MOLECULAR DIAGNOSTICS IN THE CLINICAL PRACTICE – "RESEARCH CENTRE CETAC" CASERTA (ITALY) THE 23/24 MAY 2014

R. DI FRANCIA[1], O. CATAPANO[2], A. PEZONE[3], G. LUS[4], A. TOMMASELLI[1], M. BERRETTA[5], L. DEL PUP[6], P.A. TOMMASELLI[7], D. DE LUCIA[8]

[1]National Cancer Institute "Fondazione G. Pascale", Naples, Italy
[2]MeriGen Molecular Biology Laboratory, Naples, Italy
[3]Department of Molecular Medicine & Biotechnology, University "Federico II", Naples, Italy
[4]Centro per la Sclerosi Multipla, II Clinica Neurologica, Second University of Naples, Italy
[5]Department of Medical Oncology, National Cancer Institute, Aviano (PN), Italy
[6]Department of Surgical Oncology, National Cancer Institute, Aviano (PN) Italy
[7]Department of Radiology, National Cancer Institute "Fondazione G. Pascale", Naples, Italy
[8]Azienda Policlinico at Second University of Naples, Italy

INTRODUCTION

Over the past 30 years, the clinical laboratory has evolved into a complex, technology-driven enterprise whose main purposes are diagnosing and screening for disease, monitoring health and therapeutic response, and gauging deviations from normal physiology in humans.

Advances in diagnostic medicine, have been achieved through the application of science and technology as a result of a synergic effort among Universities, industries, governments, and private institutions. We are now entering the era of Molecular Diagnostics, which is bringing forth the newest and most powerful science and technology available for the modern-day practice of diagnostic laboratory medicine. Among the numerous important areas to consider with molecular diagnostics there are the emerging issues concerning the development of genetic assays and their use for testing individual patient responses for personalized pharmaceutical therapy. Here, we highlight the most relevant discussion presented by speakers in the conference "Molecular Diagnostics in the clinical practice" held in Caserta at "Research Centre CETAC on May 23-24 2014 (Figure 1). At the above faculty have been presented the latest reports from literature in the fields of newest genomic test suitable in clinical practice.

Knowledge-Base session

Dr. Oriana Catapano introduced the knowledge-base of *"Old and new techniques in molecular biology."*

In the last seventy years, many improvements have been made onto DNA techniques.

Karyotype, FISH, PCR, Real-Time PCR, MLPA, Sanger sequencing are actually used to diagnose genetics syndromes, tumours, viral or bacterial infections, to predict or to follow up pharmacological therapies and for many others applications. Many technologies like Array technologies (aCGH, Gene Expression Profiling, SNP-array, etc.) and Next Generation Sequencing platforms, developed into research laboratories, are now largely used in clinical diagnostics. They have the advantage to give more information in less time than traditional techniques, giving a higher detection rate and resolution power, allowing to identify unknown genetic alterations and improving knowledge on molecular pathological mechanisms[1]. However, the reagents high costs and data management are the main limitations to the diffusion of these techniques in all diagnostics laboratories[2].

Dr. Antonio Pezone clarified the Knowledge-base of *"DNA methylation is a "scar" induced by damaged repaired DNA and remodelled by transcription."*

Figure 1. Conference scene.

DNA is modified by methylation, which is layered on the primary genetic information and alters 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. 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, p16 CDKN2A methylation traits in myeloid leukemia, we have monitored the progression of the disease from the onset to the end. We are currently able, to detect patient-specific signatures (corresponding to DNA damage events experienced by the cells) and partially, disease-specific marks. 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 cancer history and treatment[5].

Molecular 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 has led to identify it as a complex disorder that can be further subdivided into different subtypes, each possibly characterized by a common pathophysiological molecular mechanisms 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, it is still in the early stages[6]. The identification of anti-aquaporin-4 as a specific other form of demyelinating disease of the central nervous system, such as the neuromyelitis optica (NMO), is by far the most successful example of diagnostic biomarkers. 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 provide greater predictability to the prognosis of MS[7].

Prof Domenico De Lucia highlighted the suitable *"Laboratory Markers in the Diagnosis of Venous Thromboembolis."*

The purpose of this brief review is to discuss the value of selected blood tests that may be useful in the diagnosis of venous thromboembolism (VTE), or in the identification of a congenital or acquired defect associated with the development of VTE. It is extremely important in today's cost-conscious world to make sure that each test that is ordered will be clinically useful in the management of the patient. This approach is emphasized in this review.

Selected blood tests may be useful in the diagnosis of venous thromboembolism (VTE), or in the identification of a congenital or acquired defect as-

sociated with the development of VTE. Several studies have shown the D-dimer assay to have a high negative predictive value but poor specificity when used in the detection of VTE.

The presence of such genetic thrombophilia markers as factor V Leiden, prothrombin 20210A mutation, and antiphospholipid antibodies significantly increases patient's risk of a thrombotic event. Other markers such as hyperhomocysteinemia and deficiencies of antithrombin, protein C, or protein S, when combined with the previous mutations, also significantly increase patient's risk of a thrombotic event.

We feel that it is important to identify these ultra-high-risk patients to provide adequate counseling about the risk of thrombosis before elective surgical procedures. Often, lifelong anticoagulation may be needed as these patients and family members may need testing before taking birth control pills or hormonal replacement.

Pharmacogenomics and molecular oncology session

Dr Raffaele Di Francia reported a pilot study on *"Cost-effectiveness of pharmacogenomics testing in Fluoropyrmidine/oxaliplatin-based therapy."*

In general, there are three main types of economic analysis for genotyping that differ primarily in the evaluation of health outcome: cost-effectiveness, cost-utility and cost-benefit parameters. The primary aim of a cost-effectiveness analysis is to provide sufficiently robust information for decision-makers to allocate resources to healthcare interventions. Overviews of cost-effectiveness studies on PGx technologies are now available; all the reviews until 2012, used different inclusion criteria and assessed the quality of analyzed studies using different approaches. Sutton et al[8] reports that different meta-analysis methods used to evaluate the accuracy of diagnostic tests can affect and interfere with the economic evaluation. Recently, several methods to assess the quality of cost-effectiveness, cost-utility and cost-benefit of PGx tests have become available. A relevant example is the National Institute for Health and Clinical Excellence (NICE). NICE forms a Diagnostic Advisory committee, which is willing to stimulate Pharma and Academic communities to produce a robust set of data, including the design and data source in economic models of healthcare[9]. It is well known that PGx tests, performed before drug treatment, lower overall medical costs and provide higher quality and longer life expectancy[10].

The methods and cost of genotyping specific germline polymorphisms in four drug-metaboliz-ing genes, associated with Fluropyrimidine/oxaliplatin-based therapy, have been reported. Relative costs of these PGx tests have been evaluated by "subjective" criteria due to lack of specific guidelines.

The aim of this study is to provide information on the advantages and limitations, in terms of costs of the most common available methods for molecular detection of variations of *DYPD*, *TYMS MTHFR* and *GSTP1* genes[11].

The retrospective and prospective trials evaluating the pharmacoeconomic impact of genotyping testing in Fluoropyrimidine/oxaliplatin-based-therapy will provide answers on the possibility to incorporate PGx testing into routine clinical practice.

Dr Antonio Pasquale Tommaselli clarified the important role of *"Integrated Thyroid diagnostics –cytologic, immunohistochemical, biomolecular- of fine needle aspiration."*

Thyroid cancer is the most common malignant tumor of the endocrine system. These tumors frequently have genetic alterations leading to the activation of the mitogen-activated protein kinase (MAPK) signaling pathway.

Most common mutations in papillary carcinomas are point mutations of the *BRAF* and K*RAS* genes and *RET/PTC* rearrangement. These genetic alterations are found in >70% of papillary carcinomas and they rarely overlap in the same tumor[12].

In follicular carcinomas, the second most common type of thyroid malignancy, include *RAS* mutations and *PAX8-PPAR*□ rearrangement. *RET* point mutations are crucial for the development of medullary thyroid carcinomas.

The traditional diagnostic approach to this clinical situation is ultrasound-guided fine-needle aspiration (FNA) of the thyroid nodule followed by cytologic examination, which together reliably establish the diagnosis in 70% to 80% of cases. In the rest of nodules the presence of cancer cannot be ruled out by FNA cytology. 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[13].

Dr. Massimiliano Berretta introduced the known *"Molecular markers of colorectal cancer."*

Over the past 30 years, there has been a great interest in clinical and molecular prognostic factors in metastatic colorectal cancer (CRC).

This interest is even greater today with the advent of molecularly targeted agents that have changed dramatically the treatment algorithms and the survival for patients with metastatic CRC.

CRC is one of the most commonly diagnosed cancers in the world and remains the second leading cause of cancer death in Western countries.

Survival for patients with metastatic CRC has improved dramatically over the past decade. In the mid 1990s, the median overall survival (OS) for patients with metastatic CRC treated with a 5-fluorouracil (5-FU)-based regimen was only about 12 months, increased to approximately 18 months with the addition of irinotecan and oxaliplatin[14]. The introduction of biologic agents has led to a substantial jump in OS, approaching 30 months in some studies and allowing significant advances in the study of CRC prognosis and outcome.

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

Molecular analyses have shown that not all CRCs have the same natural history[15].

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.

The selection of the most beneficial treatment regimes in CRC 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.

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.

CONCLUSIONS 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 Pharmacoge-nomics testing. As a result, the hospital-based clinical laboratory is the logical site to perform routine molecular testing.

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[16].

The rapidly growing area of molecular diagnostics is ideally suited to clinical laboratories and suitable testing is a necessary and critical step to move personalized medicine into practice.

Hopefully, the future implementation of the methods for genotyping, will result in personalized treatments and eventually, in shifting the balance from disease relapse towards disease eradication. It is important and quite timely for the clinical diagnostic industry to step up, assuming these new responsibilities, and pharmaceutical and biotechnology companies should join each other, in order to develop a commercial test suitable for routine molecular diagnostics.

Duality/conflict of interest:
None declared/applicable.

References

1. Riegel M. Human molecular cytogenetics: From cells to nucleotides. Genet Mol Biol 2014; 37(1 Suppl): 194-220.
2. Voelkerding KV, Dames SA, Durtschi JD. Next-generation sequencing: from basic research to diagnostics. Clin Chem 2009; 55: 641-645.
3. Cuozzo C, Porcellini A, Angrisano T, Morano A, Lee B, Di Pardo A, Messina S, Iuliano R, Fusco A, Santillo MR, Muller MT, Chiariotti L, Gottesman ME, Avvedimento EV. DNA damage, homology-directed repair, and DNA methylation. PLoS Genet 2007; 3: e110.
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. 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.
6. Comabella M, Montalban X. Body fluid biomarkers in multiple sclerosis. Lancet Neurol 2014; 13: 113-126.
7. Fernández O. Integrating the tools for an individualized prognosis in multiple sclerosis. J Neurol Sci 2013; 331: 10-13.
8. Sutton A, Ades AE, Cooper N, Abrams K. Use of indirect and mixed treatment comparisons for technology assessment. Pharmacoeconomics 2008; 26: 753-767.

9. Dhalla IA, Garner S, Chalkidou K, Littlejohns P. Perspectives on the National Institute for Health and Clinical Excellence's recommendations to use health technologies only in research. Int J Technol Assess Health Care 2009; 25: 272-280.

10. Berretta M, Di Francia R, Tirelli U. Editorial – The new oncologic challenges in the 3RD millennium. WCRJ 2014; 1: e133.

11. 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.

12. 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.

13. Nikiforov YE. Molecular diagnostics of thyroid tumors. Arch Pathol Lab Med 2011; 135: 569-577.

14. Berretta M, Cappellani A, Fiorica F, Nasti G, Frustaci S, Fisichella R, Bearz A, Talamini R, Lleshi A, Tambaro R, Cocciolo A, Ristagno M, Bolognese A, Basile F, Meneguzzo N, Berretta S, Tirelli U. FOLFOX4 in the treatment of metastatic colorectal cancer in elderly patients: a prospective study. Arch Gerontol Geriatr 2011; 52: 89-93.

15. 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.

16. 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.