Master Thesis

OVERVIEW ON THE REQUIREMENTS AND LIMITATIONS FOR THE IMPLEMENTATION OF MOLECULAR GENETIC TESTS IN MEDICAL LABORATORIES

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Abstract

This master thesis objectives to explore the interplay between research, regulatory agencies and clinical applications of new genetic tests for cancer care. New strategies in the treatment of tumors have been made possible due to advances in genetic testing research in the last decade. Nonetheless, medical laboratories have been encountering several obstacles for establishing the use of these new technologies, once specific implementation directives have not yet been developed by controlling bodies in many countries. The increasing demand for these tests creates the call for health systems to include genetic tests in their offer. Considering the intrinsic nature of clinical genetic tests and current research, deep modifications on health services implementation models should be considered in order to systematic benefit patients with clinical indications and scientific investigations simultaneously. The purpose of this work is to highlight the current difficulties for genetic testing implementation by medical laboratories and health systems along with indications of possible solutions and pipelines for an efficient health service operation.

Zusammenfassung

Ziel dieser Masterarbeit ist es, das Zusammenspiel von Forschung, Zulassungsbehörden und klinischen Anwendungen neuer genetischen Tests für die Krebsversorgung zu untersuchen. Neue Strategien zur Behandlung von Tumoren wurden durch Fortschritte genetischer Tests in den letzten zehn Jahren ermöglicht. Dennoch stießen medizinische Labore auf mehrere Hindernisse im Rahmen Einführung dieser neuen Technologien, nachdem in vielen Ländern noch keine spezifischen Durchführungsrichtlinien von Kontrollstellen ausgearbeitet worden waren. Die steigende Nachfrage nach diesen Tests führt zu der Forderung, dass die Gesundheitssysteme genetische Tests in ihr Angebot aufnehmen. Angesichts des intrinsischen Charakters klinischer Gentests und der derzeitigen Forschungslage sollten tiefgreifende Änderungen an Umsetzungsmodellen für Gesundheitsdienste in Betracht gezogen werden, um Patientinnen und Patienten mit klinischen Indikationen und wissenschaftlichen Untersuchungen gleichzeitig einen systematischen Nutzen zu verschaffen. Ziel dieser Arbeit ist es, die derzeitigen Schwierigkeiten für die Umsetzung durch medizinische Labors und

Gesundheitssysteme sowie Hinweise auf mögliche Lösungen und Pipelines für einen effizienten Betrieb des Gesundheitsdienstes aufzuzeigen.

Declaration in Lieu of Oath

I hereby declare, under oath, that this master thesis has been my independent work and has not been aided with any prohibited mean. I declare, to the best of my knowledge and belief, that all passages taken from published and unpublished sources or documents have been reproduced whether as original, slightly altered or in thought, have been declared as such at the corresponding places of the thesis, by citation, where the extent of the original quotes is indicated.

The paper has neither been submitted for evaluation to another examination authority nor has been published in this form or another.

Graz, 15 June 2022

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Abbreviations

Technical

NGS – Next Generation Sequencing
FFPE - Formalin-Fixed Paraffin-Embedded Tissue
SOPs - Standard Operating Procedures
CNV – Copy Number Variation
SNP – Single Nucleotide Variation
MSI – Microsatellite Instability
HRD – Homologous Recombination Deficiency
LIS – Laboratory Information Systems
LIMS - Information Management Systems
HIS - Hospital Information System
QA – Quality Assurance
QM – Quality Management
PCR - Polymerase Chain Reaction
NCs - Non-Compliances
FISH - Fluorescence *in Situ* Hybridization
VAF - Variant Allele Frequency
IT - Information Technology
HPO - Human Phenotype Ontology
VUS - Variants of Uncertain Significance
PDCA - Plan, Do, Check and Act
HCPS - Cancer Hereditary Syndromes

Institutions, Authorities and Associations

HGVS – Human Genome Variation Society
ISO – International Organization for Standardization
NCI - The National Cancer Institute
ACS - The American Cancer Society
CAP - College of American Pathologists
CLIA - Clinical Laboratory Improvement Amendments
IQNPath ABSL - International Quality Network for Pathology
NAB - national accreditation body
EA - European Cooperation for Accreditation
OECD - Organization for Economic Co-operation and Development
WHO – World Health Organization
CLIS - Clinical Laboratory Improvement Amendments
IQNPath ABSL - International Quality Network for Pathology
ESP - European Society of Pathology
FDA – Food and Drug Administration
EMA - European Medicines Agency
cIQC - Canadian Immunohistochemistry Quality Control Program

RCPAQAP - Royal College of Pathologists of Australasia Quality Assurance Programs

EA - European Cooperation for Accreditation

OECD - Organization for Economic Cooperation and Development

ESMO - European Society for Medical Oncology

AMP - Association for Molecular Pathology

NCT - *Nationales Centrum für Tumorerkrankungen* from the University of Heidelberg

RIMGC - Roberts Individualized Medical Genetics Center

SUS – *Sistema Único de Saúde*

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1 Introduction

This master thesis aims to explore the interplay between research, regulatory agencies and clinical applications of new genetic tests for cancer care. New strategies in the treatment of tumors have been made possible due to advances in genetic testing research in the last decade.

According to the World Health Organization (WHO), “Cancer is a leading cause of death worldwide, accounting for nearly 10 million deaths in 2020, or nearly one in six deaths” (1). Data from the institution reveals most common cancers are breast, lung and colon/rectum; and the majority of deaths are found among lung, colon and liver cancers. Table 1 shows current figures worldwide:

Most common cancers in 2020	
Breast	2.26 million cases
Lung	2.21 million cases
Colon and rectum	1.93 million cases
prostate	1.41 million cases
Skin (non-melanoma)	1.20 million cases
Stomach	1.09 million cases
Most common cancer deaths in 2020	
Lung	1.80 million deaths
Colon and rectum	916.000 deaths
Liver	830.000 deaths
Stomach	769.000 deaths
Breast	685.000 deaths

Table 1 - Most common cancers and cancer deaths in 2020 according to the WHO (1)

WHO states this adversity can be reduced in 30 – 50% by preventing new cases through reduction of risk factor exposure and new screening methods. This rate can also be improved by early detection and appropriate treatment, many tumors are possible to be cured if timely detection and correct treatment are carried out. (1) A wide range of possibilities have been brought by genetic testing, and multiple gene tests are a reality in cancer care.

1.1 Genetic Testing

The National Cancer Institute (2) defined genetic testing as “The process of analyzing cells or tissue to look for changes in genes, chromosomes, or proteins that may be a sign of a disease or condition, such as cancer. These changes may also be a sign that a person has an increased risk of developing a specific disease or condition. Genetic testing may be done on tumor tissue to help diagnose cancer, plan treatment, or find out how well treatment is working (2).” Mutations can be of a germline or a somatic nature. Germline variants are mutations occurring in germ cells, and this modification can be passed to next generations. Somatic variants are mutations in any other cells and are not able to be transmitted. As it can be implied, on one side germline mutations are relevant considering pathogenicity, and care is oriented for screening and prevention. On the other hand, somatic mutations impact clinical treatment directly (3). The American Cancer Society (ACS) divides genetic testing for cancer in predictive genetic testing and testing cancer cells for genetic changes. Predictive testing comprehends the tests searching for inherited gene mutations that represent a higher risk of developing specific types of cancer. They are recommended for people with peculiar familiar history, for diagnosed patients with a familiar history or certain peculiarities (i. g. early onset) and family members of these last two cases. Testing cancer cells for genetic changes comprehend medical indications by a physician to search for specific mutations in a biopsy. Results will guide treatment and prognosis (4).

Genetic variations can cause a range of consequences in the biology of a cell. Table 2, based on data from Li, Datto, Duncavage et al. (3), structures examples of such modifications in respect with their effects:

Activating mutations		
Gain of function of protein	Consequence: autophosphorylation and loss of downstream regulation leading to uncontrolled proliferation	Example: missense mutation in kinase protein domain
Inactivating mutations		
Loss of function	Consequence: loss of function of tumor-suppressor genes	Example: Nonsense, splice-site, frameshift, indels

Table 2 - Mutations and their effects. Data from Li, Datto, Duncavage et al. (3).

Therefore, the types of mutations are of relevance in testing for genetic alterations. Table 3, with data from Li, Datto, Duncavage et al. (3), displays the type of mutations searched in genetic testing:

Mutation type	Consequence	Clinical examples:
Single Nucleotide Variants (SNVs)	Missense, silent, non-sense amino acid substitution; slice-site	Oncogene *NTRK3* in lung and colon cancers
Copy Number Variations (CNVs)	Loss (deletion) of a gene, gain (amplification) of an oncogene	Loss of tumor suppressor gene *RB1* in retinoblastoma; Gain of oncogene *ERBB2* in invasive breast cancer
Structural rearrangements	Chromosomal translocations, deletions, duplications and inversions	Gene fusions and fusion proteins such as *EML4-ALK* in non-small cell lung carcinoma

Table 3 - Types of mutations in genetic testing. Data from Li, Datto, Duncavage et al. (3) and He, He, Liu et al. (5)

Further aspects searched by oncologic genetic testing are microsatellite instability (MSI) or the homologous recombination deficiency (HRD), which indicate a susceptibility in tumors possible to be pharmacologically targeted (6).

1.2 Genetic Testing and Medical Laboratories

Medical laboratories have been encountering several obstacles for establishing the use of these new technologies, once specific implementation directives have not yet been developed by controlling bodies in many countries. Laboratories habitually undergo several quality control processes, such as external quality assessment programs, accreditation etc., however, against a background of continuous technological updates, validation of procedures represents a complex challenge.

Among the several possibilities to perform genetic testing, this text will consider the novel methods that emerged in recent years relevant for the oncological area. In other words, the consolidation of testes performed by Next Generation Sequencing (NGS) platforms will be the main topic approached in these pages given its increasing implementation. This technology has been growing in preference by laboratories due to its many technological, economical and well-timed advantages.

Experts currently have been discussing the elaboration of laboratorial guidelines that cover specific tests aspects, such as sensitivity, errors, pre-analytical conditions, data storage and interpretations (7)-(13). Chapter one will present an overview on the phases sequencing tests go through in medical laboratories including request, pre-analytical, analytical and post-analytical phase, and reporting. Furthermore, the chapter will discuss laboratory standardization and quality management considering the deficits in regulations for specific sequencing laboratorial procedures. Institutions such as the College of American Pathologists (CAP), the Clinical Laboratory Improvement Amendments (CLIS) and the International Quality Network for Pathology (IQNPath ABSL) provide recommendations to cover this area (10) (11).

1.3 Molecular Pathology

Molecular tests for cancer diagnostics and other biomarkers are a concrete part of oncological care, for instance, immunohistochemistry. Molecular pathology laboratories analyze cancer samples collected from biopsies and screen cells for such biomarkers and, more recently, they build a genetic profile of the tumors. Molecular pathology genetic testing has been increasingly offering genetic analyzes through individual genes analysis and gene panels, the later offered by a sequencing platform or composed by the laboratory itself. The work with NGS, as a novel test method, generates a demand for validated tests, which must comprise: sample handling, choice of correct platform considering divergent sensitivities, among other parameters (14). The consideration of medical indication and observation of guidelines by oncological societies is also of relevance (15). Chapter two will present these topics in more detail. Finally, interpretation of variants configures a key aspect in molecular pathology. The clinical relevance of a mutation is, in many cases, undetermined because investigations are still on course. For this reason, they must be interpreted with aid of many resources and need professionals with this know-how. A discussion about the

use of these resources and the necessity for qualified professionals in the area will be included in chapter two as well (16).

1.4 Health Systems

The increasing demand for these tests creates the call for health systems to include genetic tests in their offer. This matter is the subject presented in chapter three. In order to effect this implementation, concepts of medical utility and awareness about the genetic testing functioning, along with its implications, must be converted into a dynamic system that considers continuous research findings as well (17). Moreover, the present chapter will go through the necessary transformations in health systems in order to include molecular pathology genetic tests in their coverage. Considering the intrinsic nature of clinical genetic tests and ongoing research, deep modifications on health services implementation models should be considered in order to systematic benefit patients with clinical indications and scientific investigations (17).

1.5 Methods

This master thesis presents an overview from current literature on the topics aforementioned. Relevant articles were chosen from search on PubMed engine from 2017 to 2021.[1] Articles from previous years were included if the primary subject was not found in earlier publications. Official guidelines, reports and other regulatory documents were included as the latest versions. Conclusively, the purpose of this work is to highlight the current difficulties for genetic testing implementation by medical laboratories and health systems along with indications of possible solutions and pipelines for an efficient health service operation.

[1] Key words: laboratories genetic testing, quality management genetic testing, molecular pathology genetic testing, genetic testing health systems.

2 External Quality Assessment (EQA) and Guidelines for Laboratories for Genetic Testing

This chapter will present overviews on technical aspects of genetic tests performed by medical laboratories. The content will go through a brief discussion on liquid biopsy as a promising method and already indicated in some clinical cases (as instance, when a biopsy is not possible or high patient morbidity). The chapter will describe NGS functioning, once this technology is currently preferred in parallel analysis of multiple genes. Secondly, the text will visit requirements for laboratory management, considering the work flow from referral to report issuing, presenting also observations on costs. The next section will go through quality standards. Both those related to new genetic test implementation, i. e., validation and verification, as well as accreditation and certification.

2.1 Genetic Tests in Medical Laboratories

The improvement of sequencing techniques over the last ten years brought unlimited possibilities in oncology in what concerns new diagnostic methods, administration of target therapies, prognostic markers, treatment response predictability and adaptation. Oncologists have now modern tools to determine a tumor´s molecular and genetic profile by ordering genetic tests such as single gene tests and gene panels from medical laboratories. Testing laboratories are currently facing challenges to provide qualitative molecular pathology genetic tests, once recent findings develop in a fast pace, demand specialization and correct choice of equipment and platform. In this context, NGS represents the most frequent method adopted by laboratories, although bringing its own obstacles concerning technique, standardization and correct interpretation of results.

2.2 NGS Technique

NGS analysis occurs in four steps that go from sample preparation and library preparation, go through sequencing and, finally, data analysis.

Sample preparation must ensure non-necrotic material containing enough cells. It is usually fixated with formalin-fixed paraffin-embedded tissue (FFPE), while for

hematological cancers, cell count or cytometric flow data, in the case of bone marrow, can be performed for the right fraction of cells. Additionally, solid tumor samples must go through a pathologic microscopic examination.

Library preparation can be performed with two different systems: hybrid capture-based or amplification-based method. Both methods present differences considering error-prone genomic sites and sequence fragment sizes. Data generated by NGS is unique under many points of view: type, organization of sequence information, volume and interpretation approaches (13).

DNA is sequenced by fluorescence signal detection and a process denominated base calling, i.e., nucleotides are identified with a specific software. The particular nucleotide sequences found are organized in short fragments, called reads. The reads, in turn, are evaluated with a score, the Phred-Score, according to their quality levels. Both of these information parts are saved in a file known as FASTQ. Next, another program, in other words a software known as read mapper or short read aligner, will compare the reads with a reference genome and determine the positions of each read in the genome. This process is designated as sequence alignment. The file generated at this point is the BAM, a binary aligned map, although laboratories might also work with CRAM or SECRAM files, the compressed and encrypted forms of BAM, respectively.

There are two other steps included in this phase, which are relevant for the quality of the genetic testing. The annotation will describe the reads position in a chromosome, in a gene, in a conserved or not conserved area, classify if it configures a variation and if it constitutes a repetition of elements or centromere. The “mappability” evaluates the probability the sequence was matched to its correct position, according to its “uniqueness”, given same sequences can appear in different parts of the genome.

Once variants are found, they need to be identified, filtered, in order to remove false-positive and artefacts, annotated and prioritized. Variant calling is the process for a correct identification of single nucleotide variants (SNVs), indels, copy number variants (CNVs) as well as larger scaled alterations in the chromosome structure. This process is structured through algorithms and depends strongly on the reads quality, which must be realigned in this step (12). Table 4 shows some tools for variant calling:

SNVs, indels and called variants	SAMtools	https://github.com/samtools/
Representation and storage	gvcftools	https://sites.google.com/site/gvcftools/home
Representation and storage	Sequence Ontology Genome Variation Format	https://github.com/The-Sequence-Ontology/Specifications/blob/master/gvf.md
Representation and storage	Human Genome Variation Society (HGVS)	http://varnomen.hgvs.org/bg-material/simple/
Representation and storage	Health GAfGa File Formats	https://www.ga4gh.org
Representation and storage	Genome Analysis Toolkit	https://bio.tools/gatk
Representation and storage	Atlas 2	https://www.hgsc.bcm.edu/software/atlas-2

Table 4 - Tools for variant calling based on (12)

Finally, when variants are prioritized, i.e., identified according to its clinical relevance, laboratories must apply hard filters or use a data dictionary. This step includes algorithms that must be revised to meet the clinical relevant criteria, especially considering the constant changes and updates characteristics of this type of data (13).

2.3 Genetic Tests and Laboratory Management

Laboratories face many challenges to ensure new implemented genetic tests are medically relevant, deliver patients benefits, have adequate turnaround times while finding effective cost and schedule management solutions. Launching new genetic test options demands choosing adequate technologies and their kits, which must meet clinical requirements. With respect to those, laboratories must take in consideration the workflow starting from patients' needs to results reporting, having in mind the

requirements of each phase, in order to develop a patient oriented laboratory routine organization.

Laboratory tests pass through three steps, namely, pre-analytical, analytical, and post-analytical phases. The pre-analytical phase covers samples entry, identification and processing until initiation of analysis protocol; the analytical phase covers performance of protocol analysis and results, being the phase most targeted by EQA programs. Finally, the post-analytical phase refers to readouts interpretation and results reporting (11).

There are several aspects to be observed in this course, and laboratories must ensure admissible test requests, pre-analytical sample handling, choice of adequate equipment, materials and reagents and kits for gene panels or customized testing, enough personal expertise and availability and, finally, correct reporting. Moreover, all processing phases should adopt internal and external quality assessments, pursue validation of its methods and subsequently undertake a strategy for accreditation in the long term (11). Figure 1 presents a diagram of this workflow:

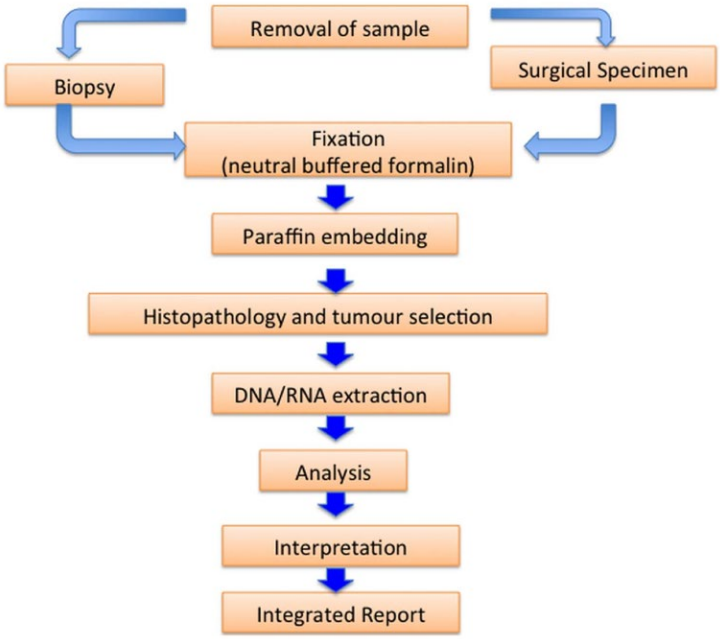

Figure 1 - Phases of molecular pathology tests from Cree, Deans, Ligtenberg et al. (10)

2.3.1 Request and Pre-Analytical Phase

Laboratories must be available to fill the increasing demand for genetic tests and, at the same time, beware of overtesting. Laboratories need to coordinate communication with referring clinics and clinicians, multiple samples entry and storage, turnaround times and test urgency with a management solution. A strategical plan to store samples in order to perform multiple tests simultaneously, in cases when specimens are ideal for such procedures, can configure an alternative for routine management. That would apply in case the test indication is categorized as health check or monitoring. On the contrary, tests for diagnostic or treatment purposes usually must be fast delivered, for instance, as stated by the Austrian Gene Technology Act (18).

Pre-analytical phases have been reviewed by Cree et al. (10), the article provides an overview on samples entry and labeling, tissue fixation, handling, transportation, and summarizes the standard operating procedures (SOPs). The totality of these steps are covered by the norm ISO 15189, and instructions for labeling are also included in the norm ISO 9001 (International Organization for Standardization), relevant for European laboratories. Labeling should be numbered making possible easy location and identification. Patient identification should include at least four codes, such as name, surname, date of birth and hospital. According to Dufraing, Fenizia, Torlakovic et al. (11), tissue samples fixation must be controlled by the laboratory performing the test. Smaller samples can be easily fixated with formalin, however, larger samples require a fixation control step. In this context, transportation should take place under vacuum specially for durations above one hour. It is essential to avoid cold ischemia, which occurs in the meantime between the biopsy extraction and the fixation in the laboratory. Cold ischemia alters the levels of DNA expression both in RNA and protein levels. Moreover, decrease of RNA or DNA levels through decalcification, cross contamination must always be avoided (11).

Finally, attention should be paid to macro or microdissection in what concerns the delimitation of the area to be analyzed, having in mind frequently it is necessary to work with multiple areas in order to investigate tumor heterogeneity. Laser capture microdissection is still considered a tool used mostly for research purposes and has not reached laboratory routine yet (19).

2.3.2 Analytical Phase

DNA extraction should be validated, performed with standard controlled reagents (CE-marked, for Europe). Manual and automated methods can be used for this step, although automated processes can reduce staff workload and prevent mistakes. Quantification can be performed also with a variety of methods such as spectrophometry, fluorometry and PCR. Finally, storage of extracted DNA, PCR products and libraries should be kept in -20º. That is necessary for performing same test multiple runs, one tendencies of sequencing kits indicate an increase of gene panels to screen for actionable genes. It is recommended laboratories in Europe prefer CE marked kits and reagents and test those materials always when starting new batches (10).

Panel design should attend some requirements based on its intended use, considering diagnostic and prognostic or therapeutic targets. The first two, being more specific according to the tumor being analyzed, usually require a coverage of less genes, the ones directly related to the tumor. Indications to design a gene panel are strongly related to scientific literature and must be documented in validation steps. Laboratories also need to consider costs implications, test and interpretation complexity, as well as necessary time and resources while deciding which genes to include in the panels. Similar considerations are valid for the election on which sequencing platform and method, as different technologies will diverge on turnaround times, sensitivity, bioinformatics support, among others. The selected technology must attend these specific requirements and be compatible type of samples received, complexity of genes tested and volume of tests. Finally, depth of coverage is a decisive parameter to assure meaningful genetic testing. Briefly, the depth of coverage relates to the number of reads in a determined sequencing position, being >250 reads the recommended depth coverage (20). This parameter can be influenced, among others, by the sequencing platform or the complexity of the intended region to be analyzed (20).

2.3.3 Results Report

A proper results report is the last stage of consistent gene test, once it represents the document that assures good communication between laboratory staff, pathologists, oncologists and other physicians and health care professionals and patients. Many international institutions, such as the College of American Pathologists (CAP) and the European Society of Pathology (ESP) have defined recommendations for a pertinent report to guide laboratories (11) (10). They comprise general items such as sufficient patient identification, easy-to-read layout, concise and clearly presented information, spellcheck. They include requirements for more specific information such as also possible limitations, information about number of cells or percentage of sample when relevant, type and extent of molecular analysis. An adequate report must allow identification and contact of the professional in charge and their authorization, the same for the responsible pathologist, where legally required. Genotyping should be registered by using the Human Genome Variation Society nomenclature rules (HGVS nomenclature). Lastly, the report should include results interpretation. An integration in a health system or electronic record is recommended in order to allow easy access by all professionals in care team. Good communication between treating oncologist and laboratory is essential in order to allow the integration of information that can influence results and mutation interpretation. Comparatively, information on previous diagnostics, treatment phases or previous treatments and interventions etc. should be communicated to the laboratory.

Finally, laboratories need to achieve a certain number of samples and patients, including a solution for rare mutations and special cases, in order to be able to establish a management system that is cost effective. Laboratories usually work with gene panels and must take into account costs for tests falling out of a “common” category, developing a logistic solution (10).

Tack, Dufraing, Deans et al. (21) states laboratory information systems (LIS) and laboratory information management systems (LIMS) are developing a role of increasing relevance in health records. The authors differentiate the tasks each of these software perform, the first patient centered, and the second, specimen-centered. Usually the LIS is integrated with the hospital information system (HIS). The authors discuss the importance of using a centralized informatic system to not only store data, but to facilitate its integration and aid interpretation as well. LIMS store clinical, anatomical and molecular descriptions, besides patient data. The software can also

read different formats of imported files and export a variety of reports. They can incorporate further resources, such as risk scores, classification tables, therapy schemes etc.) and images.

The current obstacle pointed out by the authors consist in the integration of new types of molecular data into the system, demanding that laboratory staff rely on paper document formats or spreadsheets. These resources have a high risk of error, are time consuming and hinder data tracking. Furthermore, challenges extend to the integration with formats used in sequencing machines and other equipment, such as PCR curves (21).

2.4 Laboratory Quality

Laboratories are controlled by specific institutions in charge of establishing the standardization of molecular tests. For the scope of this work, most relevant institutions are the ones active in Europe and in the USA:

- USA: Clinical Laboratory Improvement Amendments (CLIA) – 1988 – created by the federal government and implemented by the Center of Disease Control and Prevention. Laboratories must have CLIA certification, or approval by the Food and Drug Administration (FDA) or state authorization (attending similar criteria) in order to perform services for Medicare or Medicaid (federal insurance programs for disabled, elderly, low-income populations). Accreditation of pathology laboratories is an affair of the College of American Pathologists (CAP).
- Europe: ISO 15189 (2012), DIN EN 17020, National Accreditation Bodies, Certification for In Vitro Diagnostic Medical Devices Regulation (EU) 2017/746 (IVDR), substituting the Directive 98/79/EC on in vitro diagnostic medical devices in Europe as off May 26 (similar to the FDA approval). Each country is free to regulate their genetic tests; in Austria genetic testing is regulated by the Austrian Gene Technology Act of 1995.

The European Medicines Agency (EMA) covered topics such as good practices in pharmacogenomics, genomic sampling and management of genomic data for clinical trials and guidelines for use and follow-up of treatment with gene therapies (22) (23) (24) (25) (22). The ICH Guideline E18 on Genomic Sampling and Management of

Genomic Data (26) comprises genomic sampling from collection to storage and curation of inventory, covering also quality parameters and transportation. The guidelines also include information on generation, processing and storage of genomic data. The document aims to help new drug development based on genomic data. The Genomics, Genetics, Transcriptomics and Epigenetics Subgroup Report objectives the "characterization and mapping of a type of 'big data', namely genomics data, including epigenetics and transcriptomics data" (25). The report also includes a possible applicability of genomic data in regulatory processes. Furthermore, the report states genomic data held by pharmacological industries should be made available to regulatory agencies for the same intention. The Agency recognizes "a vast number of initiatives are now being performed which do link genomics data to clinical data" (25). The report affirms the access to genomic data would contribute to more individualized patient-based assessment. This availability would facilitate the use of genomic data both for purposes and regulatory purposes. Furthermore, the authors of this report defend the collaboration with academic groups, which is encouraged by theirs participation in the access of such data. Finally, the report indicates the necessity of a system to be accessible from both parts, which is supplied with research data and information on clinical utility. Finally, the report concludes, among other items, the shortage of regulatory guidance on genomic data should be approached. The suggestions include:

- "Consider the need to provide guidance on technical validation of advanced genomics (e.g. sequencing) methods because current guidance is limited.
- Consider the need to provide further guidance on standardization of genomics analysis and data processing techniques, as well as for standardization of data formats for genomics data and/or clinical outcome data linked to genomics data; because currently there is limited guidance available on these topics (25)."

2.4.1 External Quality Assurance

Genetic testing for oncology patients can influence diagnostic, treatment choice and adaptation, as well as monitoring. In case of laboratory errors, consequences can lead to a wrong or delayed diagnostic, suboptimal treatment and false recurring status.

Having that in mind, testing laboratories should have a quality assurance system including internal and external procedures, complying with international standards, which are represented in Europe by the ISO norm, as well as those from international referenced institutions such as the College of American Pathologists (CAP), the Clinical Laboratory Improvement Amendments (CLIA) and the International Quality Network for Pathology (IQNPath ABSL). The latter is an umbrella organization funded by an initiative of the European Society of Pathology (ASP) and gathering institutions all over the world such as the Canadian Immunohistochemistry Quality Control Program (cIQC), the Royal College of Pathologists of Australasia Quality Assurance Programs (RCPAQAP) and the CAP, among others. “External Quality Assurance (EQA), one important component of QA, is mostly a service to diagnostic laboratories by assessment of their testing procedures compared with their peers and/or ´designated true value´. Participation in EQA is highly recommended and often mandated (13).” The IQNPath ABSL has reviewed several EQA programs publishing and updating a guideline with the utmost uniformed requirements for EQAs. Details on necessary requirements and compliance for organizations performing EQA are out of the scope of this work. The table below provides an overview of EQA program items based on Dufraing, Fenizia, Torlakovic et al (11):

1.1	The format of an EQA program should depend on the purpose of the program (integrated approach vs. “test performance characteristic”
1.2	The program should be planned and organized by the EQA coordinator considering advice from experts: medical and technical experts and assessors.
1.3	The time to return results must be pre-defined and monitored.
1.4	ISO/IEC 17043 accreditation is strongly recommended.
2.1	Samples should be fit for purpose in terms of the investigated TPCs.
	Targets should be present in a clinically relevant reportable range, unless pre-determined otherwise.
2.2	If possible, sample matrices should be identical to routine samples. Otherwise, substitute matrices could be used.
2.3	Results for challenging cases should be included in the total performance score, unless more than a pre-defined fraction of laboratories had an incorrect result.
2.4	The EQA provider is responsible for validation procedures and for the selection of validating laboratories where the validation is conducted. The EQA provider should assess the competence of all laboratories chosen to validate EQA materials.

	Validation of EQA samples is defined as reproducibility of the results in at least two laboratories or by different techniques; one laboratory is always a “designated reference laboratory.” This is the required minimum, but the final validation procedures could be more elaborate and may include other TPCs if deemed necessary by the EQA provider.
3.1	Testing of the pre-analytical phase is generally out of scope of these EQAs.
3.2	For scoring of the analytical phase, a two-tiered system can be used.
	EQA providers should define and monitor “technical malfunctions” and “laboratories with frequent technical malfunctions.”
3.3	In schemes with a TPC-focused approach, the following elements should be scored as a minimum: name of the test, sensitivity of the test, and the variants tested. Quality metrics might be scored, depending on the specific methods used for analysis.
	In schemes with an integrated approach or TPC-focused schemes where interpretation accuracy is a TPC, the presence and correctness of the interpretation should be scored in relation to the clinical and methodological information. The test interpretation should be written in a general and directive way, unless national guidelines stipulate alternative requirements.
4.	EQA providers will report (persistent) poor performers to governmental bodies, if these bodies are available. Where such bodies are not available, it is suggested that EQA providers should perform follow-up studies (e.g., request root cause analysis by the participants) or have to rely on national accreditation bodies for suggestions for improvement and/or could perform additional follow-up studies.
5.	The EQA provider should make efforts for clear communication with laboratories before (e.g., scheme purpose), during (e.g., sample handling), and after result submission (individual results, general report, and appeal phase).

Table 5 - Recommendations for EQA programs, adapted from Dufraing, Fenizia, Torlakovic (11)

**TPC – Test Performance Characteristic*

The next figure shows most relevant EQA providers active in Europe:

Agence française de sécurité sanitaire des produits de santé - Afssaps
www.afssaps.fr

Association des Cytogénéticiens de Langue Française - ACLF
www.eaclf.org

Berufsverband Deutscher Humangenetiker e.V. - BVDH
www.bvdh.de

Cystic Fibrosis European Network - CF Network
www.cf.eqascheme.org

Cytogenetics European Quality Assessment - CEQA
www.ceqa-cyto.eu

European Molecular Genetics Quality Network - EMQN
www.emqn.org

European Research Network for evaluation and improvement of screening, Diagnosis and treatment of Inherited disorders of Metabolism -ERNDIM
www.erndim.unibas.ch

External quality assurance in laboratory medicine in Sweden - EQUALIS
www.equalis.se

German Society for Clinical Chemistry and Laboratory Medicine - DGKL
www.dgkl.de

Instand e.V.
www.instandev.de

Italian External Quality Assessment - IEQA-ISS
www.iss.it

Labquality Ltd.
www.labquality.fi

QualiGene
www.qualigene.co.il

Swiss Quality Control Centre -CSCQ
www.cscq.ch

United Kingdom National External Quality Assessment Service for Clinical Cytogenetics - UKNEQAS
www.ccneqas.org.uk/cyton

United Kingdom National External Quality Assessment Service for Molecular Genetics - UKNEQAS
www.ukneqas-molgen.org.uk

Figure 2 - EQA providers in Europe from Berwouts, Morris and Dequeker (9)

Usually, EQAs are performed by sending validated test samples and a survey to participating laboratories. General and specific objectives, as well as test phases included must be clearly defined at the beginning of the EQA program. Evaluated areas include diagnostic, prognostic, and predictive biomarkers for various types of cancer and with different technologies, such as PCR, NGS in situ hybridization and others. Laboratories then operate their analyses and send their results and answers. The EQA provider evaluates al these data and provides a feedback on analyses quality. Moreover, EQA institutions report overviews of the results pointing out common obstacles faced by laboratories, improvement needs, as well as general recommendations in documents structured by laboratory sizes and locations, usually employing a performance score (11).

2.4.2 Accreditation

Quality control procedures have gained increased relevance in medical laboratories and distinctively in institutions offering new developed genetic tests. The International Organization for Standardization (ISO) established standards for testing laboratory procedures in two known documents, namely, ISO 17025, for laboratories in general, and ISO 15189, for medical laboratories. There are no available specific standards for genetic testing laboratories, and accreditation is accomplished based on ISO 17025. That means particularities for genetic testing methods standardization are based on the interpretation efforts for this specific context by the various countries, being independently developed by their own accreditation standards (9).

Figure 3 - Accreditation authorities in different countries from Keppens, Dequeker, Patton et al. (27)

Laboratories aspiring to become accredited usually must first contact their national accreditation body (NAB) and follow their independent instructions for application, definition of accreditation scope, once the laboratory has the choice accredit only specific tests, and accreditation control procedures. The NAB will indicate a technical assessor (team) to run audits and follow up non-compliances (NCs) corrections. Accreditation is usually granted for 3 to 5 years with a regular audit to ensure compliance each 1 to 2 years. Renewing accreditation usually takes the same efforts as the initial procedure, and laboratories are encouraged to expand accreditation scope. Main aspects covered by an accreditation according to ISO 15189

include a quality manual with definitions of SOPs and their documentation, definition of objectives and policies (27). Quality system is formed by two substantial sections: requirements of management and technical part. The next table shows items comprised in each of these parts:

Management	Document control, identification of NC points, implementation of continuous improvement strategies, internal audits, complaints management, supervision of external service providers and suppliers and referral laboratories, contracts and licenses.
Technical	Staff training, spatial arrangement, equipment adequacy, validation, EQAs, maintenance and calibration.

Table 6 - Items in quality system. Data from Keppens, Dequeker, Patton et al. (27)

NABs have been aligning themselves and signing cooperation agreements with major bodies, for instance the European Cooperation for Accreditation (EA), which, in turn, collaborate with the International Laboratory Accreditation Cooperation.

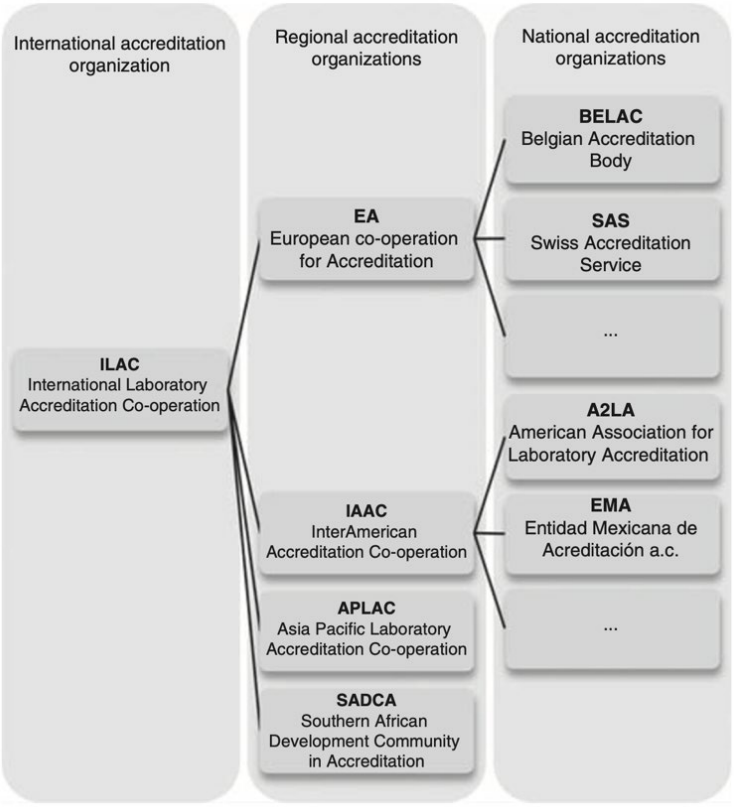

Figure 4 - Organization of accrediting authorities from Berwouts, Morris and Dequeker (9)

Verification and validation constitute important requirements for an accreditation of a laboratory. According to ISO, verification is outlined as the process that confirms test requirements have been fulfilled through proper evidence observation, in other words, if the test procedures are appropriate. Validation is understood as the process that confirms the requirements for a determined intended use or application have been fulfilled by observing objective evidence, which means correct choice of a test for a given intent (8).

Essentially, before a test is implemented, the laboratory must ensure its technical competence and have a justification for tests objective. This course of a test implementation should consider test design, including evaluation of methodology, protocol, reagents and controls, as well as comparisons with gold standards, for instance. The test objects, i. e., the genes and variations being analyzed, must be clinically relevant. Furthermore, both aspects must match, meaning technical conditions should ensure a test is giving *de facto* the clinically relevant results intended. In this respect, evaluation of tests observe an interval of critical parameters, which signifies tests falling out of this limitations cannot be implemented before a phase of necessary methodological corrections. An applicable example for this situation would be, e.g. DNA concentrations or protocols for DNA extraction (8).

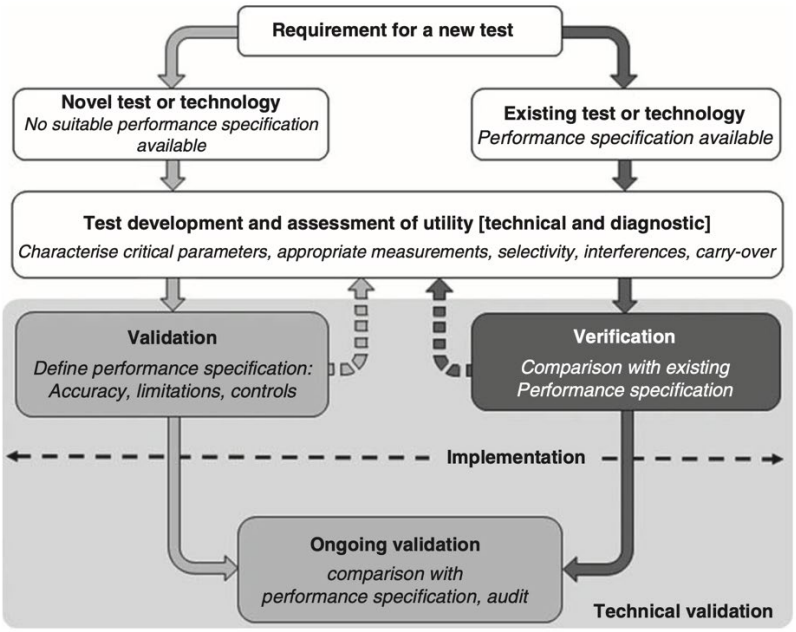

Figure 5 - Validation and verification from Mattocks, Morris, Matthijs et al. (8)

In the beginning of the 2000´s, the Organization for Economic Cooperation and Development (OECD) has observed increasing genetic tests being performed in participating countries with practices that included international exchange of data and samples. This created an environment of potential juridic disparities across borders, considering the variation in accreditation, licensing and certification regulations in member countries. In turn, those were not defined for the specific genetic context (28). The OECD has recognized the necessity of a best practices document along with guidelines comprehending quality of genetic testing procedures, as well as data protection and sample handling. The OECD Guidelines for Quality Assurance in Molecular Genetic Testing was issued in 2007 and, although it does not compose a formal regulator, it is a pertinent document in what concerns genetic testing specificities standardization. It covers quality assurance, EQA, results reporting and staff training (9) (29).

Tack, Schuuring, Keppens et al. (7) analyzed the performance of several laboratories in many countries which took part on EQAs organized by the European Society of Pathology (ESP) with the goal to investigate which laboratory aspects impact quality in genetic testing. The experts collected information on laboratory attributes and classification and measured the percentage of errors and failures. Finally, they performed an analysis to associate EQA results with this data. The study focused on tests for confirmation of *KRAS* and *NRAS* in cases of metastatic colorectal cancer (mCRC) and active variation of EGFR in cases of non-small-cell lung cancer (NSCLC). *ALK* and *ROS1* were also analyzed, as they are relevant for treatment with tyrosine kinase inhibitors (TKI) as well. Table 7 shows the number of participant laboratories and results obtained from 2010 to 2015.

EQA scheme	Scheme year	Number of samples distributed	Number of participants	Number of successful laboratories (≥90%)	Percentage analysis errors	Percentage technical errors
Colon (*KRAS/NRAS*)	2010	10	103	598	4.3%	1.4%
	2011	10	124			
	2012	10	105			
	2013	10	131			
	2014	10	125			
	2016	10	123			
EGFR	2013	4	106	280	8.7%	5.0%
	2014	9	144			
	2015	9	114			
	2016	10	97			
ALK FISH	2012	5	54	379	3.2%	4.3%
	2013	5	100			
	2014	8	116			
	2015	10	111			
ALK IHC	2012	8	29	296	4.8%	1.2%
	2013	12	48			
	2014	9	96			
	2015	5	95			
ROS1 FISH	2014	8	56	92	2.6%	7.4%
	2015	9	68			
ROS1 IHC	2014	10	31	42	8.9%	0.2%
	2015	5	31			

Educational cases were excluded, as they were not taken into account to determine the EQA score
EQA external quality assessment

Table 7 - Laboratories and results in EQA programs from Tack, Schuuring, Keppens et al. (7)

The publication showed “The insufficient performance of laboratories in EQA together with the increasing number of biomarker tests are subjects of concern (7).” In reference to accreditation status and volume of samples processed, the authors concluded: “First, laboratory accreditation is needed to ensure high quality and reliable implementation of new diagnostic molecular biomarkers. Second, university and research laboratories reach more optimal results. Third, larger number of samples tested per year proved to be an indicator for good EQA results, implying more centralization of biomarker testing to reach sufficiently high volumes (7).”

3 Molecular Pathology

This chapter will discuss the main area in oncology where genetic testing has been increasingly finding applications, Molecular Pathology. The first part will go through a general overview on Molecular Pathology and how it has been transformed by the arrival of analytical possibilities enabled by NGS. Afterwards, the focus will be given to technical aspects to be observed while performing NGS testing in order to offer adequate analysis to patients. Further, interpretation of mutations and actual clinical indications will be discussed taking into consideration the scenario of intense research. Concepts for new training programs for professionals in this area are the subject of some discussions in the literature, which will be approached directly after. Lastly, this chapter will introduce the discussion on the emergence of personalized medicine taking into account.

3.1 Brief overview on Molecular Pathology

Since the finding of chromosomal alterations in cancer cells by the scientist Theodor Boveri in 1905, many discoveries have been unfolded, which brought further elucidation of malignant diseases mechanisms and consequently allowed the emergence of new diagnostic and therapeutic methods. “Molecular oncology is a research area investigating molecular principles of cancerous diseases. It is assumed that new and effective diagnostic and therapies can be developed once molecular principles are understood (30).” In this framework, medical laboratories witnessed the emergence and consolidation of a new area, coined under the term “Molecular Pathology”. It comprehends diagnostics through molecular analysis of different samples, such as organs, tissues and bodily fluids. On its turn, Molecular Pathology is undergoing more impacting transformations with the arrival of several sequencing technologies. Included in this scope, the most recent findings in oncogenetics unveiled a range of possibilities in molecular diagnostics additionally to other already consolidated methods, such as immunohistochemistry. Over the last decades, clinical routine and quality of health care in oncology have been deeply transformed. Diagnostic processes now include a range of pathogenic mutations in accordance to updated oncological guidelines. The following table, from Di Sanzo, Cipolloni, Borro et

al. (31), illustrates the large cipher of approved targeted therapies in the USA based on genetic diagnostic and configuring a personalized medicine option.

Gene	Tumor Type	Targeted Agent
HER2	Breast cancer and gastric cancer	Trastuzumab
BRAF	Melanoma, colorectal cancer and thyroid cancer	Vemurafenib
BCR-ABL	Chronic myleloid leukemia, GIST and myeloproliferative disorders	Imatinib
KDR	Gastric cancer, colorectal cancer and lung cancer	Ramucirumab
SMO	Basal cell carcinoma	Vismodegib, Sonidegib
CD274	Bladder cancer	Atezolizumab
VEGF	Brain cancer, cervical cancer, colorectal cancer, kidney cancer, lung cancer, ovarian epithelial cancer, fallopian tube cancer and primary peritoneal cancer	Bevacizumab
MTOR	Brain cancer, breast cancer, kidney cancer and pancreatic cancer	Everolimus
HER1/HER2	Breast cancer	Lapatinib
HER2	Breast cancer	Pertuzumab
CDK4/CDK6	Breast cancer	Palbociclib
EGFR	Colorectal cancer, head and neck cancer	Cetuximab
EGFR	Colorectal cancer	Panitumumab
PDCD1	Kidney cancer, lung cancer, lymphoma and melanoma	Nivolumab
MS4A1	Leukemia and lymphoma	Rituximab
BCR-ABL	Leukemia	Dasatinib
CD52	Leukemia	Alemtuzumab
EGFR	Lung cancer	Gefitinib
EGFR	Lung cancer and pancreatic cancer	Erlotinib
CD38	Multiple myeloma	Daratumumab

Table 8 - Targeted therapies approved by the FDA from Di Sanzo, Cipolloni, Borro et al. (31)

**Abb.: GIST: gastrointestinal stromal tumor*

From another perspective, efforts to organize resources for oncological care and research, such as online accessible databases and biobanks configure another transformation in the field.

“The Cancer Genome Atlas (TCGA) represents one of several international consortia dedicated to performing comprehensive genomic and epigenomic analyses of selected tumor types […] Thirty-three tumor types (selected by histology or tissue of origin, to include both common and rare diseases), comprising >11.000 specimens, were subjected to DNA sequencing, copy number and methylation analysis, and transcriptomic, proteomic and histological evaluation. Each cancer type was analyzed individually to identify tissue-specific alterations, and make correlations across different molecular platforms (32).”

A platform with multiple resources was released from this effort and can be accessed online on https://portal.gdc.cancer.gov. The platform considers pathological specification of these tumors and their correlations to molecular and genomic profiles, counting with a digital tissue library.

Among relevant project´s main findings for understanding and treating different types of tumors is that some cancers will present a very specific molecular profile, such as clear cell renal cell carcinoma, and indicates the constitution a group of its own. Opposed to this, other types of cancer, such as breast invasive ductal carcinoma, present a large variation of molecular profiles within the same histological group. A third observation is molecular profiles are kept in other histological tumor groups that appear in different anatomical locations. Moreover, it was observed gene expression and methylation profiles correspond to the tissue of origin over genomic alterations, leading to the relevance of cellular context. These knowledges influence the search for points to target while treating these cancers.

Projects results also pointed out two categories of oncogenic processes: copy number-driven and mutation-driven. The first resulting from early mutations in *TP53*, highlighting the importance of genome stability regulation. Further somatic copy number mutations include the amplification of following genomic sites, singularly in epigenomic areas:

- Sites containing oncogenes: *CCND1*, *CCNE1*, *MYC*, epidermal growth factor receptor (EGFR), *ERBB2*, *MCL1*, and *MDM2*
- Telomere, histone, chromatin maintenance: *TERC*, *RMRP*, *WHSC1L1*, *BRD4*, *KAT6A*, *KAT6B*, *NSD1*, and *PHF1*
- Hot spot mutations in chromatin regulator genes: *ARID1* and *CTCF*.

On the other hand, strategies to analyze mutation-driven tumors branch in other two ways: study of driver mutations or study of overall genomic structure and cancer features (32).

Di Sanzo, Cipolloni, Borro et al. (31) evaluated the recent transformations in different clinical areas, elaborating diagrams for treatment taking into consideration the moment to prescribe tests for drug response and resistance:

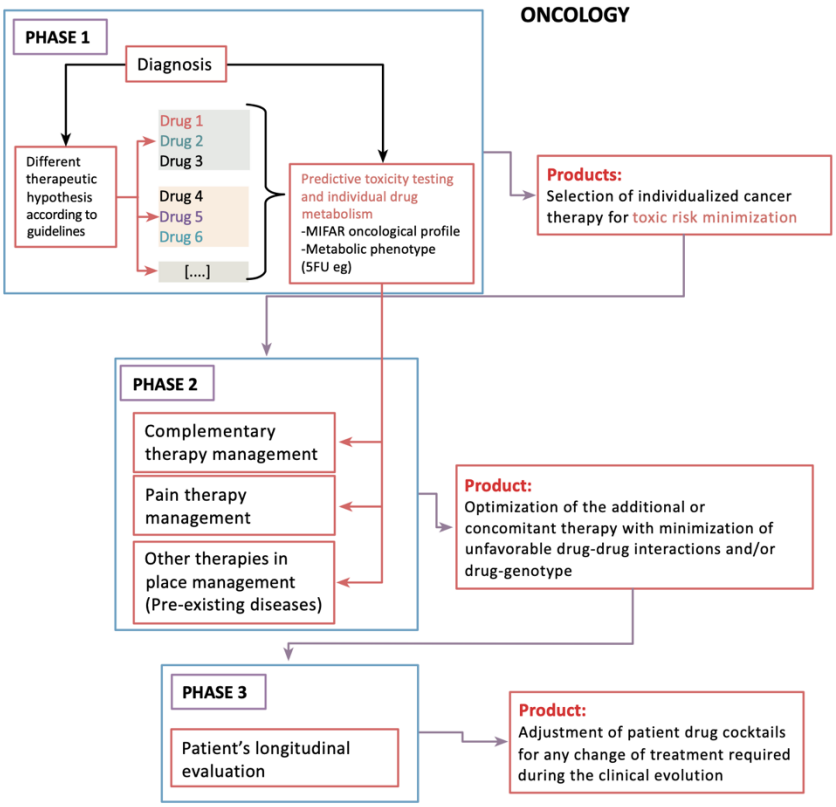

Table 9 - Workflow in oncology with genomic data strategy from Di Sanzo, Cipolloni, Borro et al. (31)

3.2 NGS in Molecular Pathology

The exhaustive analysis of multiple genes (gene panels) and molecular profiling are the main development brought by the recent progress in sequencing technologies. The advances in NGS platforms and their cost reduction per analysis over the last years has transformed this equipment into the main system for the performance of such tests, although consolidated technologies, such as Sanger sequencing or FISH, are still in broad use. Testing for large mutations such as chromosomal aberrations, or performing qualitative analyses of few genes without a need to deep explore molecular mechanisms present in such mutations configure the domain of Fluorescence in situ hybridization (FISH) and Sanger sequencing, according to recommendations of oncology guidelines. (33)-(34) Notwithstanding this framework, NGS applications offer a more detailed level of analysis and also enables multiple genes testing at a time. Consequently, NGS platforms have been increasingly favored by medical laboratories and gained a firm ground in Molecular Pathology (35). Fiorentino, Scarpelli, Lopez-Beltran et al. (36) anticipate that NGS will in the long run take over all testing functions.

Voelkerding, Dames and Durtschi (37) affirm that Sanger sequencing offers a significantly lower analysis cost, however, the expanding scope of gene analyses, namely the capacity to process great amounts of sequence information with high sensitivity, led laboratories to increasingly adopt NGS, especially in areas dealing with tumor cells. Challenges for the clinical use of NGS remain in its streamlining process and in the validation to ensure accuracy and the bioinformatic pipeline for the clinical interpretation of results (37).

The arrival of new genetic diagnostic and prognostic markers tested from tissue samples imposes challenges for an adequate laboratory analysis. One of the changes brought to the processing of samples consist in a significant decrease of amount of material, nonetheless, sample handling is of crucial importance for sequencing correctness. Fassan (35) has gathered relevant steps, i. e., a molecular assessment, that should be observed while dealing with samples for molecular pathological analysis. The sample must configure an authentic representation of the case, which means, besides checking for sampling errors and selection of a proper area, the sample must be tested for the presence of necrotic and/or inflammatory cells, histopathology must be confirmed. Another step configures the choice of correct technology according to the test sensitivity, including elimination of artifacts. Finally, tumor heterogeneity must also be taken into consideration. In what refers to the proper samples handling, Fassan (35) writes a validation strategy must be striven by observation of analytic sensitivity and specificity properties as well as consideration of only necessary genes specified in guidelines. Deans, Costa, Cree et al. (10) examined fundamental features for the implementation of valid genetic tests in diagnostic laboratories. Namely, the definition of analysis range (genome region at issue), technical coverage and accuracy, regular test efficiency parameters such as repeatability, reproducibility and analytic sensitivity and specificity. The table below shows in greater detail aspects involved in these features.

Test performance characteristic	TPC applied to NGS	Metrics and notes on assessment
Reportable range	The region of the genome in which a sequence of acceptable quality can be derived (may not be a contiguous region)	Reporting range must be confirmed during test validation
Reference range	The spectrum of normal variation of sequence within the population that the assay is designed to detect.	Test results outside this range may be clinically significant and require additional investigation.
Limit of detection (LOD)	The lowest allele frequency to which the assay can detect with an acceptable quality to enable confidence in a result i.e. the LOD is within the reporting range, (establishes the detection limit for sequence variants)	Minimum and maximum amount of DNA for 95 % test runs with adequate "no call" rate Allelic read percentage Sensitivity of the assay must be defined within the reporting range of allele frequencies and amount on input DNA for which the LOD was defined.
Repeatability	Concordance of variant detection between runs from the same sample under the same conditions e.g. prepare different libraries from the same samples run at the same time with the same operator and same instrument (within-run or intra-batch variability)	Analyse adequate number of runs depth of coverage Uniformity of coverage Transition/transversion ratio Pair-wise agreement
Reproducibility	Consistency of results from the same sample under different variations in conditions e.g. between different runs, different sample/library preparations, by different operators, or using different instruments (between-run or inter-batch variability).	Analyse adequate number of runs Depth of coverage Uniformity of coverage Transition/transversion ratio Pair-wise agreement
Accuracy (if reporting VAF)	The degree of agreement between the nucleic acid sequences derived from the NGS assay and a reference sequence (a measure of sequencing accuracy and error rates)	Adequate depth of coverage Uniformity of coverage Positive percent agreement Negative percent agreement Technical positive predictive value Rate of "no call" Allelic read fraction (number of independent reads assessed when calling a variant)
Precision (if reporting VAF)	The degree of agreement between replicate measurements of the same material across users and runs (a combination of reproducibility and repeatability)	Analyse adequate number of samples Depth of coverage Uniformity of coverage Transition/transversion ratio
Analytic sensitivity	The proportion of samples that test positive for a sequence variation and are correctly classified as positive (=TP / (TP + FN) (false-negative rate)	Depth of coverage Number of independent reads used to make a base call. This is dependent upon the amplifiability of the template DNA in the assay. Evaluation of base quality scores and signal-to-noise ratios
Analytic specificity	The proportion of samples that test negative for a sequence variation and are correctly identified as negative (=TN / (TN + FP) (false-positive rate) Some laboratories establish specificity by calculating the number of false positives per assay run.	Coverage (read depth and completeness) Number of independent reads assessed when making a base call Evaluation of base quality scores and signal-to-noise ratios. Potential for cross-reactivity and interfering substances Cross-contamination
Sequencing depth and allelic frequency cut-offs	The minimum sequencing coverage necessary for confident detection and variant calling (established for different variants)	

Table 10 - Test performance characteristics (TPC) of NGS from Endrullat, Glökler, Franke et al. (14)

**VAF - Variant allele frequency*

Uzun and Sarioglu (38) show the importance of correct sample selection for molecular molecular pathology analysis, demonstrating also the meaning the cell count, size sample and this ratio can have in a diagnostic aproach. Their figures, reproduced in figures 1 and 2 present clearly this argument:

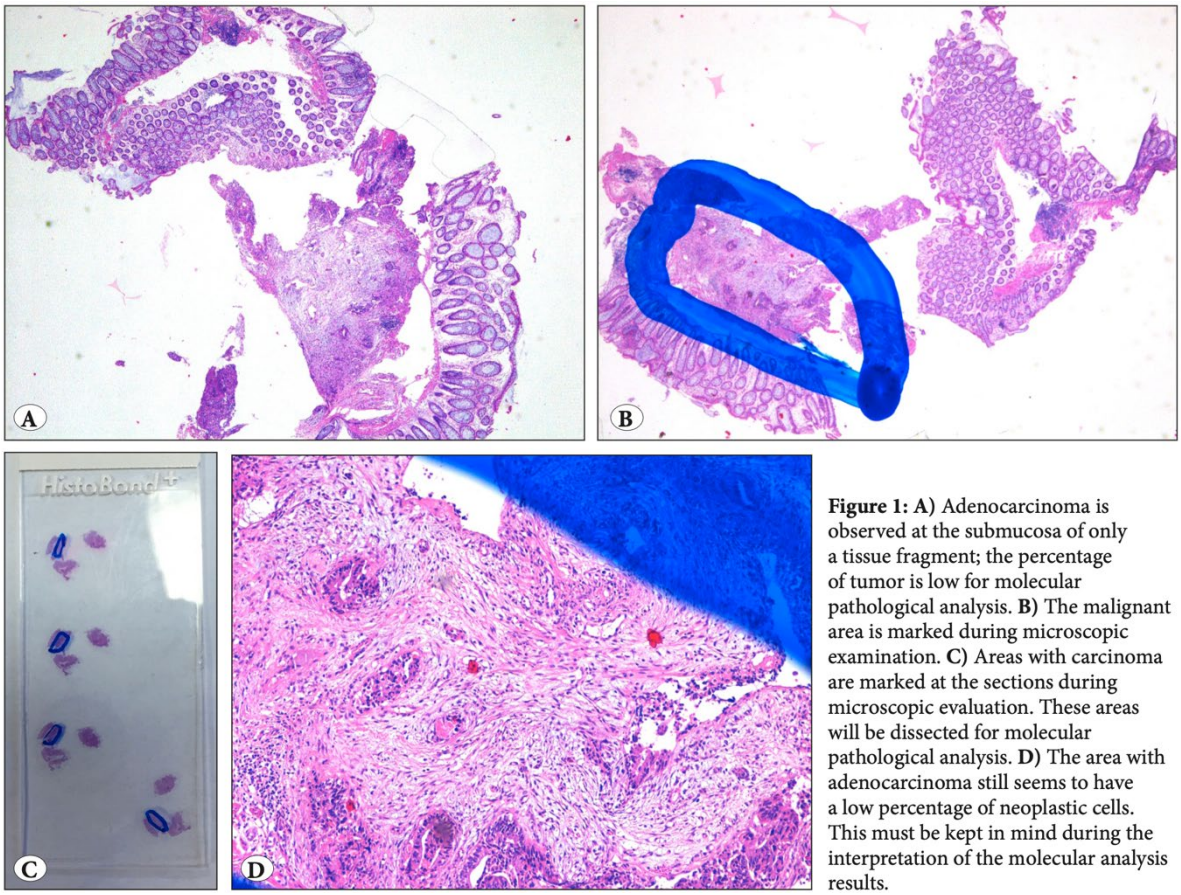

Figure 1: A) Adenocarcinoma is observed at the submucosa of only a tissue fragment; the percentage of tumor is low for molecular pathological analysis. **B)** The malignant area is marked during microscopic examination. **C)** Areas with carcinoma are marked at the sections during microscopic evaluation. These areas will be dissected for molecular pathological analysis. **D)** The area with adenocarcinoma still seems to have a low percentage of neoplastic cells. This must be kept in mind during the interpretation of the molecular analysis results.

Figure 6 - Sample selection for molecular pathology analysis from Uzun and Sarioglu (38)

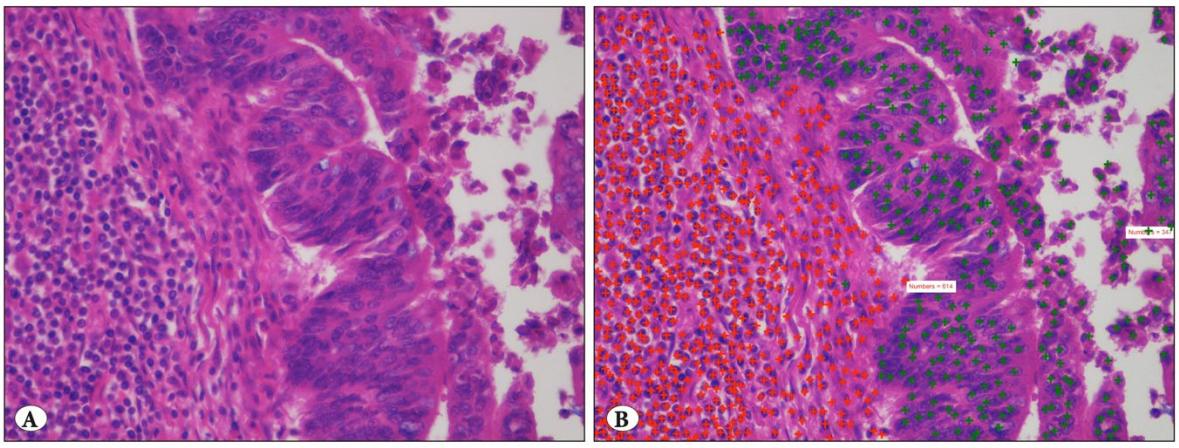

Figure 2: A) Colon adenocarcinoma; half of the area of the image is covered by the neoplastic lesion. **B)** Nuclei are counted using the image analysis program allowing the pathologist to click to select each point; the adenocarcinoma nuclei count was 347 but the non-neoplastic nuclei count was 614. These image and point counting results highlight the discrepancy between area and tumor nuclei percentage.

Figure 7 - Sample selection for molecular pathology analysis from Uzun and Sarioglu (38)

Specialists on behalf of IQN Path ASBL examined the implementation of NGS in laboratories performing molecular pathology tests with respect to testing strategy, implementation of testing within clinical service, sample requirements, data analysis

and reporting of results (10). The authors reviewed extensively the main points to be considered for laboratories to competent offer sequencing tests in oncological area.

Tissue sample should be assessed to meet criteria of enough material provided, minimum of neoplastic cells, adequate preprocessing, fixation (such as FFPE) and transport.

Preparation of nucleic acids must observe quality (concentration, purity) and quantity. Nucleic acid with purity levels below acceptance criteria, might not react properly, making sequencing quality poor (such as lower number of reads). This will produce a sub-optimal test performance, given such tests have a requirement in order to fulfil validity criteria. A non-observance of both requirements could bring false negative results, which in turn can lead to sub-standard or false treatment and test will most likely have to be repeated.

Sample identification: samples must be completely traceable, a failure in the identification can result in false treatment, delay of care. In cases when a sample is not correctly identified, the laboratory has to order the to repeat of sample collection and testing. A check of laboratory equipment and reagents is important to assure quality management and SOPs. Reagents must be verified in respect to their expiring date, storage, and equipment should be properly calibrated and undergo maintenances. Compromising reagents will have reduced enzyme activity and poor ligation properties.

NGS libraries are another topic that can influence sequencing test results and should have a minimum concentration and a library preparation quality control system. Each of the preparations must be checked for quality, otherwise the test will be performed with inaccurate coverage or bias. The choice of another method, such as PCR, can help cross check if the results are adequate.

The sequencing should occur with the expected depth, quality assessment metrics, quality management (QM) of base calling and sequencing accuracy (Q score). In case sufficient depth is given for the relevant sequenced area (the actionable mutations), the test might be accepted for reporting, however in cases of unclear data, the whole test might have to be repeated. Comparable criteria must also be observed for variant detection and reporting: sufficient average read length, gene coverage and sequence depth. Further factors to be contemplated to orient interpretation are the following: allelic frequency, presence of variant in both strands, comparison to reference genome with help of validated software and databases.

Finally, the bioinformatic section also ought to obey some key points such as software adequateness and necessary updates, reference sequence check. If these last two steps are not compliant, variants can be omitted or called inadequately.

Sometimes exceptions to protocols are acceptable, as long as they have been conformingly documented and their impact is assessed. Quality management and compliance should be kept as close to the standards as possible. Moreover, Fiorentino, Scarpelli, Lopez-Beltran et al. (36) forecast a laboratory information system that will comprehend the integrity of the molecular pathology workflow from sample collection to reporting.

The first item includes purpose of testing, which means, medical indication for a gene panel must be clear. Relevant world organizations such as the Association for Molecular Pathology (AMP) and the European Society for Medical Oncology (ESMO) currently recommend testing of only those groups of genes for each type of tumor that are actionable and that have demonstrated its relevance on therapy algorithms and a gain for the patient. Nonetheless, laboratories offers include testing for a number of mutations still being analyzed in different types of clinical trials and genetical studies. According to Deans, Costa, Cree et al. Guideline´s (10) those should be only tested in the scope of clinical studies and against adequate patient stratification.

The expansion of both individual gene and panel gene testing for tumor diagnostic can be seen in the updated oncological guidelines from the European Society of Medical Oncology (ESMO). The following table summarizes the current indications for genetic testing in the diagnostics or prognostic of many tumors. A key point to be observed, many cancers can be caused by virological agents, whose presence can be verified through further genetic testing. Such methods lay beyond the scope of this project. Guidelines also mention relevant genes which can be associated with a disease, but still lack enough scientific and translational evidence to assume the function of a marker. Those can be diagnostic, or prognostic, significant in the future, and must be taken into consideration in the clinical interpretation of found variants (39).

Endocrinology			
Cancer Type		Indication	Specific cases
Thyroid Cancer	Differentiated and poorly	- *BRAF, RAS* Profile for *FTC, NIFTP, PCTs*	

	differentiated thyroid cancer	- *RET* Fusion for Follow-up	
	Anaplastic thyroid cancer	- *BRAF*	
	Medullary thyroid cancer	- *RET* Fusion, *RAS*	
Gastroendopancreatic neuroendocrine neoplasms		- *DAXX/ATRS*, *P53/RB* for NET stage G3	
Upper Gastrointestinal Cancer			
GIST		- *BRAF*, *KIT*, *NF1*, *PDGFRA*, *SDHA*, *SDHB*, *SDHC*, *SDHD*, *PIK3CA* - Fusion genes	
Gastric Cancer		- Family history - MSI - EBV	*ATM*, *BRCA* 1 and 2, *EGFR*, *ERBB2/3*, *FGFR2*, *MET*, *NTRK1*, *NTRK2*, *NTRK3*, *PIK3CA*, *RET*, *ROS1*
Hepatocelular Carcinoma		- HCV	
Pancreatic Cancer		*GNAS*, *KRAS*	hENT1
Lower Gastrointestinal Cancer			
Metastatical Colorectal Cancer		*RAS*, *KRAS*/*NRAS* for treatment with anti-EGFR *BRAF* MSI	*ERCC1*
Melanoma			
Metastatic Melanoma		*BRAF*, *NRAS*, *CKIT*, *GNAM*, *GNAQ*	Mandatory if unresectable, stage

			III, IV; and resectable, stages IIc and IIIb-c
Head and Neck			
Squamous Cell Carcinoma	(oral cavity, larynx, oropharynx, hypopharynx)		HPV, if positive: *PI3KCA* *TP53*, *CDKN2*
Nasopharyngeal Carcinoma		EBV, HPV	
Breast Cancer			
Early Breast Cancer		*BRCA1* and *BRCA2* if familiar cases and high risk patients	Familiar cases and high risk patients
Advanced Breast Cancer		*PIK3CA* PDL if triple neg. MSI MMR *NTKR* Fusion	
BRCA (Familiar Syndrome)		- If more than 3 familiar cases of Breast and Ovarian Cancer in women younger than 50 y. - 2 familiar cases of Breast Cancer in women under 40 y. - Ethnic Ashkenazi - Gynecomastia - If early onset/bilateral Ovarian Cancer	
Gynecological Malignancies			
Epithelial Ovarian Carcinoma		*BRCA*, HRD	
Non-Epithelial Ovarian Cancer		*FOXL2*, *DICER1*	

Cervical Cancer	HPV	
Endometrial Cancer	*PTEN*, *PAX2*, *WT1*, *PIK3*, *AKT*/mTOR, *RAS-MAPK* HRD, MSI	
Lung and Chest Tumors		
Early and Loc. Advanced Non-small Cell Lung Cancer	*EGFR*, *ALK*	
Metastatic Non-small Cell Lung Cancer	*EGFR* (Exon 19/21 – *L858R*) *ALK* – FISH *ROS1* – FISH or NGS *BRAF, NTRK* If reincident*: T790M*	
Small-cell Lung Cancer	TMB (Tumor Mutation Burden)	
Malignant Pleural Mesothelioma		Del p16
Thymic Epithelial Tumors	*MEN1* - if familiar	
Leukaemia and Myeloma		
Acute Lymphoblastic Leukaemia (ALL)	Philadelphia Chromosome / *BCR-ABL* (RT-PCR or FISH) TKI Sensitivity Further: *MLL*; *TP53*, *CREBBP*, *RAS/NRAS/KRAS*, *FLT3*, *NF1*	
Acute Myeloblastic Leukaemias (AML)	t(15;17) – (q22;q12)	

	t(8;21) – (q22;q12) inv(16)(p13.1q22) oder t(16;16)(p13.1q22) - RT-PCR or FISH *CEBPalpha*, *FLT3ITD*, *NPM1*	
Chronic Myeloid Leukaemia (CML)	Philadelphia Chromosome / *BCR-ABL* (RT-PCR oder FISH) TKI Sensitivity	
Multiple Myeloma	del (17p) t(4;14) t(14;16)	
Urogenital Cancers		
Prostate Cancer	If familiar and metastatic: *BRCA1* and *BRCA2*	For risk patients: *DDR*
Renal Cell Carcinoma	*MET*	*EGFR*, *SET*, *CDKN2A*, *TFE3*, *TP53*, *VHL*, *PBRM*1, *BRCA1*, *BAP1*
Testicular Seminoma and Non-seminoma	Isochromosome 12p miR 371a-3p	
Lymphomas		
Diffuse Large B Cell Lymphoma	HCV	
Mantle Cell Lymphoma	t(11;14)(q13;q32) Cyclin D1 - If neg.: *SOX11*	
Waldenström´s Microglobulinaemia	*MYD88* *CD79B*	

Primary Cutaneous Lymphomas	*MYD88*	
Peripheral T Cell Lymphomas	HIV, EBV	
Extranodal DLBCL and PMBCL	*REL*, *TNFAIP3*, *SOCS1*, *MYD88*, *CD79a*, *B2M*, *CHTA*, *FOXP1* del (6p21.32) – *HLA* *BCL2* und *BCL6* HCV, HIV	
Hairy Cell Leukaemia	*BRAF* (Exon 15)	
Marginal Zone Lymphomas	*MYD88* – PCR t(11:18)(p21;p21) – FISH *NOTCH2*, *KLF2*, *TP53*	
Follicular Lymphoma	t(14:18) HCV, HIV	
Chronic Lymphocytic Leukaemia	*CD23*: RT-PCR for Cyclin D21 t(11:14) IGHV - Sanger or NGS *CD* 80 del (17p) - if neg.: *TP53* – Sanger or FISH HCV, CMV, HIV	
Sarcoma		
Soft Tissue and Visceral Sarcomas	*NTRK*, *TP53* (for patients under 46 y.)	
Bone Sarcomas	*TP53* if familiar and age under 46 y.	

	If multiple onset and under 46 y. Mandaroty for differentiation between Ewing and RCSs: *EWSR1*, *ETS* Fusion, *CIC*, *BCOR*	
Central Nervous Systems Tumors		
Gliomas (Astrocytomas, Oligodendroglioma, Glioblastoma)	For tumors from WHO Stage II *IDH1*, *IDH2* ○ If confirmed mutations: 1p/19q, *TP53*, *ATRX* and *LOH* 1p/19q ○ If wild typ: *TERT*, *EGFR*, *TP53* und *H3F3A*, *HIST1H3B*/*C*, *TP53*, *ATRX* CNVs MGMT Promoter methylation	

Table 11 - Current indications for gene testing from ESMO Guidelines (53)-(90)

3.3 Variant Interpretation

Laboratories must perform variants interpretation and report these. A number of resources, such as databases, recommendations by societies and publications should assist this step. However, significant differences in variant interpretation methodology are still found between diverse laboratories (3). Li, Datto, Duncavage et al. (3) published standards and guidelines for variant interpretation and reporting pursuing the standardization of this step. Authors organized the key points to be observed while making use of genomic databases. Laboratories should be able to comprehend

databases content and how information is added and processed. There are limitations in the scope of each database that must be understood. Laboratories must access updated documentation of the database and verify the corresponding literature. Attention should also be paid to the versions of human genome in use, as well as genomic coordinates. The quality of the genomic information being accessed must also be critically evaluated with help of literature, deepness of studies, other databases, number of entries, molecular mechanisms and drug response (3). Special attention must be given to In Silico tools. These algorithms assist predicting if a mutation will influence the structure and function of the protein. Nonetheless, they have a moderate specificity (60-80%) and can lead to over-prediction of negative influence. The interpretation of typical cancer mutations while applying In Silico tools is a complex process needing adequate expertise and can lead to equivocal results. Li, Datto, Duncavage et al. (3) exemplify the common mutation *BRAF V600E*, which can appear as benign in some algorithms. Therefore, laboratories should be that aware the use of this tool must be reinforced with other resources.

Finally, variants interpretation should include a categorization, considering many mutations are still being researched and their impact on a patients disease and treatment is still not clear. There are different systems of classification, that have been reviewed by Horak, Leichsenring, Kreutzfeldt et al. (6). The ESMO Scale for Clinical Actionability of Molecular Targets (ESCAT) is oriented by drugs approved by the EMA, however, due to its complexity, has not been upheld by clinicians (6). Another relevant classification system is the “NCT-Klassifikation” developed by the *Nationales Centrum für Tumorerkrankungen* (NCT) from the University of Heidelberg. This classification was developed for advanced tumors, resistant to therapies, and does not follow guidelines, drug approval nor criteria for clinical trials (6). Li, Datto, Duncavage et al. (3) present the Joint Consensus Recommendation with four categories to report variations illustrated in the table below:

Level	Explanation
A	Prediction of response or resistance to approved drugs or have been included in professional guidelines.
B	Prediction of response or resistance to approved drugs, and or have diagnostic or prognostic value based on well-powered studies, with consensus from experts.

C	Prediction of response or resistance to drugs with off-label, and or have diagnostic or prognostic value based on small studies, use should serve as criteria to the inclusion in clinical trials.
D	Plausible therapeutic significance based on preclinical studies, and or have diagnostic or prognostic potential value based on small studies, multiple case reports and have no consensus.

Table 12 - Joint Consensus Recommendation. Data from Li, Datto, Duncavage et al. (3)

Furthermore, categories A and B imply a strong clinical significance, composing a tier (level of evidence) I, and C and D, with potential clinical significance, tier II. The authors also mention variants of unknown significance as a class of its own, corresponding to tier III. Finally, benign variants comprise those with a high allele frequency but with no evident association with a cancer, they compose tier IV (3). Another point to be observed is the classification of variants as germline or somatic mutations. This identification is of relevance because it can lead to clinical care implications. Li, Datto, Duncavage et al. (3) point out that laboratories can use methods such as a matched control DNA, variant allele frequency (VAF) check, referring to reviewed literature. Moreover, the necessity of patient consent for disclosure and reporting is highlighted by the authors, once a germline mutation has other implications that can even escape the scope of the current clinical disease.

As it can be observed, variant interpretation remains a greatly complex step. Genomic variations can represent modifications of diagnostic, prognostic, therapeutic selection and monitoring, yet evidence based measures are only present in specific cases. There are many resources to assist laboratories in this task, such as publications, guidelines and databases. Accordingly, sphere of competences should be enclosed in the scope of molecular pathologists activities (3).

Other than specific gene testing or gene panels, laboratories also offer whole genome sequencing and whole exome sequencing. Deans, Costa, Cree et al. (10) recognize these strategies as an option which requires longer turnaround times and technically not yet proper for samples of variant DNA and RNA. It presents a lower read depth, what translates into problematic sensitivity, once the proportion of cells with mutation might be smaller and have a greater variety due to tumor heterogeneity. Frequently, variants of unknow significance are detected, which demand a comparison to a simultaneous germline testing, and imposes a difficulty for the clinical interpretation. Lastly, reporting must be signed by competent staff member, and staff

should receive trainings for adequacy of performance. Trainings should be regular and documented.

In order to evaluate the clinical importance of a variant, it is necessary to consider the updated approval status for therapies, diagnostic or prognostic markers. Secondly, variants being currently analyzed in trials and less specific mutations, but known to be part of oncogenesis, should also be taken into consideration.

According to Fiorentino, Scarpelli, Lopez-Beltran et al. (36) molecular tests should be prescribed only under strict indications recommended by the CAP and the Society of Clinical Oncology, additional testing can be provided under the scope of clinical trials. In spite of that, a considerable amount of tests are prescribed, generating expenses to health systems. The authors emphasize the need of reviewing the correspondent tests for each case and the specific therapies.

3.4 Molecular Pathology Training

Fassan (35) advises the work of a pathologist to combine the morphological profile with the molecular profile in order to assure the best method is applied, avoiding extra processing and analysis, which could also make the process more expensive. In this scenario, pathologists need to follow molecular testing guidelines to offer all patients the recommended tests, considering reflex testing in this point, independently of health system´s plans.

The author highlights the requirement for pathologists to be able to handle molecular information, have the know-how to perform a sample assessment and have awareness for matching technology with sample analysis, subsequently delivering diagnostic results that include pathological and molecular information. The author points out the demand for a pathology curriculum that includes molecular concepts and clinical interpretation skills.

The field has indeed gained a new subsection called Molecular Genetic Pathology, with specific training needs addressed by Rosenbaum, Berry, Church et al. (40). In this conception, the committee assume a professional in this area should have the special competencies to interpret and categorize outcomes of a broad scope of genomic analyses, as well as building and validating further methods observing official recommendations and applicable regulations. The fellowship comprises a training of one year in diagnostic of inherited genetic diseases, somatic disease (both solid and

hematological), nonneoplastic somatic disease, infectious disease, immunogenetics and bioinformatics. Weichert (41) indicates curriculum changes in Pathology to enable these professionals to deal with molecular information, which should be also implemented in Germany, despite obstacles related to a lack of “clinician scientists” for pathology laboratories. Moreover, Fiorentino, Scarpelli, Lopez-Beltran et al. (36) see the shift in this area as an opportunity to up enhance the profession, defending the inclusion of molecular pathology in residency programs.

In the current scenario of personalized medicine, the implementation agenda by the National Health Service in the UK, Jones, Oien, Lee et al. (16) emphasize the need to update pathology curricula and training programs in order to integrate new genetic and molecular analysis knowledge. A non-integration could lead to a fragmentation of the field, resulting in a deficit in the development of applicable molecular methods, hindering the full potential, which molecular pathology can bring to personalized medicine.

Moreover, the authors state further areas originated from the emergence of molecular pathology, such as professional biobanking, digital pathology, pathology bioinformatics, and information management. Furthermore, pathological epidemiology need as well to be incorporated into traditional pathology given their potential role to be played in future translational studies.

Kim, Park, Kim et al. (42) indicate that there is only a small count of clinically validated and approved biomarkers, both for predictive and also for prognostic tests. They see the intensified investigation of tumor genetics in clinical trials, with its subsequent accumulation of genomic data, will bring changes to cancer diagnostics and treatment. The number and range of genes to be analyzed depend also on the condition of the patient. In the authors perspective, larger gene panels will be required to the detection of multiple genes and actionable mutations in the future. However, the diagnostic use must keep within the boundaries of updated clinical indications, while broader scopes of genes should be reserved to analysis in trials.

3.5 Liquid Biopsy

Tissue samples depend majorly on tumor anatomical location, i.e., some biopsies can only be performed with a high level of difficulty or are not achievable at all. Another obstacle to tissue samples is the inclusion different cell populations in order to guarantee tumor heterogeneity is covered by analysis. Liquid biopsy, the procedure where circulating tumor cells or DNA fragments contained in peripheral blood are analyzed, represent an alternative to these challenges. Actual research has put efforts into making this non-invasive procedure a possible choice for early tumor detection, cases where tissue biopsy is not possible and oncological monitoring.

Analysis of peripheral blood can concentrate, among other metabolites, on:

- Circulating tumor cells (CTCs)
- Circulating cell-free DNA (cfDNA)
- Circulating tumor DNA (ctDNA)
- Circulating cell-free RNA (cfRNA).

These objects allow conclusions on tumor DNA mutations as well as tumor evolution, progression and metastatic status, and also primary tumor. Nonetheless, liquid biopsy techniques still face obstacles before being adequate to clinical use. The mechanism by which ctDNA is set free in the blood is not fully comprehended, being a topic for further research over the next years. Origin of CTCs and other bodies must be tumoral, which has to be first proved by plasma analysis; tumor heterogeneity coverage, sensitivity and specificity are other issues which are not clear in a possible clinical routine use of ctDNA. Analysis sensitivity is majorly influenced by amount of material available for analysis, i.e., sampling volume, number of cells, tumor fraction (43).

Meddeb et al. (19) published guidelines for preanalytical conditions for cfDNA in 2019 on a systematic review pointing out the main critical points while using cfDNA as a diagnostic tool and the obstacles that occur in the pre-analytical phase. Main issues in the analysis of cfDNA include detection of normal sequences and genetic and epigenetic alterations, quantifying cfDNA and determining fragmentation levels. Heterogeneous blood composition represents a difficulty for cfDNA isolation, moreover, it has a short duration in what concerns enzymatic degradation and clotting. cfDNA is only present in very low quantities in the blood, an attribute that can be

influenced by many different factors, namely, demographic (gender, ethnicity), biological (age, menopause, medication and drugs intake, diet, exposition to toxic substances) and also laboratorial handling procedures (collection tube and time, food intake). In addition, cfDNA can be contaminated by blood cells DNA during cfDNA isolation.

Liquid biopsy can be performed with samples extracted into EDTA tubes, virus and bacteria can be separated by applying buffy coats methods, serum analysis is not recommended, once leucocytes DNA is present. Lithium heparin tubes are not recommended because lithium could influence PCR. Lastly, cf-DNA offers a possibility for mutation analysis, nonetheless, it still has not been incorporated to routine practice yet (19).

Keppens et al. (44) organized in 2020 a scheme for EQA for ctDNA tests based on European EQA programs participating on IQN Path. They monitored 32 laboratories testing of 5 EGFR and *KRAS* and *NRAS* samples each, many of those performing ctDNA tests for some years. Their study found out error rate is still high, above 20%; laboratories experienced issues detecting wild types and providing details over cfDNA amounts used. Some of the laboratories manifested troubles in cfDNA extraction as well as its associated reduced sensitivity. Deans et al. (29) carried out a similar study, accompanying 167 laboratories in Europe, observing a major divergence in methods and platforms, emphasizing the need for accurate EQA concept in what concerns ctDNA testing (29).

3.6 Molecular Pathology, NGS and the Emergence of Personalized Medicine

The relevance of the area, other than the actual applications of molecular methodologies, can be attested through its strict connection to clinicians activities and patients treatment, both configuring areas managed and afforded by health systems. The introduction of such novel technologies in clinical routine hustled and brought to the agenda discussions about means to be established as a plausible and cost-effective offer of personalized medicine.

Fiorentino, Scarpelli, Lopez-Beltran et al. (36) argument there has been a perspective change, meaning that molecular pathology tests are no longer viewed as an additional cost, but rather a source of income. Health systems and insurance are

adopting means to cover those service. Also pharmacological companies developed an interest in investing in molecular pathology studies and their implementation, equally considering the large bio-banks kept by pathology institutions.

Di Sanzo, Cipolloni, Borro et al. (31) analyzed the transformations personalized medicine went through with the arrival of genetic testing for diagnostics and screening. Personalized medicine can have different definitions and approaches, and the authors pointed out the most relevant. According to the European Parliamentary Research Service (45), 'Personalised medicine' is a multi-faceted term without a clear-cut definition. It refers to an emerging approach to medicine that uses scientific insights into the genetic and molecular basis of health and disease brought on by the sequencing of the human genome, to guide decisions with regard to the prediction, prevention, diagnosis and treatment of disease. The aim of personalized medicine is generally perceived to be the 'right treatment for the right person at the right time'. The document explores the “4P” concept of personalized medicine that stands for Predictive, Preventive, Personalized and Participatory.

Furthermore, the authors discuss the role played by the societal factor in the construction of a personalized medicine that can be offered to patients in an egalitarian and ethical form, founded on mature research. Nevertheless, public awareness for this new conception still needs to be achieved, bearing in mind that patients are not just the part interested in new and more effective diagnostic opportunities and treatments, but they bring also their “perceptions, values and expectations”. They also represent the main funders for this recent conceived system, and therefore their understanding of the new methods is crucial.

The several challenges for the definitive application of NGS tests in molecular pathology include, as presented earlier, the analytic validity, not yet well established EQA programs and obstacles to a clear interpretation of mutations. Wilson, Miller and Rousseau (15) stress the gap between the usability of new generated genetic information and the actual possible clinical uses of it. Aside from technical limitations of the different platforms (coverage, error rates, algorithms and databases for selection and interpretation of mutations), there is a large step that must be taken to build unbiased evidence for medical applicability. Furthermore, the authors also see as critical the rise of extensive information that, given its premature research state, contribute to the emergency of a domain, the so called “incidental findings”, which have uncertain clinical significance and weak epidemiological meaning. Besides the costs

implicated in the investigation of this extra data, the analysis of such secondary genomic information can lead to consequences such as overtesting, if the prevalence of a disease is not taken into consideration previously, as well as the risk for individuals to actually develop a treatable condition. The chance to produce false positive results can repercuss in over treatment, generating, in its turn, more expenses.

In this article, the specialists highlight the need to keep genetic testing under the limits of medical indications based on proved clinical benefits, which are at the moment not as large as the volume of genomic targets being investigated. This will enable costs to be affordable by health systems and stakeholders, shielding those resources of being streamed to an uncertain area. Proceeding this way, will also protect patients from purposeless procedures and eventual overtreatments.

As pointed out previously, the need for validation processes and development of specific EQA programs and laboratorial guidelines for molecular pathology and genetic testing are topics in the agenda of major societies and association responsible to assure and approve quality diagnostic tests. Moreover, bioinformatic methods, genetic databases and a possible change in the curriculum of pathology educational programs are being improved and discussed in order to structure a method for such interpretations, form professionals able to deal with this novel type of medical data and, finally, set the base for another step in the construction of a personalized medicine offer to reach all oncological patients concerned.

4 Health Systems

4.1 Health systems and the arrival of NGS testing

According to the World Health Organization, “health systems are responsible for delivering services that improve, maintain or restore the health of individuals and their communities. This includes the care provided by hospitals and family doctors, but also less visible tasks such as the prevention and control of communicable disease, health promotion, health workforce planning and improving the social, economic or environmental conditions in which people live. Health systems are also responsible for the careful management (or stewardship) of these services to ensure that they reach everyone equally, are responsive to individual needs and vulnerabilities, and do not impose an excessive financial burden on individuals or families (46).”

These institutions, originated form public, private or combined initiatives present a wide range of funding models: governmental taxation, insurances (national, public or private), private payments (known as “out of pocket”) and, lastly, philanthropic activities and organizations for non-profit must be taken into consideration. Among health centers, hospitals and various types of clinics participating in a health system, cancer centers, specific hospitals and laboratories are also active in research and are many times associated with universities and research centers.

Traditionally, novel health services are implemented by a health system after critical evaluations on public health necessity, return on investments and generation of economic value. In this process, several stakeholders may take part of funding, notably private corporations in health systems with an opened market participation (47) (17). Trosman, Weldon, Gradishar et al. (17) published an overview on insurance coverage models for NGS in oncology. According to the authors, health systems apply the concept of “medically necessary” or “experimental/investigational” to categorize health services that have the potential to be integrated in the scope covered medical services. The article reveals that many NGS tests are being currently performed in cancer centers in the US, but without (formal) coverage of private and public insurances. The authors criticized the current concepts of “medically necessary” and “experimental/investigational” used by health care systems to classify and enable coverage of certain services. They argument “Next Generation Sequencing blurs the boundaries between these two concepts, making coverage decisions difficult” (47).

They indicate the need for adaptation of this concept in what concerns developing and adopting novel models of coverage for NGTS (next generation tumor sequencing). This technology brings to light genetic findings that are well established and known in oncology, but at the same time opens possibilities for detecting variants newly identified and those still being researched. Therefore, NGS can contribute simultaneously to both clinical uses and investigational purposes, facilitating adequate referrals to clinical trials.

Furthermore, the authors point out, in the latter case, patients often are directed to experimental treatments, which can represent a favorable solution for certain complex cases, advanced or recurrent cancers. Health systems should recognize how this choice configures not only an experimental clinical approach, but at the same time represents a “medically necessary” alternative. Equally, the “off-label” use of certain drugs fit to this same situation, according to the authors. The detection of such variants in an earlier tumor stage can also be beneficial and more economic viable as a means of avoiding additional procedures or non-efficient treatments.

Moreover, they propose a model for understanding well known variants, newly discovered variants and variants still in phase of research. Their conception fits these different categories of variations in a systematic flow, where variants transit from the lower categories (still in research phase) to upper categories (well known) based on novel research findings and evidence.

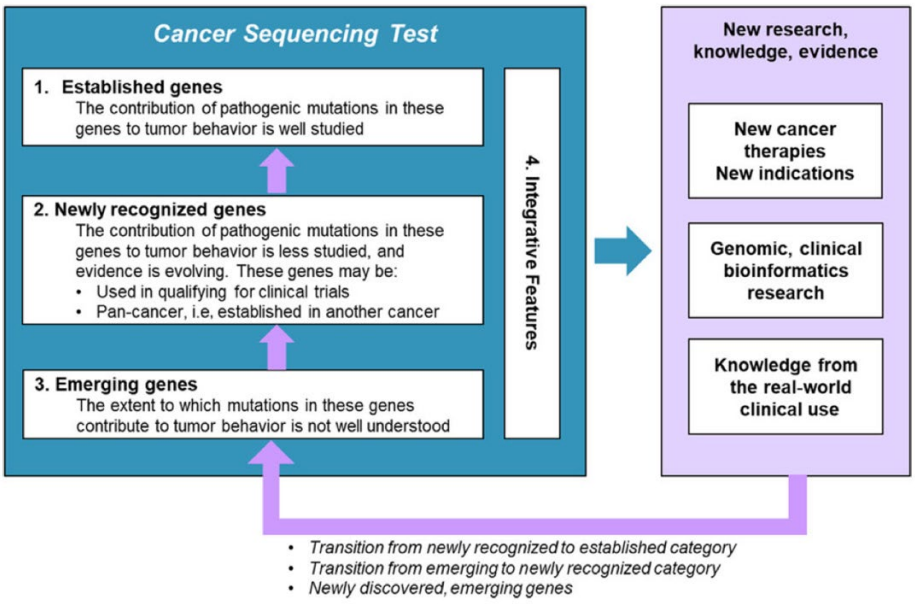

Figure 8 - Model for variants classification from Trosman, Weldon, Gradishar et al. (17)

The composition of gene panels, which is stated by Trosman, Weldon, Gradishar et al., (17) is not static and does not qualify to the same decision pathway for coverage as other clinical services. Additionally, waiting for research to reveal and classify novel variants in a first place in order to approve coverage could delay the detection of these variants for many patients who would profit of clinical trials. Also their families would take advantage of screening in case of germline variants, which can be found. By including analysis of not established genes in a panel, and by taking advantage of electronic integration in local and international databases, the investigational process can be accelerated, bringing benefits for research and helping the cooperation between scientists globally.

Ginsburg and Philips (48) observed that health care systems continue to face the challenge to manage an economic strategy for the crescent implementation of approved laboratory genetic testing. The authors stress the narrow comprehension of the area while analyzing possibilities of coverage. The article points out the need of demonstrated economic value and affordability. Nonetheless, the increasing number of economic evaluations in precision medicine indicate how precision medicine interventions present the same efficiency and cost-benefit assessment.

4.2 Key Elements in the Conception of Genetic Testing in Health Systems

Studies analyzing the implementation of genetic testing in hospitals and medical centers pointed out innumerous challenges reaching from the conception of new services until the realistic integration of genetic testing in a clinical routine. The literature considers not only the benefits for patients, but also the gains for research. The reviews and reports considered in this chapter outline the obstacles, possible solutions and examples and its application in specific medical centers. The key elements observed by authors in this area constitute know-how difficulties for the staff, understanding of the patients, clinical interpretation and application of genetic information, integration of medical records in an electronical system for case overview and solutions to integrate this new information in databases to be used in research as well.

O´Shea, Taylor, Crook et al. (49) synthesized in a comprehensive table the items to be approached in the process of organizing a new genetic health service to

be offered by health systems. They summarize solutions for staff education, interdisciplinarity, documentation and electronic systems.

Complex intervention [24] type	Implementation strategies [23]
Education (health professional or patient)	Face to face education
	Online education
	Written information
	Family history collection proforma
Interdisciplinary practice	Genetic counsellor at multidisciplinary team (MDT) meeting
	Embedded Genetic counsellor in oncology
	Genetic counsellor or oncologist facilitates communication
	Genetics or oncologist led referral pathway
	Patient navigators
Documentation (GC referral, GT outcomes and written information to facilitate mainstreaming)	Use of electronic medical record (EMR) or MDT proforma
	Testing protocol
	Pathway or checklist
	Standardised letters for results
	Consent form
Systems (electronic or process)	Smart text for EMR or pathology reporting
	Synchronous scheduling of GC appointments
	Shared GC referral or review e-mail inbox
	E-mail alerts
	E-mail notifications for referral
	EMR GC referral
	Result tracking

MDT multidisciplinary team, *EMR* electronic medical record, *GC* Genetic Counselling, *GT* Genetic testing.

Table 13 - Elements for organizing genetic testing in Health Systems from O´Shea, Taylor, Crook (49)

Morgan, Hanna and Yousef (50) analyzed different methods to bring new clinical practices into health systems structures. Mostly, aspects to be considered in order to achieve this under the perspective of, among other, molecular pathology and oncology are three: patient oriented decision making, professional training and resources of health systems. The authors mention the habitual long way between the empirical finding of new medical procedures and their realistic implementation as health care offer. They point out, for instance, the need of analytical standardization and careful selection for genomic targets.

Patient care is a topic demanding attention for several factors. Besides personal aspects of each patient, such as specific diagnose and treatment needs, psychological and social factors ought to be considered as well. They influence strongly how patients deal with their conditions and also their responses and cooperation during tests, treatment and follow up. Authors stress also that these elements influence sometimes

an entire family, distinctively in the context of a familial syndromes and if there are minors in risk. Considering a wider level, social factors must also be seen under the perspective of possible insurance segregation.

According to the authors, an action plan to integrate novel tests in a health system must go firstly through the knowledge creation, i. e., interpretation of clinical trials results according to a clinical context. Data must be exchanged between research centers internationally and different professionals in a wide multidisciplinary cooperation. A supportive communication between academic experts and decision makers from health systems must be established. Finally, a key element in this planning phase is patient engagement. A second phase constitutes in the implementation of the actual services, including the design of the new products and services and analysis of their distribution. This means developing a concept of a product introduction, assessment and viability. In this process compliance to already existing regulations must be observed, and if necessary, new policies should be pursued.

An interesting perspective is described by the authors, the dissemination of information and announcement of new products goes frequently over social media, configuring nontraditional communication platforms. They offer the possibility of sharing research results, asking for patients feedback and generating engagement, as well as measuring new products outcomes. This can be explored for more dynamic and faster results. The decision making must be a shared process between all the involved groups. Finally, based outcomes and feedback, adjustments must be implemented and measures for facilitating the acknowledgment of the new structure.

The graph below shows a detailed scheme for implementing a new health service, according to the conceptualization proposed by the authors. It summarizes all items belonging in each step, from discovery, to product creation (knowledge translation) and achieving clinical practice.

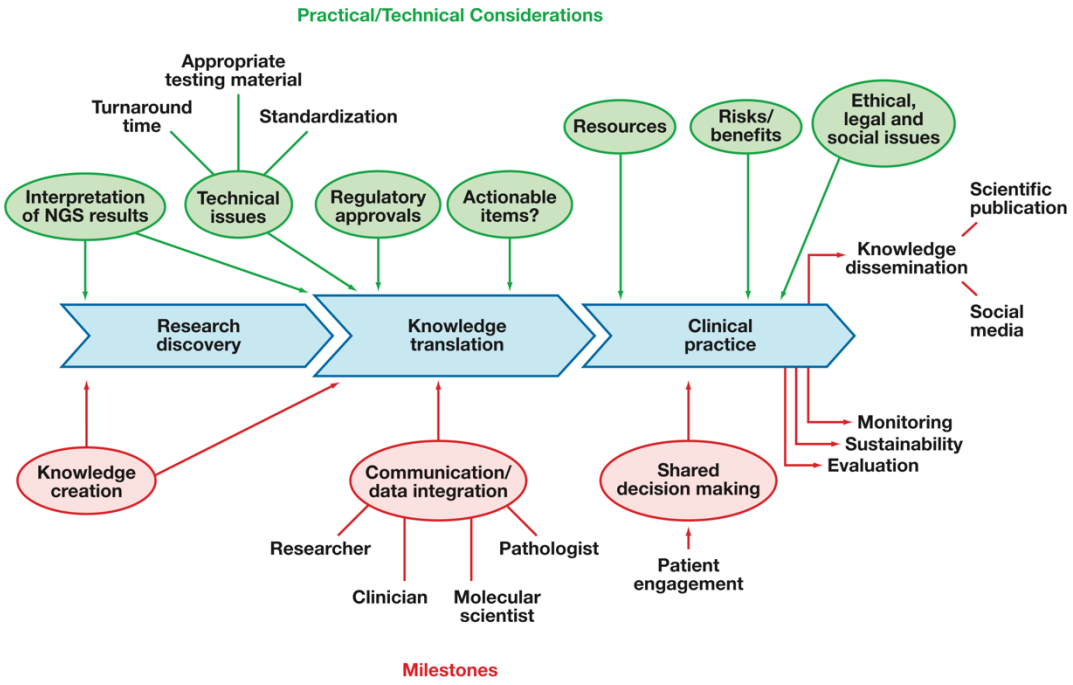

Figure 9 - Elements for implementation of new health service from Morgan, Hanna and Yousef (50)

Ginsburg and Philips (48) investigated the interplay between informatics, analyses and precision medicine in the context of health systems conceptualization, under a perspective where research is integrated in clinical routine and which tools can enable such approach.

The first aspect described by the authors highlights the importance of electronic medical records and information technology (IT). The great volume of data generated by genetic tests which can give origin to new findings when cross-referenced with bio databases, patient information, inclusive family history. This information can be used not only for patient care, but also for knowledge bases in laboratories. Further, this type of databases can be combined with international networks, contributing to the dissemination of knowledge. In turn, these references can be used as basis for improvements of health systems. The authors defend a complementary interaction between research, clinical and laboratorial practices and health systems. These agents should operate contributing to the constant update one of another. In order to achieve this concept, data systems must be programed to attend these needs. In this process, patient engagement and informed contentment must be an integrated part of such systems.

The researchers point out that the combination of information systems can assume the function of powering the identification and interpretation of new genetic

variants. “The power of genomics-enabled research and health care is proportional to the amount of data that can be accessed and analyzed (48).”

Health care systems can use genomic integrated data to establish methods for improving population health, heath care and orientation to professionals, through updated guidelines. The applications of such data extend also to strategies for policies and regulations, as well as economic planning.

Grant, MPhil, Langlois et al. (51) estimate that there are around 75,000 genetic tests on the market, and around 10 new tests arrive every day. This poses a challenge for health systems and regulatory bodies, that are set against the need to assess constant test innovations for viability of coverage and update of guidelines and regulations.

Decision making process in health care demands proof of clinical utility, resulting in the denial of approval for many new tests that still lack medical evidence. Furthermore, different types of healthcare plans, implicating different payments, and geographical location are factors to be included in the strategical implementation of novel tests. The article considered studies measuring health care systems genetic tests implementation in the USA and Canada for different genetic diseases from 1993 to 2020, among those, cancer diseases from 1996 to 2020. From the patients perspective, as reported by the authors, a discouragement has been found if the test must be self-financed, even with the possibility for later reimbursements. They concluded that patients who were referred for oncological genetic tests did not undergo the exam alleging majorly economic issues in the past. Nevertheless, authors detect a changing trend with patients willing and being more interested in such diagnostic methods given a possibility of insurance coverage.

The authors conclude that it is necessary to act against the inconsistent referral of genetic tests, improve patient and clinicians and health care providers´ comprehension. Training, regulations and guidelines are the resources to be applied and aid the adequate resource management for accessible genetic testing.

Biswas, Medne, Devkota et al. (52) published their experience in the Children´s Hospital of Philadelphia. This facility was fully adapted to integrate genetic testing in its clinical routine, as well as allowing the use of this data for research and health care purposes. The project lasted for 4 years and analyzed more than 3400 patients referred to genetic testing. This report is the first project in a pediatric hospital patient setting.

The authors listed their challenges starting from the lack of specialized staff and the difficulties and lack of time experienced by clinicians and other care providers dealing with such information. Other early obstacles comprised the lack of confidence genetic information, which would contribute with positive results to health care, return on investment, integration of new actionable findings in the electronic health register, communication of test results, management of secondary findings, and the follow-up for patients and family members.

The hospital implemented an orientation support for clinicians providing overviews on adequate genetic testing based on symptoms, findings, family history and examination. Aid on clinical interpretation was also available. The support included resources for patient education and consent and insurance authorizations as well.

The hospital established a body in charge of addressing these issues. The Roberts Individualized Medical Genetics Center (RIMGC) implemented a sequencing pipeline gathering phenotype information from physical examinations, generating HPO (human phenotype ontology) terms, clinical associations and sequencing results and interpretation. The center also elaborated metrics for quality improvement.

The following graph represents all the divisions of the new structured hospital listing all their functions.

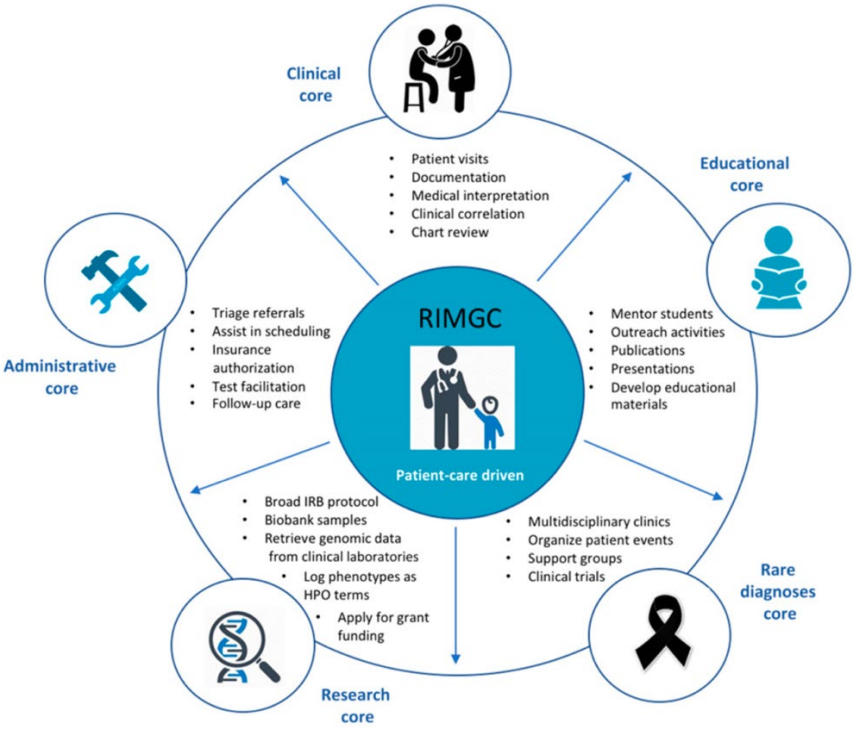

Figure 10 - RIMGC structure from Biswas (52), Medne, Devkota et al.

Evans and Manchanda (53) point out the lasting inequality and underutilization of genetic testing, resulting in the dissipation of possibilities, which these technologies may bring for precision medicine.

The researchers also studied comparisons between different methods of genetic counseling: per DVD, telephone, group and other distance communications. Conclusions were that all of these methods showed noninferior efficiency in relation to the standard personal counseling. This opens the possibilities to genetic counseling in rural and distant locations.

The authors indicate the need for studies on ethical and legal impact of genetic testing, as well as the influences on mental health, quality of life and other social consequences. The narrow health system approach to diagnostic and treatment, not focusing on prevention, is criticized by the authors. Prevention of cancerous diseases is a crucial factor, configuring an obstacle to heath systems. Therefore, they stress the necessity of translational studies for genetic screening, suggesting further comprehension on variants of uncertain significance (VUS), which can help to stratify the population and enable an efficient screening. Finally, dissemination of guidelines into clinical routine should be pursued through health campaigns and education programs.

In the long run, if health systems organize their structure and manage to build a system including the mentioned factors, considering additionally the increasing test costs drops, the authors predict an efficient implementation of genetic testing for large populations, inclusively in emerging countries.

Nogueira, Silva-Fernandes, Albuquerque et al. (54) analyzed the genetic testing implementation in a health system for the first time in the North-East Region of Brazil. The implementation phase was ran for one year, providing genetic testing services to over 300 families. The project was implemented with the PDCA (Plan, Do, Check and Act) model, known too by the denomination of Shewhart cycle or Deming wheel. The plan was elected given its management efficiency in 4 phases bringing possibilities to verify and implement improvements during the process. The method contributed not only with handling organizational difficulties but as well brought administrative and professional experience to the staff. The authors describe a behavior change was necessary to deal with implementation steps, and the Check phase lead to an overview on the differences between expected and achieved results. On its turn, this method allowed the application of correcting and improving measures.

Another obstacle exposed by this study reveals the problems with accessing patient information to include them in the trial. Due to differences in health care systems, patient data were often incomplete, leading to the exclusion of participants presenting histopathological findings that would qualify them for the trial.

The authors observed about 90-95% of tumors were sporadic, while the remainder was linked to cancer hereditary syndromes (HCPS). Screening of patients with neoplasms and HCPS risk was found to improve mortality and morbidity rates after choosing preventive care means. Despite the international recommendation from the National Comprehensive Cancer Network (NCCN) genetic testing is offered with genetic counseling, the region was not able to provide this service in all cases, give limited resources.

The article concluded “some of the patients and/or family members diagnosed with pathogenic mutations as part of the project did not meet SUS (national health system – “*Sistema Único de Saúde”*) criteria (for example, with respect to age) for free-of-charge access to medical imaging. This evidenced the need for changes in both institutional and public policies (54).”

Moreover, the study showed the necessity of further investigations for cost-benefit assessment, considering the probable cost reduction in primary and secondary prevention, in other words, a minor incidence rate and earlier detection. These reductions, in their turn, leading to fewer advanced treatments, longer survival rate, which translates into lower overall costs. The authors outlined a possible strategy for a definitive implementation of the project after the research financed phase. In order to achieve a long term economic system, the authors pointed out the necessity to train the staff, with focus on detection of pathogenic variations. At the same time, on the financial context, they outlined the need for partnerships with the State Department of Health and other health care providers (54).

5 Discussion and Conclusion

5.1 Discussion

5.1.1 Laboratory Management

The arrival of genetic tests as biomarkers for diagnostics, prognose and targeted therapy planning has to be recognized as an innovative clinical strategy with distinctive characteristics in what concerns research and medical applications. Essentially, the investigational aspect must be seen as indissociable from the clinical use. Along with this qualification, medical and technical research and equipment advances are continuous and raise challenges for clinicians, laboratories and legislators to uphold the latest status.

Medical laboratories providing genetic services to clinics and patients face obstacles to design tests that can be validated and formally approved, keep updated with both methodological and technical new advances and organize regular staff training.

Pre-analytical and analytical difficulties start at the sample processing level. They consist, firstly, in sample entry, identification, tissue fixation, transportation. Further, there is DNA extraction, quantification, storage, libraries preparation and storage (10) (11). Finally, results should be reported with correct identification, concise and include the necessary information, interpretation, as well as possible limitations. The nomenclature should follow the HGVS rule. Lastly, availability in a LIS and LIMS is recommended in order to facilitate access to all clinical team, but also for research purposes (10). The next challenges are encountered while developing systems for prioritizing variants, a step that needs to include multidisciplinary efforts to interpret clinical relevance, updated literature and adequate evaluation of coverage (12). Furthermore, hassles are also found in the management level considering both external requirements for regulatory approvals and EQA programs, as well as developing internal quality assessment systems. In addition to that, medical laboratories must be able to find an in-house cost-effective schedule, consider turnaround times, choose adequate equipment and kits in a context of continuously advancing technologies (11). In addition to that, laboratories must ensure staff goes

through constant training, and the necessity for specialized professionals represents an increasing demand (9).

A further complicating element for laboratories is the panel design. Laboratories can use and offer panels already designed by sequencing suppliers or have the option to develop their own. The key factor in this procedure is the consideration of updated literature, as well as documentation and validation. Additionally, the design of own panels involve cost implications, interpretation complexity and time (20).

5.1.2 Molecular Pathology

Among the changes brought by genetic testing, the area of Molecular Pathology has been through considerable transformations. Oncology care, considering current guidelines from oncological and hematological societies, has included innumerous mutations for prevention, diagnostic, targeted therapy and follow-up. (31) The establishment of NGS as equipment and method of choice for gene panels represents another challenge in this area, both of technical and medical relevance. The list below briefly summarizes the technical aspects in the development of NGS gene panels:

- Sensitivity
- Specificity
- Elimination of artifacts
- Solutions for tumor heterogeneity and sample handling
- Analysis range
- Coverage and accuracy
- Repeatability, reproducibility (35).

On the other hand, variant interpretation configures the step where technical and medical challenges gather and demands high expertise in order to deliver clinical relevant test results. Although a number of scientific resources are accessible online, evaluation of variants currently does not configure a standardized process (3). In this step, it is relevant to consider updated publications on analyzed genes, tumor in question and recommendations of oncological societies and guidelines. Genetic online databases are often tools which require a specialized background for its use. They

must be comprehended in their scope, limitations, and functioning, i. e., how and which information is added and processed (3).

Different schemes for categorizing variants have been formulated by experts bodies, the most relevant are the ESMO Scale for Clinical Actionability of Molecular Targets (ESCAT), the NCT-Klassifikation developed by the *Nationales Centrum für Tumorerkrankungen* from the University of Heidelberg and the Joint Consensus Recommendation (6). These classifications should be used to separate mutations that are considered actionable, i. e., can influence a treatment, follow-up and other types of care, and mutations that are still being investigated, which could potentially reduce test sensitivity. In its turn, the use of such classifications is also pertinent as it contributes to ongoing research.

As observed, molecular pathology tests, under this perspective, gained a new range of information to be considered in tests performed for oncological patients. Professionals acting in this area are now responsible for the morphological characterization of the different types of tumors, the molecular profile and the integration of both fields of data in a result report alongside with its clinical interpretation. For this reason, authorities such as the Association for Molecular Pathology, endorse a curriculum transformation in order to train a new generation of pathologists in these competences (40). Experts recognize the increasing demand for qualified staff, and point out the lack of this development could lead to a fragmentation in the area and more difficulties in the integration of knowledge that is needed for modern cancer research, according to Jones, Oien, Lee et al. (16). Other than that, and alongside with all technological advances, molecular pathology laboratories saw the emergence of innovations in auxiliary areas such as biobanking, bioinformatics, digital pathology databases and pathological epidemiology (15).

5.1.3 Considerations on Genetic Molecular Pathology and the Emergence of Personalized Medicine

Novel methodologies applied for oncological care gather at their core a multidisciplinary interaction between researchers, clinicians, medical laboratories, patients and, finally, health care systems. This modality of health service must be approached as personalized medicine applicability, bringing different management demands for its implementation.

Personalized medicine, briefly reiterated, is a clinical approach that makes uses of genetic and molecular findings to prevention in risk populations, prognostic estimations and to direct treatment. It acknowledges the individual genetic and biochemical profiles, seeking the best fit treatment and care for each person (45). In order for such concept to be implemented by health systems it must be cost-effective and equalitarian, therefore, multiple changes are necessary in health care conception, increasing awareness on genetic testing specific knowledge and a patient oriented care.

5.1.4 Health systems

One of the essential transformations in health systems consist in changing the current paradigms on necessity of clinical services. As visited in Chapter 3, the arrival of genetic testing in oncology brought not only innovative methods for cancer treatment, but also altered the perspective of integrated research and care.

Models proposed by both by molecular pathology and translational studies to classify variants present a structure with different levels. As exemplified by Trosman, Weldon, Gradishar et al. (17), variants are divided as less known, or recently found; being object of investigations; or are well recognized as pathological, configuring a clinical indication for testing. Based on this conception, health system coverage would not depend on research confirmation beforehand but could fund testing of variants from the first two categories aforementioned. Objectively, patients could profit from experimental trials and use of “off-label” drugs, on the other hand, research databases could gain information and contribute to the scientific investigation of such variants.

Patient oriented decision making should take into account the consequences of the understanding level patients have about their condition, the possibilities offered by a genetic testing, i.e., how it influences their treatment and possible treatment outcomes. Moreover, this understanding leads to better cooperation from the patients, as demonstrated by Morgan, Hanna and Yousef (50). Furthermore, other influencing factors such as social and psychological conditions are equally relevant and can also affect patient cooperation. Finally, in cases when a familiar syndrome is detected, patients often show concerning other family members might develop a tumor, especially children (50).

Different types of resources for genetic counseling directed to patients are available with comparable positive results. Personal consulting, DVD recordings, telephone and video-call appointments are some of the means that have proved to inform patients and increase comprehension levels. Distance channels count with the advantage to reach geographically distant populations and have an including effect in the offering of new health care services (53).

Patient engagement is essential for the implementation of genetic testing by health systems, since they represent a fraction of health care stakeholders as well. If the treated and tested population does not comprehend how the new service can contribute to their condition and is not aware of the health care conceptions being offered, risks of an inefficient treatment increase. Repercussions of this lack of knowledge consist in incomplete or delayed diagnostics, inadequate treatment and, thus, increasing negative outcomes, morbidity and mortality. Secondly, as patients must be recognized as participating decision makers. They are responsible for generating health care demands and sociopolitical pressure towards these.

Considering the aforementioned, challenges imposed to medical laboratories, the transformations molecular pathology is undergoing and, finally, the new concepts to be considered by health systems, originated a literature supporting an integrated model of research, clinical routine and information exchange. Efforts already carried with this approach, such as the Children´s Hospital of Philadelphia (52) indicate the benefits for all agents participating on this interplay. A unified system where treating physicians can access most recent research results and recommendations and guidelines from oncological societies assure health care consistency and efficiency of treatment. On the research level, data on less known variants can be gained and contribute to more dynamic scientific investigations. Conclusively, once health systems operate with this approach, decision makers in this area can also benefit from data demonstrating advantages for patients, and which, in turn, make possible estimates for further system improvements (52).

5.2 Conclusion

Genetic testing is not only a technological arrival bringing various transformations in oncology care, but also represents a novel approach for medical routine itself. Targeted therapy options, precision molecular biomarkers for diagnostics, prognostics and follow-up constitute innovative clinical means unveiling interesting possibilities, however, its use implicate some peculiarities. Those are, as pointed out above, the necessity for laboratorial regulations and guidelines in order to standardize genetic tests and enable a qualitative health service. Secondly, the need of transformations in molecular pathology laboratories that approach the handling of new technologies and the level of expertise. Finally, personalized medicine is an exclusive concept that allows the application of genetic test for clinicians, and its implementation, in turn, must also be considered along with its specificities as a unique health care system concept. Additionally, the utilization of sequencing information in the clinical routine implicates its simultaneous role played in ongoing research.

On health care providers side, economic models for both implementation and integration of these plans have to be studied under the perspective of cost-effectiveness, especially in the long-run with the help of translational studies.

Finally, the totality of these aspects must be deeply investigated in order to find solutions to current technical and medical challenges, as well as solutions for the integration of the peculiarities aforementioned. Finally, this analysis must occur along with the consideration of results of already implemented efforts. As these examples have already demonstrated, modern oncology care provided with edge genetic and molecular findings, accessible to all patients with a clear indication, qualifies for prompt implementation.

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