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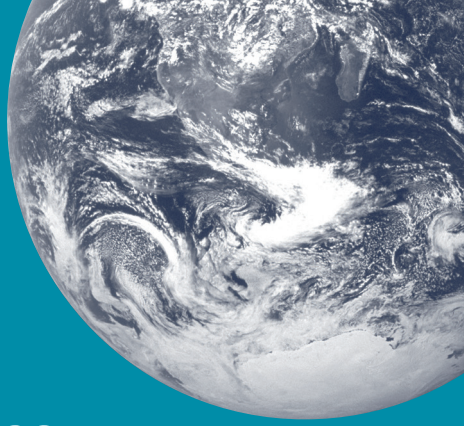

COSTS AND QUALITY OF GENOMICS TESTS IN THE ONCOLOGY FIELD

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Abstract – *The role of genomics and genotyping has occupied a primary position in the oncological scenario, leading to an era in which treatments are tailored to each patient's genetic profile. This allows to lower the risks of drug-dependent adverse effects and, consequently, the overall healthcare costs. In parallel with this, new high-throughput platforms are spreading in the routine of clinical diagnostics, encouraged by the progressive lowering of their costs, the strong cost-effectiveness, and their high sensitivity/specificity. Despite this technological advance, an important issue is represented by the validation and quality check of these tests, since they have extremely high standards of sensitivity and specificity. With appropriate controls, these assays can represent a powerful tool in the hands of oncologists for the continuous improvement of standard care for oncologic patients.*

KEYWORDS: *Genotyping methods, Molecular diagnostics, Healthy economy, Clinical laboratory, Cost-effectiveness.*

Abbreviations: SNP: Single Nucleotide Polymorphism; RFLP: Restriction Fragment Length Polymorphism; ASA: Allele Specific Amplification; SSCP: Single-Strand Conformation Polymorphism; FRET: Fluorescence Resonance Energy Transfer; TPMT: thiopurine methyltransferase; UGT1A1: UDP-glucuronosyltransferase 1A1; MDR1: multidrug resistance 1; TYMS: thymidylate synthase; DHFR: Dihydrofolate reductase; MTHFR: 5,10-methylenetetrahydrofolate reductase; EGFR: Epidermal Grow Factor Receptor; 5-FU: 5-fluorouracil; 6-MP: 6-mercaptopurine; GIST: Gastrointestinal Stromal Tumor; AML: Acute Myeloid Leukemia; NSCLC: Non-Small Cell Lung Cancer; CML: Chronic Myeloid Leukemia

INTRODUCTION

In the last fifteen years, cancer research has made enormous progresses in understanding the molecular mechanisms that allow the development of malignancies. A great revolution was the concept of "personalized medicine": each patient is different, though the type of neoplasm is the same, because the genomic profiles are different in themselves (SNP; RFLP; repeated DNA, etc.)[1]. This has led to the development of pharmacogenetics, which has its roots in the 1950s, and it had a burst at the beginning of the new millennium[2]. We are in an era in which genotype testing is more a must rather than a need. This is true, particularly in the oncological field. A lot of polymorphisms are known to be involved in drugs metabolism and then in resistance or toxicity towards one or more therapies. This fact, together with the progressive reduction of the costs for genotype testing, has promoted a very slow, but constant diffusion of genomics-based platforms in clinical laboratories[3]. It must be said that in parallel with this, it is getting higher and higher the risk of wrongly interpret the enormous amount of data obtained by this type of testing with especially dramatic consequences where the test becomes commercially available to the public. Nowadays it is possible to analyze the whole genome of an individual in less than 24 hours thanks to Next Generation Sequencing (NGS) techniques. Thus, specialized figures are absolutely needed to interpret and manage data[4].

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Although clinical laboratories can entrust these genotype tests to custom service or academic referenced laboratories, it is still possible to develop and validate in-house tests, and work with them as a laboratory service, leading to a further reduction of the costs to perform these analysis[5,6].

Pharmaceutical and Biotech companies could have an important role in this scenario. Since they are required to have validated assays for human clinical trials, they frequently develop their own clinical pharmacokinetic and pharmacodynamic tests for new drugs studies (in accordance to international standards from FDA and EMEA). These companies should be involved in the initial setup of genetic tests because they have primary data and information necessary for this stage of assays development. However, when they have to be applied in the clinical practice, this development activity should be transferred to external laboratories, such as clinical core laboratories in academic health centers, or established Clinical Research Organizations (CRO), because these independent sites are able to handle this function, as well as they are the actuals end users of these products.

The last hot issue about genotyping costs, that is already creating controversy among health organizations, healthcare providers, and the patient is who have to pay the cost of the test in itself (and consequently the possibility of reimbursement)[7,8]. Health insurance companies are deeply interested in genotyping assays in order to document the proper dosing of expensive drugs and hence reducing the incidence of adverse e drug reactions (Table 1). This could lead to a substitution of the current trial-and-error approach with a cost-effective alternative that is precise dosage scheduling based on an individual's genomic profile.

CURRENT GENOTYPING METHODS FOR KNOWN MUTATIONS

Nowadays, there are different methods and technologies that allow a fast, precise and reliable genotyping, improving outcomes and decreasing costs of care typical of a trial-and-error approach8.

A large number of platforms have been developed through years[5,9,10]. Table 2 lists the most popular ones, used for the detection of known SNPs, comparing the mean costs for instrument and reagents and the time labour needed for a single SNP analysis (details in the legend).

TABLE 1. Most common genetic abnormalities in cancer genes and their effect in chemotherapy outcomes.

GENE	*Polymorphism (nucleotide translation)*	*Molecular effect*	*Drug*	*Effect on therapy*
ABL	T(9;22) BCR/ABL fusion gene	Constitutive signal activation	Imatinib Dasatinib Nilotinib	Good response in CML
ABL	T315I M351T		Imatinib	Drug resistance
TPMT2, 3A, 3C	Various Polymorphism	Rapid degradation	6-MP Thioguanine	Hematopoietic toxicity
UGT1A 28	TA repeats in 5' promoter	Low expression	Irinotecan	Neutropenia toxicity
MDR1	(C3435T)	Low expression	Various	Drug resistance
TYMS	3 tandem repeats	High expression	5-FU, Methotrexate	Drug resistance
DHFR	(T91C)	Increase enzyme activity	Methotrexate	Drug resistance
MTHFR	(C677T)	Decreased enzyme activity	Methotrexate	Toxicity
c-KIT	(T1982C) (T81421A)	Constitutive signal activation	Imatinib	Desensitizes activity in GIST
c-KIT	D816V		Imatinib Semaxinib	Good response in t(8;21)-positive AML
EGFR	L858R		Gefitinib Erlotinib	Good response in NSCLC
B-RAF	V600E	Constitutive signal activation	Vemurafenib	Good response in melanoma patients carring V600 E mutations
RARα	T(15;17) PML/RARα fusion gene	Block of maturation of Myeloid cells	All Trans Retinoic acid (ATRA)	Good response in AML-M3 subtypes

TABLE 2. Current genotyping methods for known mutations detection.

Genotyping methods to detect known SNP	*Instrument mean costs[§]*	*Reagent costs per SNP[$]*	*Approximate time-labour per SNP[#]*
Maldi-TOF	++++	Moderate	Moderate
SSCP	+	Moderate	Moderate
Allele Specific Amplification (ASA)	+	Very Low	Moderate
Restriction Fragment Length Polymorphism (RFLP)	+	Low	Laborious
FRET probe Allelic Discrimination (Hyb Probe® TaqMan®, Beacons® Scorpions®)	++	Low	Moderate
Locked Nucleic Acid (LNA) probe	++	Very low	Very Fast
Oligo ligation assay (SNPlex®)	+++	Moderate	Very Laborious
PCR-Invader® Assay	+++	Moderate	Moderate
High resolution melting (HRM)	++	Very low	Fast
Pyrosequencing*	+++	Very high	Very Fast/ Fast
Peptide nucleic acid-mediated Clamping PCR*	+	Low	Moderate
Gene Chip technology (LabOnChip)*	+++/++++	Very high	Very Laborious
Conventional sequencing*	++	High	Moderate
Next Generation sequencing	++++	Very high	Laborious
D-HPLC	++++	Moderate	Very Fast
PGM™ Ion Torrent	++/+++	High	Fast

[§]Approximate instrumentation list price was scored as + (<10,000€); ++ (<50,000€); +++ (<100,000€), ++++ (>100,000€)
[$]Reagent costs were scored as very low (<5€), low (<10€), moderate (<30€), high (<50€), very high (>50€).
[#]Time-labour refers input needed to perform a single test of multiple samples. It was scored as very fast (<1 hour), fast (<4 hours), moderate (<1 day), laborious (<2 day) very laborious (>2 working day).
*No address to allelic discrimination (mutant vs wild type).

PCR-based techniques are the most common tools used in the context of clinical laboratories for allelic discrimination and genotyping[5]. They include: I) PCR-based protocols without fluorescent probes (ASA, SSCP and RFLP); II) PCR methods combined with hybridization probes (FRET-based platforms, Invader assay and LNA Probes); III) PCR-based with intercalating fluorescent dye as High Resolution Melting (HRM); VI) Denaturing-High Performance Liquid Chromatography (D-HPLC) (PCR required only for sample pre-treatment). Notable in this setting is MALDI-ToF, a mass spectrometry technique that is able to detect and discriminate the alleles with the highest sensitivity and specificity.

New generation and high-throughput platforms are constantly emerging on this scenario, also because of their precision (consensus base accuracy ≥ 99.99%) and long-term cost-effectiveness (especially taking into consideration the time labor)[11-13]. This is particularly evident looking at the enormous interest of Biotech companies for them, resulting in the creation of new patented platforms such as 1G Genetic Analyzer by Illumina/Solexa (Cambridge, UK), Ion PGM™ System by Thermo Fisher Scientific (Waltham, MA USA) and Genome Sequencer FLX by Roche Diagnostics (Branford, CT, USA).

In addition, some new high-throughput sequencing technologies are able to directly genotype genomic DNA without PCR amplification: Golden Gate® Assay and DNA chip-based microarray are the latest developed. Anyway, these are not widely used in the ordinary clinical laboratory setting compared to PCR-based methods[5].

Lastly, it is possible to detect mutant alleles in a given DNA thanks to methods like Pyrosequencing (which require a PCR pre-treatment), PNA-mediated clamping-PCR, gene chip technologies or conventional sequencing methods.

ANALYTICAL VALIDATION AND QUALITY CONTROLS

Analytical assessment

The constant technological improvement allows the introduction of advanced genotyping techniques into the field of clinical diagnostic routine. The subsequently increased availability of these platforms to a growing number of laboratories requires an increased attention in matters of analytical and quality controls. Validation methods have to keep up with this transition.

To use a genotype test in the clinical practice, it must be standardized and validated under the aspects of specificity, sensitivity, reproducibility, accuracy, and sample stability[14]. In the US, diagnostics these products are regulated by the Food and Drug Administration (FDA), while diagnostic services are under the rules of the

Clinical Laboratory Improvement Act (CLIA)[15]. In Europe, it is applied the In Vitro Diagnostic (IVD) policy, without the distinction between commercial products (used by laboratories) and diagnostics service.

Two types of analytical controls must be performed: the internal control (intra-laboratory), checking the whole system from the instruments in itself to reagents and all the equipment needed to perform a given assay, including a periodic analysis of blanks, calibrators and control samples, in order to verify that the obtained data are precise and accurate in every moment; the external control (inter-laboratories), that allows to uniform results, regardless of the laboratory where the test is performed. In this sense. Ahmad-Nejad et al[16], in cooperation with the European Communities Confederation of Clinical Chemistry and Laboratory Medicine, focused the attention on the External Quality Assurance Programs (EQAPs) with the aim of rising the technical and standard quality of genotyping assays and molecular diagnostics.

Quality Assessment

In addition to the analytical validation criteria, no data can be assumed as valid without Quality Controls (QCs). For genotyping assays, QC samples typically consist of positive and negative controls

To accept analytical data from a run of clinical samples, all positive QC samples (bearing each of the potential genotypes of interest) must be assigned to the correct genotype, and the negative QC samples (water/non-PCR amplified templates) must give no genotype results, demonstrating the lack of spurious genotype calls in DNA samples, which indicates the absence of contaminating genomic DNA.

Various QC samples are included in each analytical run. This allows to monitor constantly the method performances, the occurrence of cross-contaminations and the ability of the assay to identify wild-type, heterozygous and homozygous allele combinations. In general, QC samples can represent up to the 10% of the entire analyzed samples in a given analytical run. QCs are subjected to the same criteria used to determine acceptable genotyping calls in the clinical samples and if QC samples fail, the entire analytical run must be repeated.

As PCR is required in almost all the genotyping methods, it represents a crucial step in the overall protocol. The main risk during the process of PCR is contamination. The frequency of these events can be drastically lowered using simple precautions such as wear clean lab coat and gloves, work in a dedicated clean area, using dedicated equipment (periodically decontaminated), storing reagents in aliquots and using of closed-tube homogeneous assays. Anyway, the risk of contamination can never be completely eliminated.

When performing fluorescent-based detection methods, well-to-well variations can be normalized (within-run) in reference to a fluorescent dye, which remains constant throughout amplification, or even using a third fluorescent dye which does not take place in the amplification reaction (e.g. Rhodamine ROX). A genotyping test can be applied to the analysis of clinical samples only after it has been validated, approved and documented.

GENOTYPING COSTS

A crucial issue related to genotype testing regards its costs. Despite at the beginning of the genomic era the effort to perform a genotype assay was very high, with the progression of technologies, hence the introduction of high throughput methods, these costs are drastically lowered. In this sense, diverse cost-effectiveness studies were performed, showing that there is sufficiently robust information for decision-makers to allocate resources to promote the diffusion and the use of genotype testing.

We report some examples of these studies: Aquilante et al[17] compared the accuracy, rapidity, and costs of two methodologies used for genotyping a single variant in the cytochrome P450 (*CYP*) 2C9 gene: the cost per sample for a single SNP detection was estimated to be $1.90 using PCR-Pyrosequencing and $3.14 by RFLP. Another group studied thiopurine S-methyltransferase (TPMT) genotyping before the 6-mercaptopurine treatment in pediatric Acute Lymphoblastic Leukaemia (ALL) estimating the cost for genotyping of TMPT mutation around €150[18]. The role of genotype testing in the clinical practice is also underlined by Plumpton et al[19] which have recently reviewed several papers about the economic evaluations of genotype tests prior treatment with different drugs, providing robust evidence of the cost-effectiveness of this approach.

Genotyping costs can be reduced as little as ~€20 per SNP for "home brew" PCR-based tests, as long as they have been validated and standardized[20]. Although the initial expense that a laboratory has to sustain to set up a genomic-based platform is usually very high (in particular for high throughput methods), it must be said that all the efforts seem to go in the direction of genotyping diffusion for routine clinical practice.

CONCLUSION AND FUTURE PERSPECTIVES

Although there is a compelling need for further extensive studies to establish the cost and effectiveness of genotyping, its usefulness in the oncology field is globally recognized. The personalized medicine allows to drastically reduce overall medical costs as well as the incidence of adverse drug effects, providing a better quality of life.

Over the last decade, medical research has developed and improved specific tools for novel treatments and management of cancer patients. New pharmacogenomic markers are constantly being identified and validated, allowing physicians to tailor specific therapies in reference to individual genetic profiles[21]. It appears obvious that the usefulness of these variants in the clinical practice strictly depends on the improving of the diagnostic tools, as well as on a specialized genomic expertise to efficiently interpret the results of the tests[4].

The integration of genotype testing in the routine clinical practice is an ongoing process. Over the next few years, the emergence of molecular resistance to standard therapies, due to genetic polymorphisms, will lead pharmaceutical and biotechnology companies to join the efforts, towards the development of new, more innovative and cheaper tests for routine diagnostics.

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The authors stated that there are no conflicts of interest regarding the publication of this article.

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