The prevalence in Europe is nearly 1%, but it may vary geographically along a north-south gradient, ranging from 0.5% in Northern countries to 2% in Mediterranean areas. In Italy, where the overall prevalence is 3.2%, it was observed an increasing prevalence in the Southern Italy regions, especially in the elder patients.

The HCV genome is a positive, single-stranded RNA genome approximately 10 kb long, included in the genera Pestivirus and Flavivirus, which displays a considerable genetic heterogeneity among isolates. This led to its classification into six major genotypes, with different geographic, age and gender distribution, differing from each other by 30% in terms of nucleotide sequence. It has been found a greater prevalence of 1b and 2a/2c genotypes in elder patients and 1a and 3a in younger individuals, suggesting the involvement of intravenous drug use.

In Italy, around half of cases are of the 1b subtype, followed by 2a/2c subtype. Whether or not a north–south distribution gradient exists is as yet unconfirmed; however, especially in the Northern Italy, the incidence of genotype 3a seems to be increasing.

It is thought that this genetic heterogeneity may account also for some of the differences in disease outcome, evolution and response to treatment observed in HCV-infected patients. In fact, genotype 1 and 4 show more resistance as compared to genotype 2 and 3 to Pegylated–Interferon (PEG-IFN) and ribavirin combination therapy and, thus, require different treatment duration and dose[18].

Moreover, in the last two decades, some studies had showed that HCV RNA viral load could be considered as an important prognostic indicator of response to antiviral therapy and that a high pre-treatment viral load (> 6,0 x 10E^5 IU/ml) can be linked with low rates of response to therapy.

Recently, a single nucleotide polymorphism (SNP) on chromosome 19, upstream of the interleukin 28B (IL28-B) gene, has been reported as strongly associated with the spontaneous clearance of HCV or induced by pegylated-interferon plus ribavirin treatment, primarily among individuals infected with genotype 1.

Aim of this study was to describe the distribution of HCV genotypes in chronic hepatitis patients from the Campania region in southern Italy and to estimate their association with risk factors and viral load.

CONCLUSION AND FUTURE OUTLOOK

The clinical diagnostic laboratory performs testing of patient samples, provides the guidelines for standardizing test development and utilization, and is the site most likely to standardize pharmaco-genomics testing[19].

The rapidly growing area of functional genomics is ideally suited to clinical laboratories and suitable testing is a necessary and critical step to move personalized medicine into practice[20]. Furthermore, the major issues to consider for the clinical laboratories (who are responsible for providing PG services), are: i) the availability of FDA-cleared tests; ii) the current absence of public reimbursement; iii) the need for genotyping accuracy; and iv) the need to find clinical expertise to interpret laboratory data results[21].

Hopefully, the future implementation of functional genomics will result in personalized treatments and eventually, in shifting the balance from disease relapse towards disease eradication.

Duality/Conflict of Interests:
None declared/applicable.

REFERENCES

1. Riegel M. Human molecular cytogenetics: From cells to nucleotides. Genet Mol Biol 2014; 37(1 Suppl): 194-220.
2. De Monaco A, Berretta M, Pugliese S, Valente D, Ciaffarafa S, Di Francia R. Evaluation of genotyping costs. Eur Rev Med Pharm Sci 2014; 18(14): 2084-2087.
3. Morano A, Angrisano T, Russo G, Landi R, Pezone A, Bartollino S, Zuchegna C, Babbio F, Bonapace IM, Allen B, Muller MT, Chiariotti L, Gottesman ME, Porcellini A, Avvedimento EV. Targeted DNA methylation by homology-directed repair in mammalian cells. Transcription reshapes methylation on the repaired gene. Nucleic Acids Res 2014; 42: 804-821.
4. Perillo B, Ombra MN, Bertoni A, Cuozzo C, Sacchetti S, Sasso A, Chiariotti L, Malorni A, Abbondanza C, Avvedimento EV. DNA oxidation as triggered by H3K9me2 demethylation drives estrogen-induced gene expression. Science 2008; 319: 202-206.
5. Comabella M, Montalban X. Body fluid biomarkers in multiple sclerosis. Lancet Neurol 2014; 13: 113-126.
6. Fernández O. Integrating the tools for an individualized prognosis in multiple sclerosis. J Neurol Sci 2013; 331: 10-13.
7. De Monaco A, Faioli D, Di Paolo M, Catapano O, D'Orta A, Del Buono M, Del Buono R, Di Francia R. Pharmacogenomics markers for prediction response and toxicity in cancer therapy. WCRJ 2014; 1: e276.
8. Di Francia R, Siesto RS, Valente D, Del Buono A, Pugliese S, Cecere S, Cavaliere C, Nasti G, Facchini G, Berretta M. Current strategies to minimize toxicity of oxaliplatin: selection of pharmacogenomic panel tests. Anti-cancer drugs 2013; 24: 1069-1078.
9. Di Francia R, Rainone A, De Monaco A, D'Orta A, Valente D, De Lucia D. Pharmacogenomics of Cytochrome P450 Family enzymes: implications for drug-drug interaction in anticancer therapy. WCRJ 2015; 2(1): e483.
10. Nikiforov YE, Yip L, Nikiforova MN. New strategies in diagnosing cancer in thyroid nodules: impact of molecular markers. Clin Cancer Res 2013; 19: 2283-2288.
11. Nikiforov YE. Molecular diagnostics of thyroid tumors. Arch Pathol Lab Med 2011; 135: 569-577.
12. Berretta M, Di Francia R, Tirelli U. Editorial – The new oncologic challenges in the 3RD millennium. WCRJ 2014; 1: 2372-3416, e133.
13. Di Francia R, De Lucia L, Di Paolo M, Di Martino S, Del Pup L, De Monaco A, Lleshi A, Berretta M. Rational selection of predictive pharmacogenomics test for the Fluoropyrimidine/Oxaliplatin based therapy. Eur Rev Med Pharmacol Sci 2015; 19(22): 4443-4454.
14. Lleshi A, Fiorica F, Fisichella R, Spartà A, Di Vita M, Berretta S, Berretta M . Gastric cancer: prognostic aspects, predictive factors to therapy response and real impact on treatment approach. WCRJ 2014; 1(4): e395.
15. Overview of FDA-approved anti cancer drugs used for targeted therapy. WCRJ 2015; 2(3): e553.
16. Del Buono A, D'Orta A, Del Buono R, Del Buono MG, De Monaco A, Marullo MN. Relationship between diet and heavy metals in high risk of the environmental toxicity areas. Implication for cancer prevention. WCRJ 2014; 1(4): e411.
17. Catalano D, Trovato GM., Ragusa A, Martines GF., Tonzuso A, Pirri C, Buccheri MA, Trovato FM. Non-alcoholic fatty liver disease (NAFLD) and MTHFR 1298A > C gene polymorphism. Eur Rev Med Pharmacol Sci 2014; 18(2): 151-159.
18. Pinzone MR, Zanghì AM, Rapisarda L, D'Agata V, Benanti F, Spartà D, Nunnari G, Cacopardo B. Cirrhotic patients are still at risk of developing hepatocellular carcinoma despite Interferon-induced sustained virological response. Eur Rev Med Pharmacol Sci 2014; 18(2 Suppl): 11-15.
19. De Monaco A, D'Orta A, Fierro C, Di Paolo M, Cilenti L, Di Francia R. Rational selection of PCR-based platforms for pharmacogenomic testing. WCRJ 2014; 1(4): e391.
20. Sutton A, Ades AE, Cooper N, Abrams K. Use of indirect and mixed treatment comparisons for technology assessment. Pharmacoeconomics 2008; 26: 753-767.
21. Di Francia R, Valente D, Pugliese S, Del Buono A, Berretta M: What health professions in oncology needs to know about pharmacogenomics? WCRJ 2014; 1: e90.